



Universiteit
Leiden
The Netherlands

New approaches to imaging and treatment of ocular melanoma

Brouwer, N.J.

Citation

Brouwer, N. J. (2022, April 13). *New approaches to imaging and treatment of ocular melanoma*. Retrieved from <https://hdl.handle.net/1887/3283451>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3283451>

Note: To cite this publication please use the final published version (if applicable).



3.1

Conjunctival Melanoma: New Insights in Tumour Genetics and Immunology, Leading to New Therapeutic Options

Niels J. Brouwer¹, Robert M. Verdijk^{1,2,3}, Steffen Heegaard^{4,5}, Marina Marinkovic¹, Bitá Esmali⁶, Martine J. Jager^{1*}

Progress in Ret Eye Res 2022; 86:100971

- 1 Department of Ophthalmology, Leiden University Medical Center, Leiden, The Netherlands
- 2 Department of Pathology, Leiden University Medical Center, Leiden, The Netherlands
- 3 Department of Pathology, Erasmus University Medical Center, Rotterdam, The Netherlands
- 4 Department of Ophthalmology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
- 5 Department of Pathology, Eye pathology section, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark
- 6 Department of Plastic Surgery, Orbital Oncology and Ophthalmic Plastic Surgery, M.D. Anderson Cancer Center, Houston, Texas, USA

ABSTRACT

Recent developments in oncology have led to a better molecular and cellular understanding of cancer, and the introduction of novel therapies. Conjunctival melanoma (CoM) is a rare but potentially devastating disease. A better understanding of CoM, leading to the development of novel therapies, is urgently needed.

CoM is characterized by mutations that have also been identified in cutaneous melanoma, e.g. in *BRAF*, *NRAS* and *TERT*. These mutations are distinct from the mutations found in uveal melanoma (UM), affecting genes such as *GNAQ*, *GNA11*, and *BAP1*. Targeted therapies that are successful in cutaneous melanoma may therefore be useful in CoM.

A recent breakthrough in the treatment of patients with metastatic cutaneous melanoma was the development of immunotherapy. While immunotherapy is currently sparsely effective in intraocular tumours such as UM, the similarities between CoM and cutaneous melanoma (including in their immunological tumour micro environment) provide hope for the application of immunotherapy in CoM, and preliminary clinical data are indeed emerging to support this use.

This review aims to provide a comprehensive overview of the current knowledge regarding CoM, with a focus on the genetic and immunologic understanding. We elaborate on the distinct position of CoM in contrast to other types of melanoma, and explain how new insights in the pathophysiology of this disease guide the development of new, personalized, treatments.

Article Highlights

- CoM is a rare but potentially deadly extraocular tumour, with a rising incidence.
- Genetic mutations in CoM resemble those in cutaneous melanoma, but not UM.
- The presence of immune cells is important for the development and control of CoM.
- Targeted therapy and checkpoint inhibitors can be applied to treat CoM patients.

1. INTRODUCTION

Conjunctival melanoma (CoM) is a rare but potentially devastating disease. With an incidence of 0.3 – 0.8/million in Caucasian adults,¹⁻⁴ it accounts for about 5% of ocular melanoma cases.^{4,5} The estimated number of new cases per year is 130 in the USA, and 320 in Europe. CoM originates from melanocytes in the basal layers of the conjunctiva, and can develop in an area of primary acquired melanosis (PAM), in a nevus, or *de novo*.⁶ The role of ultraviolet (UV) radiation in CoM development is under discussion, following long-standing epidemiological and more recent genetic work.

Originating from the conjunctiva, CoM is a mucosal melanoma with much resemblance to melanoma of the skin.^{7,8} This may be no surprise when looking at the histological and functional similarities of these epithelial tissues. CoM is a very different entity compared to uveal melanoma (UM), which affects the choroid, ciliary body or iris,⁹ and CoM and UM have distinct aetiologies and genetic backgrounds.¹⁰

In clinical practice, CoM most typically presents as a pigmented lesion near the limbus of the eye. Any part of the conjunctiva can be affected, however, and lesions may range from amelanotic to deeply pigmented or even black (Figure 1). Localised disease is commonly treated by surgical excision and adjuvant therapy (such as cryotherapy, radiotherapy or topical chemotherapy), while widespread disease on the ocular surface or palpebral conjunctiva may need more extensive therapy such as orbital exenteration.¹¹ Despite treatment, up to 66% of CoM patients may develop local recurrences,¹² and up to 38% will die due to the disease within 10 years of primary treatment (Figure 2).^{1,13} Risk factors for metastases formation include a greater tumour thickness, non-bulbar location, low tumour pigmentation, histologic ulceration, and local invasion.^{6,14-16} A better understanding of CoM, leading to novel therapies, is therefore urgently needed.

Recent developments in the field of oncology have led to a better understanding of cancer and the introduction of novel therapies. These therapies include ‘targeted therapy’, aiming at specific cellular pathways and genetic mutations of cancer cells, and ‘immunotherapy’, that activates the patient’s own immune system to block tumour growth. Knowledge of the genetic and immunologic environment of CoM may expedite the introduction of these therapies in CoM.

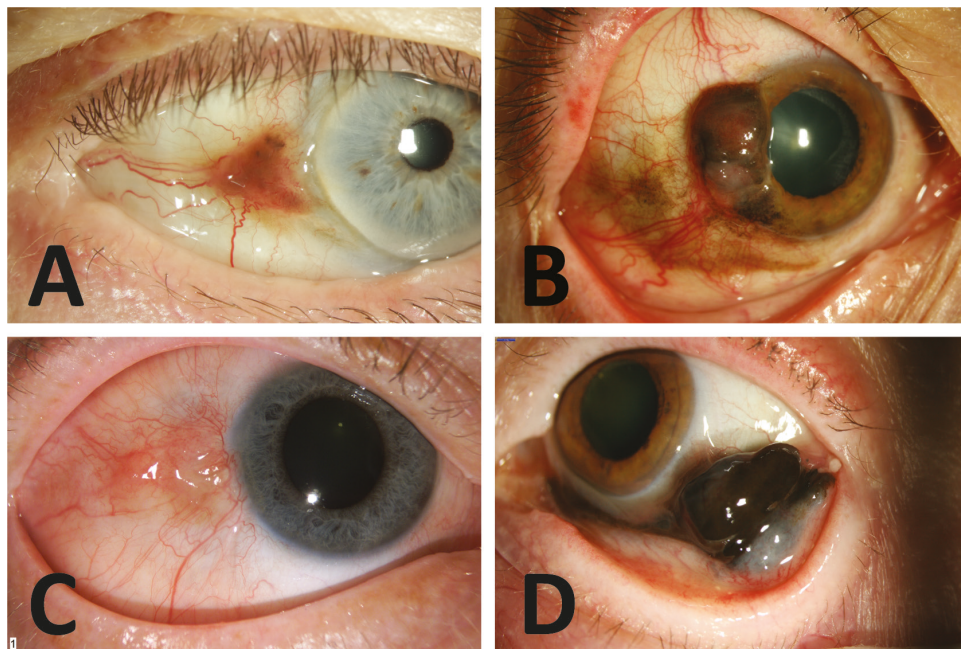


Figure 1. Conjunctival melanoma (CoM). The clinical presentation of CoM varies, as the disease can present at any part of the conjunctiva, and the colour can range from amelanotic to deeply pigmented. Treatment options are largely based on tumour size and location. (A) Localized lesion of the bulbar and limbal conjunctiva, with light-to-medium pigmentation. (B) Large pigmented lesion of the limbal conjunctiva, with an extensive area of primary acquired melanosis (PAM) on the inferior bulbar and forniceal conjunctiva. Note the marked conjunctival vessels approaching the nodular lesion. (C) Amelanotic bulbar lesion, three years after excision of an earlier CoM. (D) Large pigmented lesion, hidden in the inferior fornix of the eye. The obscured location of this lesion caused delayed presentation, which limited therapeutic options.

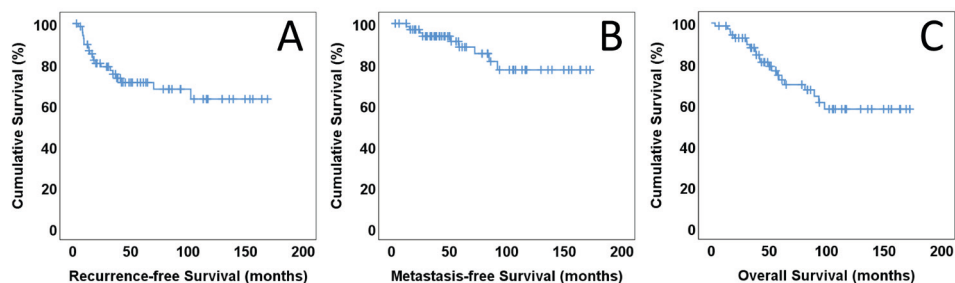


Figure 2. Clinical outcome of CoM patients. Kaplan-Meier analysis of 70 CoM patients, treated between 2001 and 2014 in The Netherlands. Included were 54 (77%) T1 tumours and 16 (23%) T2 tumours, mean tumour thickness was 2.3mm. Mean follow-up time was 70.2 months. (A) recurrence-free survival, (B) metastasis-free survival, (C) overall survival. [The cohort was reported earlier by Brouwer et al, 2018.¹³]

The genetic background of CoM is characterized by mutations in genes such as *BRAF*, *NRAS*, and *TERT*.^{17,18} These mutations are common in cutaneous melanoma as well, and distinct from the mutations that occur in UM, affecting e.g. *GNAQ*, *GNAI1*, and *BAP1*. Targeted therapies have recently been introduced successfully in the treatment of cutaneous melanoma: the use of vemurafenib (targeting the *BRAF* mutation) resulted in a better overall survival of cutaneous melanoma patients.¹⁹ New insights regarding the development of treatment resistance led to the combined therapy of *BRAF* and MEK (i.e. *Mitogen-activated ERK kinase*) inhibitors, with even better results.²⁰ Because of the molecular resemblance of CoM and cutaneous melanoma, these drug developments may be introduced to treat CoM. Some promising case studies of targeted therapy in CoM have been published recently,²¹ and further (pre-clinical) studies are being performed.

The tumour micro-environment of different types of melanoma has been studied for many decades, but is under increasing interest following the discovery of immunotherapy that enhances the body's own immune system to attack tumour cells. Examples of this breakthrough in the treatment of metastatic melanoma are checkpoint-inhibitor therapies with ipilimumab (targeting CTLA-4) and nivolumab (targeting PD-1), which via different routes activate a CD8+ T cell response. Early studies showed improved survival in patients with unresectable metastatic cutaneous melanoma who were treated with ipilimumab, compared to gp100 vaccination.²² Later studies found improved survival for nivolumab treatment compared to dacarbazine chemotherapy.²³ While the success of immunotherapy is as yet limited in intraocular UM²⁴ (possibly because of the immune privilege of the eye where tumour escape mechanisms hamper immune surveillance),²⁵ the similarities in tumour micro environment between extra-ocular CoM and cutaneous melanoma led to the belief that immunotherapy should also be applied to CoM. Promising data on small scale use of immunotherapy in CoM have been reported,^{26,27} and the evaluation of the newest therapies is awaited.

This review aims to provide an overview of the current knowledge regarding the genetic and immunologic understanding of CoM, and the implications for treatment. We touch upon similarities and differences between CoM and other types of melanoma, and explain how new insights in the pathophysiology of this disease guide the development of new therapies.

2. EPIDEMIOLOGY AND ETIOLOGY

General incidence

The incidence of CoM ranges between 0.3 and 0.8/million in Caucasian adults.^{1,3,5,12,28} It is the second most prevalent malignancy of the conjunctiva, after squamous cell carcinoma (also known as 'ocular surface squamous neoplasia, OSSN').²⁹ CoM accounts for approximately 5% of all primary ocular melanoma, being overshadowed by the far more prevalent UM.^{4,5} The incidence of CoM

increases with age: CoM mainly affects patients from the fifth/sixth decade of life onwards,^{1-4,30} and is rare in children and adolescents.³¹ The incidence can be considered equal between men and women, although some studies report a slightly higher incidence amongst males (with a male-to-female ratio of 1.26:1 and 1.29:1).^{5,32} For the USA and Europe, with a current population of 335 million and 740 million,^{33,34} the overall incidence results into an estimated 130 and 320 new cases of CoM per year, respectively.^{5,28}

The number of reports on the incidence of CoM is limited. In national registries, CoM is often classified together with other types of ocular melanoma, limiting the ability to obtain tumour-specific data.^{2,28} Current data mainly originate from North America or Europe, limiting data on population groups other than Caucasians.

Geographical and racial differences

The incidence of CoM varies between geographical areas as well as between population groups with a different racial background. This may point towards a genetic (or population-related) predisposition, as well as a role for environmental factors (such as UV-radiation) in development of CoM.

CoM is typically considered a disease of people with (northern) European ancestry, occurring most frequently in the Nordic countries and parts of North America (Figure 3); however, it can impact people of any descent. A significantly higher incidence has been observed among Non-Hispanic Whites (0.49/million) compared to Hispanics (0.33/million), Blacks (0.18/million), American Indians (0.17/million), and Asians (0.15/million) in a large American study on CoM and race.³⁵ Recent work from Canada identified a higher incidence of CoM in the eastern Canadian provinces, with presumably many inhabitants of European descent,³² corresponding with elevated incidences of cutaneous melanoma. In this study, the incidence of CoM was somewhat lower in Canada compared to the USA. This was attributed to the South-to-North gradient, with a lower occurrence of CoM in Canada due to less UV-radiation at the higher latitude.³⁶ This Canadian study demonstrated that effects from both latitude (comparing Canada and the USA) as well as ethnic background (within Canada itself) are important factors in CoM development. A recent population-based study from Europe found a CoM incidence of 0.28/million in Southern Europe, and up to 0.90/million in Northern Europe.²⁸ The highest incidences were found in Norway, The Netherlands and Switzerland, which were also the countries with the highest incidence of cutaneous melanoma. This study did not identify a significant association between the incidence of CoM and the latitude of the reported countries, which may be due to an analysis that did not stratify for racial background, levelling out an effect of latitude.

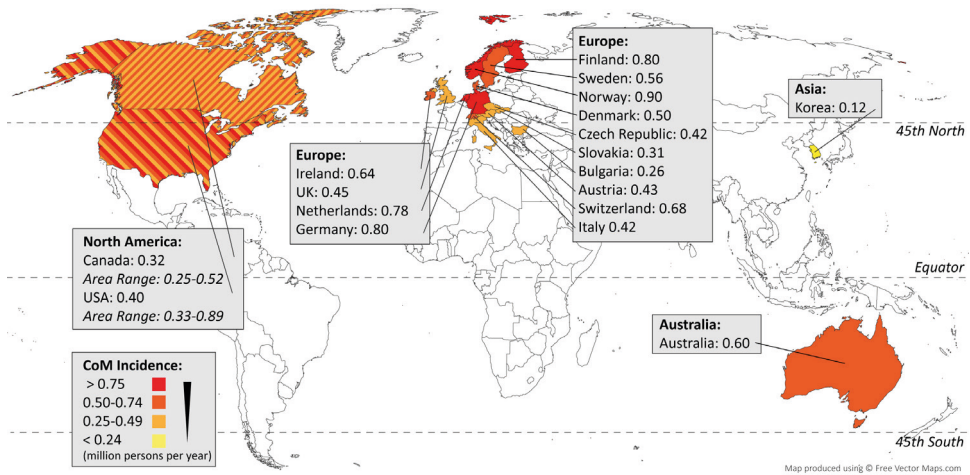


Figure 3. World map of the incidence of conjunctival melanoma. Data are depicted for countries with known incidence data based on more than 15 cases. For the USA and Canada, data are presented with a range since there is significant spread within these large countries. Incidence data source: (North America) Canada,³² USA;^{5,36} (Europe) Finland,¹ Sweden,³ Denmark,³⁰ Germany,³⁷ Ireland, UK, Netherlands, Norway, Czech Republic, Slovakia, Bulgaria, Austria, Switzerland, and Italy;²⁸ (Asia) Korea;³⁸ (Australia).³⁹

Epidemiologic observations from CoM in the US partially parallel those of UM, a disease that is most prevalent in non-Hispanic Whites (6.02/million), and less frequently seen in Hispanics (1.67/million), Asians (0.38/million) and Blacks (0.31/million).⁴⁰ Illustrating different aetiologies between CoM and UM, a significant *higher* UM incidence was observed for northern latitudes compared to other latitudes in an American³⁶ as well as a European⁴¹ study.

In non-Caucasian populations, despite the rarity in absolute numbers, CoM is relatively prevalent compared to other ocular melanomas. A registry from South Korea found that 19% of all ocular melanoma were CoM,³⁸ which is much higher than the 5% in an American data set.⁵ In the American *National Cancer Institute's Surveillance, Epidemiology and End Results* (SEER) registry, for non-Hispanic whites, an incidence ratio of 12.7 UM to every CoM was established, while this was 2.2 UM per CoM in Blacks, and 1.7 UM per CoM in Asians.³⁵ As will be further described in the chapter on genetics [chapter 3], mutations in CoM may differ between different populations, warranting studies into specific behaviour.⁴² It is promising that awareness of CoM is increasing globally: in recent years, this resulted in (a non-exhaustive list of) reports from China,^{42,43} Taiwan,⁴⁴ Japan,⁴⁵ South Korea,^{38,46} Nigeria,⁴⁷ and Mexico.⁴⁸

Time trends

The incidence of CoM has been rising during the last few decades (Table 1).^{1-3,30} Some studies report stable incidences, but this may be due to limitations in study size or a short studied time span.^{4,32,38,49,50} Between 1960 and 2005, the age-standardized incidence in Sweden has risen from 0.08 per million to 0.56 per million, with a more frequent occurrence on the bulbar parts of the conjunctiva.³ A similar pattern was observed in Denmark, with a peak incidence of 0.87 per million in 2000-2009, and an increase in bulbar lesions between 1960 to 2012.³⁰ As will be discussed later on, there may be an etiologic and genetic difference between bulbar and non-bulbar CoM. In the USA, data from the SEER database showed an overall increase in the incidence of CoM between 1973 and 1999, age-adjusted from 0.22 to 0.46 per million.² Stratified for gender, however, a clear increase was seen in the incidence amongst men, but not amongst women. The authors hypothesized that this gender difference may be caused by differences in sunlight protection and outdoor activities. A recent large European study showed no significant overall change in incidence for 1995-1998 to 2003-2007 (incidence of 0.40 to 0.43 per million) but stratified by gender, there was a significant increase for men (0.41 to 0.53 per million) and a stable incidence for women (0.39 to 0.34 per million).²⁸

The increasing incidence of CoM follows observations from cutaneous melanoma, with increasing numbers in the last decades in the USA⁵ and Europe^{1,4,51}. Similar to CoM, the increased cutaneous melanoma incidence is believed to be due to increased UV radiation exposure (specifically intermittent exposure),⁵² and is most pronounced amongst males.⁵³

Time trends that are observed in CoM are in contrast to observations from UM. Large population-based studies from the USA and Europe demonstrate no significant alterations in UM incidence for the last three decades, with overall values of 2-8/million in different regions.^{41,54} Specific analyses show minor increases in UM, however, e.g. in the white population of the US,⁵⁴ and in Canadians over the last two decades.⁵⁵

A complicating factor in the comparison of incidence rates of CoM (as well as other melanomas) between geographical areas and time periods are changing populations due to migration. As mentioned, genetic background is related to the risk for melanoma development. This may partially explain the differences between overall time-dependent rates of melanoma and numbers per subgroup in the literature. Data on race or ethnic background are not always available, calling for cautious interpretation of crude results.

Table 1. Epidemiologic studies on the incidence of CoM.

Study	Origin	Cases	Years	Overall incidence (Cases/million)	Current incidence (Cases/million)	Change over time	Race (Cases/million)
Tuomaala, 2002. ¹	Finland	85	1967-2000	All: 0.54	All: 0.80	1967 to 2000 0.40 to 0.80/million	Not reported
Yu, 2003. ²	USA	206	1973-1999		All: 0.46 M: 0.63, F: 0.32	1973/1979 to 1990/1999 0.22 to 0.46/million	Whites: 0.48, Blacks: 0.19, Others: 0.26.
Vajdic, 2003. ³⁹	Australia	37	1996-1998	All: 0.6 M: 0.8, F: 0.4	Not reported	Not reported	Not reported
Isager, 2005. ⁴	Denmark	120	1943-1997	M: 0.4, F: 0.3; NS	M: 0.3, F: 0.7; NS	1943 to 1997	Not reported
McLaughlin, 2005. ⁵	USA	324	1996-2000	All: 0.4 M: 0.4, F: 0.4	Not reported	Not reported	Not reported
Hu, 2008. ³⁵	USA	168	1992-2003	All: 0.41**		Not reported	Non-Hispanic Whites: 0.49 Blacks: 0.18 American Indians: 0.17 Asians: 0.15 Hispanics: 0.33
Triay, 2009. ³	Sweden	170	1960-2005		All: 0.56 M: 0.74, F: 0.45	1960 to 2005 0.08 to 0.56/million	Not reported
Park, 2015. ³⁸	South Korea	90	1999-2011	All: 0.12	All: 0.12	1999/2005 to 2006/2011 0.11 to 0.12/million	(Asian population)
Larsen, 2016. ³⁰	Denmark	138	1960-2012	All: 0.50 M: 0.53, F: 0.48; NS	All: 0.48	1960/1969 to 2010/2012 0.36 to 0.48/million <i>Peak: 2000/2009: 0.87</i>	Not reported
Ghazawi, 2019. ³²	Canada	190	1992-2010	All: 0.32 M: 0.35, F: 0.29	All: 0.32 M: 0.35, F: 0.29	1992 to 2010 0.32/million	Differences based on population background*
Virgili, 2020. ²⁸	Europe	714	1995-2007	All: 0.46 (crude) M: 0.48, F: 0.46 All: 0.42 (age adj.)	All: 0.43 M: 0.53, F: 0.34	1995/1998 to 2003/2007 0.37 to 0.43/million	Not reported

Abbreviations: NA, not applicable; NS, not significant. M, Male; F, Female.

*Higher rates of CoM were seen in the eastern Canadian provinces (with many people of Caucasian/European descent), lower rates were seen in the provinces Ontario and Quebec (with higher numbers of self-identified minorities).

**Overall calculation is based on the presented data per race, weighted for number of cases.

Incidence adjusted to tissue size

The absolute rarity of CoM suggests that the conjunctiva as a tissue is unlikely to develop melanoma. An interesting figure emerges, however, when the incidence of CoM is related to the small surface of the conjunctiva, as compared to cutaneous melanoma and the much larger surface size of the skin. In a large study from the USA, the incidence of CoM was estimated at 0.4/million persons per year, and that of cutaneous melanoma at 153.5/million persons per year.⁵ With an approximate skin area of 1.7 m² for a human adult,⁵⁶ and a conjunctival surface area per eye of 17.6 cm²,⁵⁷ the incidence of cutaneous melanoma can be estimated at 90 per million m² skin per year, and the CoM incidence at 113 per million m² conjunctiva per year. These figures are now well within a comparable range, which is not surprising due to the similarities between skin and conjunctiva, and their respective melanomas.⁷ To illustrate the difference with melanoma of the choroid, with a choroidal area approximating the retinal area of 1100 mm²,⁵⁸ and a choroidal melanoma incidence of 4.3/million persons per year,⁵ the incidence of choroidal melanoma can be estimated at 1955 per million m² choroid per year; this is a remarkable 20-fold higher per area unit compared to melanoma of the conjunctiva or skin. We conclude that CoM is rare in absolute numbers, but we put the rarity of CoM in perspective considering the conjunctival size. This calculation stresses the differences between intraocular and extraocular melanoma, with supposedly a different role for genetic and environmental factors (Figure 4).

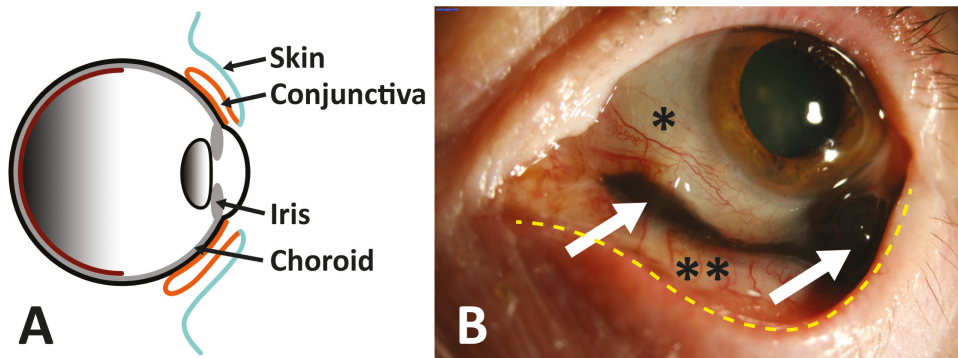


Figure 4. Ocular structures. Melanoma can affect several tissues of the ocular region. (A) There is a functional continuity between the conjunctiva and skin, opposed to the intraocular tissue of the uveal tract. (B) Note that conjunctiva can be divided into 'sun-exposed' conjunctiva (i.e. epibulbar and limbal; *) and 'covered' conjunctiva (i.e. tarsal and forniceal; **). In this patient, the melanoma extends through the fornix inferior (white arrows). The dotted yellow line indicates the eyelid margin.

Precursor lesions of CoM

Melanocytic diseases of the conjunctiva comprise a wide range of entities, based on the number and characteristics of melanocytes, and aberrations in the production of melanin. An illustrative overview of melanocytic disease was recently provided by Jakobiec.⁵⁹ Examples of conjunctival melanocytic disease include conjunctival nevi, primary acquired melanosis (PAM), complexion-associated melanosis (previously known as ‘racial pigmentation’) and CoM. By definition, CoM has invaded beyond the basement membrane into deeper tissues, but it may develop from other (non-invasive) disease. Many of the precursor lesions are far more prevalent than CoM, as was reported in an American study with population-based incidences of conjunctival nevi (50 cases per million), PAM (44 cases per million) and CoM (1.5 cases per million).⁶⁰ As with CoM, the prevalence of the precursor lesions differs between various races.

Most CoM (42-74% of cases) are believed to develop from PAM (Table 2).^{6,16,61} PAM is clinically described as a unilateral, flat, light pigmentation of the conjunctiva (resembling ‘cinnamon dust’), with a variable presence (i.e. ‘waxing and waning’). The likelihood of PAM to develop into melanoma depends on histological characteristics: while PAM ‘with atypia’ develops into CoM in 13% of cases, PAM ‘without atypia’ is considered an indolent condition that rarely ever leads to malignancy.⁶²

The histological classification of PAM is under continuous debate.⁵⁹ Grading PAM into ‘with’ or ‘without’ atypia was proposed⁶³ following systems that may have led to overacting by clinicians (using the term ‘precancerous melanosis’, by Reese) or to underestimation (using the term ‘benign acquired melanosis’, by Zimmerman). Issues remained for grouping PAM without atypia, however, and the lack of ‘melanoma-in-situ’ terminology. It can be advocated that PAM is the conjunctival equivalent of lentigo maligna of the skin, and that PAM with atypia is melanoma-in-situ, but the terminology from dermatopathology is not directly translatable. A newer system introduced ‘conjunctival melanocytic intraepithelial neoplasia’ (CMIN) on a 1-10 point score.⁶⁴ This score is increasingly being implemented. A consensus meeting for the most recent 4th ed WHO classification of tumours of the eye proposed a simplified scheme of the aforementioned PAM and CMIN terminology, using ‘low-grade conjunctival melanocytic intraepithelial lesions (CMIL)’, ‘high-grade CMIL’, and ‘melanoma-in-situ’.⁶⁵ All three scoring systems were deemed suitable and comparable in sensitivity, specificity, and accuracy; however, grading of low-risk lesions may remain difficult.⁶⁶ While the debate on histological grading may continue, it should be noted that ‘PAM’ remains a suitable term for the clinical description of lesions, however, without information on atypia.

About 7% of CoM is believed to develop from a nevus (Table 2).⁶ A range between 2 and 39% has been reported, which may be due to difficulties in histologic examination.^{12,16,61,67} Conjunctival nevi are quite common,^{60,68} and only rarely develop into melanoma: a large study from the USA

found that 3 out of 149 conjunctival nevi (2%) underwent malignant transformation.⁶⁹ Compared to CoM, nevi are seen more often in patients with a younger age (first/second decade) and often present with cysts; nevertheless, clinical differentiation can be challenging.⁷⁰

In about 11-26% of CoM cases, no precursor lesion can be identified; these CoM are considered to have developed ‘de novo’ (Table 2).^{6,12,16,61,67}

In spite of these reports, determination of the origin of CoM is controversial and imposes some difficulties. Clinical and histological findings may seem contradictory, and potential precursors may be overlooked or be impossible to determine.⁷¹ In her 1990 thesis, De Wolff-Rouendaal noted that 16 of 33 CoM that were clinically graded as ‘de novo’, showed acquired melanosis on histopathological examination, questioning the origin.⁷² As such, a co-occurring component of intra-epithelial melanocytes may be either pre-existing PAM or lateral spread of melanoma. Similarly difficult is the coexistence of potential precursors (such as PAM and nevi) making it difficult, if not impossible, to attribute melanoma outgrowth. While ‘de novo’ lesions were found to have a more unfavourable outcome compared to lesions from PAM or nevi,⁶ we hypothesize that this observation is biased, and that this (in part) can be due to rapid melanoma growth, clinically lacking an obvious precursor lesion. We advocate a thorough clinicopathological correlation, combining data from clinical and histopathological observations, ideally with mapping biopsies. In the absence of these data, we suggest cautious use of the ‘de novo’ terminology and think that a ‘de novo origin’ should be regarded as ‘uncertain origin’.

Table 2. Studies on the precursor lesions of CoM.

Study	Study size (cases)	PAM (%)	Nevus (%)	PAM and Nevus* (%)	De Novo (%)	Unknown* (%)
Shields, 2011. ⁶	382	74	7		19	
Paridaens, 1994. ⁶¹	256	57	18		22	2
Missotten, 2005. ¹²	194	57	2	4	26	11
Larsen, 2015. ¹⁶	139	62	33	2	11	
Tuomaala, 2002. ¹	85	61	30	8		
Anastassiou, 2002. ⁶⁷	69	42	39		16	3
De Potter, 1993. ⁷³	68	56	26		18	
Norregaard, 1996. ⁵⁰	42	19	21		60	

**These categories were not reported in all studies*
Abbreviations: PAM, primary acquired melanosis.

UV radiation and CoM

UV radiation is a well-established risk factor for the development of cutaneous melanoma, but has been debated in the development of CoM.⁷⁴ Several epidemiological and genetic studies indicate that UV-mediated mechanisms are involved, but the number of studies is small. Since CoM may develop at sites that are not exposed to sunlight, direct UV exposure may not be a necessity, but may be a risk factor.

Mechanisms of UV-mediated damage

Sunlight includes three classes of UV radiation: UVA (320-400nm), UVB (290-320nm), and UVC (100-280nm). UVA (95%) is more abundant than UVB (5%), while UVC is filtered by the atmosphere and hardly reaches the earth's surface. UVA and UVB have a different capacity to enter tissues, and differentially effect melanoma formation.

UVB has a direct damaging effect on DNA: photochemical reactions cause the production of cyclobutane pyrimidine dimers (CPD's) and pyrimidine pyrimidone photoproducts (PP's)⁷⁵ at locations where the pyrimidine bases (i.e. cytosine (C) or thymine (T)) are adjacent on the DNA (in sequences of CC, CT, TT, or TC). The presence of a dimer interferes with base pairing during DNA replication, leading to mutations. Both CPD's and PP's can be repaired by the nucleotide excision repair (NER) pathway, and dysregulation of NER therefore increases the risk for (cutaneous) melanoma development.⁷⁶

UVA causes production of reactive oxygen species (ROS), and to a lesser extent of CPD's.⁷⁷ Recently it was found that melanin can be involved in UVA-mediated damage: reactive oxygen and nitrogen species excite electrons in melanin, their energy is transferred to DNA and induces CPD's, hours after the initial UV exposure.⁷⁸

In the skin, UVB is predominantly absorbed in the epidermis, while UVA can reach the dermal stroma. The uvea is relatively protected from UV radiation by filtering in the cornea, lens, and other structures: only up to 1% of UV reaches the retina, most of which is UVA.⁷⁹ While the bulbar conjunctiva is sun-exposed, the tarsal and forniceal conjunctiva are not (Figure 4).

Based on the mechanism of action, an abundance of C>T and CC>TT mutations is typical for UV-mediated damage; this is called the UV 'signature' or 'footprint' in cancer development.⁸⁰ Additional effects of UV radiation on melanoma formation act via the immune system, as UV causes recruitment of macrophages and neutrophils in skin lesions.^{81,82} The role of these immune cells in melanoma are discussed further in the sections on tumour immunology [chapter 4].

Epidemiological studies

An association between UV radiation and CoM can be derived from epidemiological studies, as was mentioned in section 2.2 (on geographical incidence) and 2.3 (on time trends). In short, areas with a lower latitude (i.e. more towards the equator) are related to higher incidences of CoM,³⁶ and increased numbers of CoM in recent years are particularly due to lesions of the bulbar (sun-exposed) conjunctiva.^{3,30} Though vitamin D synthesis (following sun exposure) has been proposed as a protecting factor for cancer,⁸³ in CoM this may be overshadowed by DNA-damaging effects of UV. An Australian study on sun exposure was inconclusive regarding CoM due to low numbers (with only 19 cases reported), but CoM was related to a self-reported history of cutaneous melanoma, which could suggest either a role for sun exposure or a shared genetic susceptibility.⁸⁴

In cutaneous melanoma, intermittent exposure and sunburn are of particular importance for tumorigenesis, but cumulative exposure infers a risk as well;⁸⁵ the relation between patterns of sunlight exposure and CoM development is not known.

Genetic studies

Early work regarding the role of UV on genetic changes in CoM was limited by the techniques to detect mutations. One of these early studies (targeting mutations in the *NRAS* gene), found no aberrations in six cases of CoM and concluded on a minor role for UV.⁸⁶ The authors recognized, however, that UV may affect the immune system to create an environment that is more prone to melanoma development. A decade later, identification of a UV signature in DNA damage⁸⁰ showed direct evidence for UV-mediated mechanisms in CoM. Griewank found *TERT* promotor mutations in 12/38 (32%) CoM samples, all with the typical UV-related C>T and CC>TT changes.¹⁸ Later work confirmed the presence of this typical UV signature in a genome-wide sequencing study of two CoM⁸⁷ and five CoM⁸⁸ which were all from bulbar, (i.e. sun exposed) sites analysed by whole-exome sequencing. A recent extension to the work by Rivolta et al found that in 12/14 (86%) CoM more than 70% of the mutational load consisted of C>T changes, and the three studied CoM with the least C>T changes were tarsal and not bulbar;⁸⁹ these tarsal lesions had significantly lower amounts of single nucleotide variants than bulbar lesions. Other work, however, noticed no differences in gene expression of 161 oncology-related genes between 6 sun-exposed and 6 non-exposed CoM, suggesting less influence of UV on genetic profile.⁹⁰ Interestingly, a recent study found a UV signature in (sun-exposed) iris melanoma but not in posterior UM,⁹¹ suggesting a spectre of influence of UV rather than a strict distinction between CoM and UM; this warrants further studies in genetic similarities between CoM and iris melanoma.

The position of *BRAF* mutations in UV-mediated damage is not well understood, particularly because the most-common *BRAF* mutation lacks a UV signature.⁹² However, intermittent sun exposure of the skin (compared to either chronically or non-exposed sites) has been related to *BRAF* mutations.⁹³ Similarly, CoM at sun-exposed bulbar sites more often have *BRAF* mutations

than CoM at non-bulbar sites.³⁰ The increased frequency of *BRAF* mutations in cutaneous and conjunctival lesions may therefore be due to UV, with bulbar sites being intermittently sun-exposed; it was suggested that skin melanoma at chronically-exposed sites develops (partially) by other pathways.⁹³ A link between UV, *BRAF* mutations and melanoma is further observed since a greater exposure to UV radiation during childhood is related to the presence of more acquired nevi of the skin, carrying *BRAF* mutations, which then constitutes a risk for development of melanoma of the skin.⁹² Similarly, *BRAF* mutations are more frequently found in CoM lesions that originate from conjunctival nevi,³⁰ which harbour *BRAF* mutations as well [section 3.2.1].

In cutaneous melanoma, patients with a higher mutational burden (as seen following UV damage) may be better candidates for immunotherapy;⁹² this should be studied in CoM as well, as the use of immunotherapy is increasing [chapter 5].

Sun protection and CoM

A question that is relevant population-wide, is whether the eye needs to be protected from sunlight to diminish the risk for melanoma. Sunglasses have been suggested to prevent CoM,⁸⁷ but it will be hard to study the effects on CoM on a large scale by the rarity of the disease. Even more, as much of the UV that reaches the eye is through reflection, this may hamper good blockade.⁷⁹ Eye protection (e.g. with sunglasses) should certainly not be discouraged as it serves several purposes, but the protective effects regarding CoM should not be exaggerated in the absence of evidence.

Melanocytes and melanin

Melanin pigments have a role in the development and behaviour of different types of melanoma. This follows epidemiological data on skin and iris colour in cutaneous melanoma and UM, and is supported by the understanding of UV-mediated and UV-independent mechanisms of melanoma formation. While reports on melanin in the conjunctiva are rare, recent work suggests that tumour pigmentation is related to the behaviour of CoM,^{15,94} warranting further investigation.

Two main types of melanin pigment are reported in the eye: dark-coloured *eumelanin*, and light-coloured *pheomelanin*. The amount and ratio of these pigments determine visible traits, as a low total melanin and relative abundance of pheomelanin cause light skin colour⁹⁵ and blue iris colour⁹⁶, while a high total melanin and abundance of eumelanin causes dark skin colour and brown iris colour. Eumelanin has protective effects on melanoma formation, by shielding against UV radiation⁹⁵ and scavenging reactive oxygen species (ROS) and free radicals;⁹⁷ pheomelanin can be involved in DNA damage via UVA⁷⁸ and by itself via independent ROS formation.⁹⁸ It has been suggested that melanin also is linked to the efficacy of the immune system, partially via aspects of ROS production, as ROS inhibits CD8+ T cell function⁹⁹ and stimulates differentiation of macrophages into an M2 type,¹⁰⁰ the implications of which are discussed in the chapter on immunology [chapter 4].

Conjunctival, uveal (including iridal), and cutaneous melanocytes are derived from the same embryonic (neural crest) cells,¹⁰¹ though they migrate in different waves. Conjunctival and cutaneous melanocytes migrate to the surface ectoderm-derived epithelium and have functional similarities such as being able to transfer melanin to other cells,^{101,102} this is in contrast with uveal melanocytes that migrate into deeper mesoderm-derived tissues and do not transfer melanin. Cutaneous melanoma¹⁰³ and UM¹⁰⁴⁻¹⁰⁷ are known to occur more frequently in patients with fair skin and blue irises. No such population-based assessments exist for CoM, but as conjunctival melanocytes of light-iris eyes contain less total melanin and relatively more pheomelanin,¹⁰¹ it can be hypothesized that melanocytes in the conjunctiva of patients with light-coloured eyes are more prone to CoM development.¹⁵ Indeed, as CoM typically occur in countries with an abundance of people with light-coloured eyes, the role of melanin warrants further research.

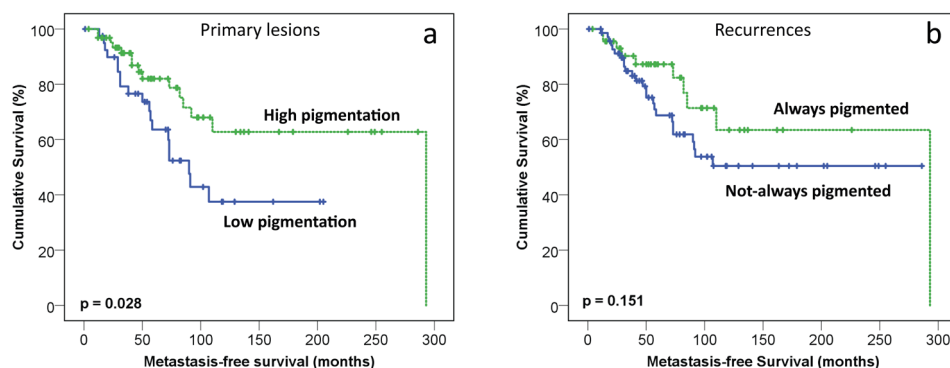


Figure 5. Kaplan-Meier analysis of metastasis-free survival based on tumour pigmentation. A combined set of Dutch and American CoM patients, of whom data on tumour pigmentation was known, was studied. (a) Patients were categorised by pigmentation of the primary lesion. A significantly worse outcome is shown for patients with low tumour pigmentation ($n = 41$) compared to high pigmentation ($n = 64$, $p = 0.028$). (b) The same group of patients is depicted, but is now categorised by the pigmentation of their recurrences. Outcome is not significantly different for those with recurrences with always high pigmentation ($n = 46$) compared to those with always low (or variable) pigmentation ($n = 71$, $p = 0.151$). [Figure re-used with permission from Brouwer et al., 2019.⁹⁴]

CoM themselves can present as amelanotic to darkly-pigmented (Figure 1). Light tumour pigmentation in CoM is related to a worse clinical outcome compared to darker lesions, with a hazard ratio for melanoma-related death of 2.42 ($p=0.020$, studied in 444 CoM patients),¹⁵ corresponding to observations from cutaneous melanoma. This could be due to direct melanocyte-related factors (such as the genotoxic/phototoxic effects of pheomelanin, and the absence of UV protection), or due to indirect effects such as late identification, insufficient treatment due to hard-to-detect tumour margins and late observation of recurrences (Figure 5).¹⁵ CoM recurrences are more often lightly pigmented compared to their primary lesion, which could be due to more

aggressive melanocytes (lacking pigment production) or treatment-related factors as clinicians may be more meticulous in assessment of melanoma-proven patients.⁹⁴ In contrast to what was seen with primary lesions, the degree of pigmentation of recurrences is not related to outcome, however (Figure 5).

As yet, no relation has been observed for iris colour and clinical outcome in CoM,¹⁵ nor for skin type and prognosis.¹⁰⁸

Conclusions (Epidemiology and Etiology)

CoM is a rare disease that accounts for 5% of all ocular melanoma. It is most prevalent in Caucasians, and is showing a rising incidence in recent decades. Adding to epidemiological studies, recent genetic work identified UV signatures in CoM, supporting the role of UV in CoM development, and suggesting different aetiologies for tarsal versus bulbar lesions. This is similar to what is seen in cutaneous melanoma. We calculated that, adjusted for tissue size, the incidence of CoM is very similar to that of cutaneous melanoma, and very different from that of UM.

It is promising that awareness of CoM increases worldwide, as improved recognition may cause earlier detection. More extensive knowledge about CoM may help clinicians to apply the appropriate treatment. Further studies are needed to determine whether CoM behaves similarly in all populations, as most current studies originate from North-America and Europe, and the genetic profile of CoM may differ between populations.

Melanin pigments (as a visible trait of melanocytes) have been related to melanoma development in the skin and uvea. A lower metastasis-free survival in CoM lacking visible pigment was observed, suggesting that the presence of pheomelanin, and absence of eumelanin, are unfavourable. Further molecular studies with quantification of melanin are warranted to understand its exact role in CoM biology.

The traditional theory of precursor lesions for CoM (i.e. being derived from PAM, nevi, or de novo) may need revision, as it is often impossible to determine a precursor, and both internal factors (such as genetics, pre-existing lesions, and melanin pigments) and external factors (such as UV radiation) are involved in melanoma development. We advocate to thoroughly study clinical data, histological data, and perform mapping biopsies to determine the origin of a lesion. It may be necessary to be cautious with 'de novo' terminology and we urge the use of 'tumour of unknown origin' in the appropriate cases.

3. GENETICS

Tumour genetics

The development of cancer is a multistep process that has been portrayed by Hanahan and Weinberg in the ‘hallmarks of cancer’.¹⁰⁹ Many of these hallmarks relate to genetics, such as sustained proliferative signalling, evasion of growth suppressors, and resistance to cell death. Simplified, cancer develops from the accumulation of genetic mutations, in addition to several epigenetic processes and interactions with the tumour micro-environment (TME). The TME is discussed in the chapter on tumour immunology [chapter 4]; here we elaborate on the genetic background of CoM, the comparison with other types of melanoma, and the implications for newly-developed targeted therapies.

An important concept in tumour genetics is that of *proto-oncogenes* and *tumour suppressor genes*. Proto-oncogenes are essentially normal genes, that, when overactive due to a mutation, contribute to malignancy. Tumour suppressor genes have an opposite role: they suppress malignancy in the normal situation, but contribute to it in case of decreased expression or mutational loss. Examples of proto-oncogenes in melanoma biology are *BRAF*, *NRAS*, and *GNAQ11*; examples of tumour suppressor genes are *NF1* and *PTEN*.

Tumour genetics - as well as normal cellular processes - act via pathways: multistep cascades of proteins, enzymes (kinases), and other cellular components that result in a certain function or effect. Two important pathways in (conjunctival) melanoma biology are the ‘MAPK’ (mitogen-activated protein kinase, also known as ‘RAS-RAF-MEK-ERK’) pathway and the ‘PI3K-AKT’ (also known as ‘PI3K-AKT-mTOR’) pathway. Overactivity of these pathways causes cell survival and proliferation. The pathways are highly complex and intertwined, but can be simplified to explain the aetiology of CoM, and the mechanisms of targeted therapy (Figure 6). Via these pathways, we will discuss how the genetic signature of CoM has several similarities to cutaneous and mucosal melanoma, while there are many differences with UM. In chapter 5.2, we will elaborate on newly-developed targeted therapies and discuss the first clinical observations of their application in CoM.

The MAPK pathway

The MAPK pathway consists of the cascade of RAS, RAF, MEK, and ERK.¹¹⁰ RAS is a small G protein, that can be activated by receptor tyrosine kinases (RTKs, a transmembrane protein) following binding by a ligand. RAS activates the cascade of protein kinases RAF, MEK, and ERK. Activated ERK (also known as MAPK) then enters the nucleus to cause expression of several proliferative genes. Mutations can occur throughout the MAPK pathway. Three different RAS genes (*NRAS*, *KRAS*, and *HRAS*) can harbour a mutation, resulting in an activated state. Among the RAF genes, a mutation in *BRAF* is the most common, resulting in increased kinase activity.¹¹¹ In

the most common *BRAF* mutation, a glutamic acid (presented by 'E') substitutes valine (presented by 'V') at the 600th amino acid, explaining the mutation terminology *V600E*.¹¹¹ Valine can be substituted by lysine (presented by 'K') as well, resulting in *V600K*; even more rare substitutions like *V600M* have been described.

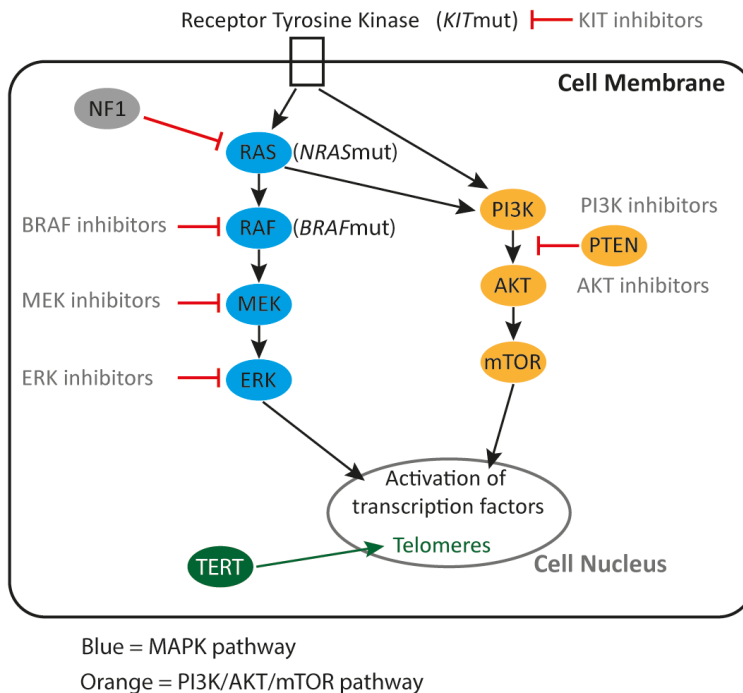


Figure 6. Cancer pathways in CoM and targets for therapy. This figure provides a simplified overview of important pathways that cause cell growth and proliferation in CoM. The MAPK pathway (in blue) consists of RAS (with possible mutations in *NRAS*), RAF (with possible mutations in *BRAF*), MEK, and ERK, which leads to activation of several proliferative factors in the nucleus. The PI3K-AKT-mTOR pathway (in orange) consists of PI3K, AKT and mTOR. NF1 (in grey) is a natural inhibitor of RAS (i.e. a tumour suppressor), by loss of this function, *NF1* mutations cause upregulation of MAPK. The receptor tyrosine kinases (with possible mutations in *KIT*) activate both MAPK and PI3K components. Another common link is RAS, that activates MAPK as well as PI3K. PTEN is a suppressor of AKT activity, acting as a tumour suppressor. TERT (in green) is involved in telomere length, causing cellular immortality.

The PI3K-AKT and other pathways

The PI3K-AKT pathway consists of phosphatidylinositol 3-kinase (PI3K), AKT, and mTOR.¹¹² PI3K can become activated by RTKs or RAS, providing a link between the MAPK and PI3K-AKT pathways. PI3K activates the protein kinase AKT, which then activates the kinase mTOR

(mammalian target of rapamycin). This is a regulator of cell proliferation and survival. *PTEN* is a natural inhibitor of PI3K, as it antagonizes its activity. Mutations or loss of *PTEN* can therefore upregulate PI3K activity.

Two actors that are proximal to the MAPK and PI3K-AKT pathway are NF1 and the RTKs. *NF1* is a gene that encodes the neurofibromin 1 protein, an inhibitor of RAS that works through GTPase activity.¹¹³ Most mutations that occur in *NF1* are loss-of-function, causing upregulation of RAS. One of the transmembrane RTKs is KIT (also known as c-KIT). Binding of the KIT ligand stem cell factor causes activation of several downstream pathways including MAPK and PI3K-AKT signalling. KIT has an important role in the function of melanocytes.¹¹⁴ Activating *KIT* mutations cause increased downstream signalling.

Apart from the MAPK and PI3K-AKT pathway, important actors in melanoma proliferation are the telomeres. Telomeres are end caps at chromosomes, that shorten with cell division to cause a limit in replication.¹¹⁵ The telomerase reverse transcriptase (*TERT*) gene encodes a catalytic subunit of the telomerase complex that is involved in preservation of these telomeres. *TERT* promoter mutations increase *TERT* expression, allowing for survival ('immortality') of malignant cells.¹¹⁶

Mutations in CoM

Most work on genetic mutations in melanoma has been performed on cutaneous melanoma, which is not surprising due to their abundance over other melanoma types. Cutaneous melanoma are often classified according to their mutational status, resulting in groups of *BRAF*-mutated, *NRAS*-mutated, *NF1*-mutated and triple-WT (wild type) melanoma.¹¹⁷ Several studies analysed the presence of mutations in CoM (Table 3), resulting in the idea that the same categorization may apply.¹¹⁸ Recent work suggests that the *NF1*-mutated group is most frequent in CoM, while the *BRAF*-mutated group is the largest in cutaneous melanoma, pointing out that differences may exist between the two tumour types.⁸⁹

BRAF mutations

BRAF mutations were first detected in cutaneous melanoma, where they occur in more than half of the cases.¹¹⁹ The most common *BRAF* mutation in cutaneous melanoma is V600E (73%), followed by V600K (19%) and some sporadical types (<5%), such as V600R, V600M or V600G.¹²⁰ The frequency of *BRAF* mutations differs between types of cutaneous melanoma, as they are seen more often in lesions without chronic sun damage (i.e. no or intermittent sun exposure) compared to chronic sun exposure.⁹³

Mutations in *BRAF* are observed in about a third of CoM,^{17,30} and similar to cutaneous lesions, the most common *BRAF* mutation is V600E (in approximately 80%), followed by V600K (in

approximately 20%).^{17,30,121} There may be racial differences in the occurrence of *BRAF* mutations, as these were less frequently observed in Asians (8%) compared to Caucasians,⁴² similar to what is observed for cutaneous melanoma.¹²²

While several authors looked for possible associations between *BRAF* mutations and clinical parameters in CoM, only a few significant relations were found, possibly due to small sample sizes. The largest series of CoM (111 cases with a known *BRAF* mutation status) showed that *BRAF* mutations (univariately) are more common in younger patients, males, lesions with an epibulbar location (compared to non-epibulbar), with absent or mixed pigmentation (compared to dark pigmentation), and lesions with a lower tumour-node-metastasis (TNM) stage.³⁰ Additionally, *BRAF* mutations are more often seen in CoM that originate from a nevus than those that originate from PAM.^{17,30}

As no relation has been observed with recurrences, metastasis or survival, the presence of a *BRAF* mutation is of limited use for prognosis in CoM.^{17,30}

During the time period 1960 to 2012, the percentage of CoM with a *BRAF* mutation has not increased, while this had been expected due to an increase in the number of bulbar CoM;³⁰ however, it may be that both demographic changes as well as an increased exposure to sunlight influenced the number of CoM, resulting in more CoM, but with an unaltered *BRAF* frequency. The relation between UV exposure and *BRAF* mutations is not fully understood however, as is presented in more detail in section 2.6.3. Importantly, the *BRAF* mutation status was not the sole predictor of MEK, ERK and AKT signalling in CoM tissue,^{121,123} implying that the MAPK and PI3K-AKT pathway are activated by other parameters as well.

BRAF mutations are very common in conjunctival nevi, where they occur in 19-56% (Table 4).^{121,123-125} While this percentage may be higher than the percentage in CoM, activation of the MAPK pathway was higher in malignant CoM than in benign nevi, again suggesting that other factors are involved in pathway activation (e.g. mutations in *NF1*).¹²³ The abundance of *BRAF* mutations in conjunctival nevi parallels that of nevi of the skin, where a study reported these in 82%.¹²⁶

BRAF mutations are rare in PAM, either with or without atypia, and most studies observed no *BRAF* mutations in PAM at all (Table 4).^{121,124} One report noted a *BRAF* mutation in 2/8 PAM lesions (with atypia), but these PAM were selected for later outgrowth of CoM, which may indicate that these have represented melanoma in situ.³⁰

Most often a pre-malignant lesion has the same *BRAF* status as its CoM outgrowth: in 19 out of 20 pairs (12 nevi, 8 PAM) the *BRAF* status concurred.³⁰ However, heterogenous lesions can

occur, as one nevus (*BRAF* mutated) later recurred into a melanoma without *BRAF* mutation, possibly indicating outgrowth of a specific strain of cells. These sequential differences have also been reported for CoM lesions and their recurrences, occurring years later.¹²⁷

BRAF mutations are less common in mucosal melanoma (other than CoM), with a likely frequency of 4-8%.^{128,129} *BRAF* mutations are not seen in UM of the choroid and ciliary body,^{119,130,131} although they have been described recently in iris melanoma in 1/30 cases (3%).¹³²

NRAS mutations

In cutaneous melanoma, mutations in *BRAF* and *NRAS* are generally mutually exclusive, with fewer than 1% carrying both.¹³³ *NRAS* mutations occur less frequently than those in *BRAF*, with a frequency in cutaneous melanoma of 12-27%.¹³³⁻¹³⁶

In CoM, *NRAS* mutations are also mutually exclusive with *BRAF*,¹⁷ and occur with a frequency of 0-18%.^{17,134} *NRAS* makes up almost all mutations in *RAS* genes in CoM; activating mutations in *KRAS* are rare, those in *HRAS* (with unknown consequences) are reported hardly at all.¹¹⁸ Though *NRAS* mutations are common in conjunctival nevi, with 39%,¹²⁵ their occurrence in PAM is unknown. There is no clear association between tumour origin and CoM *NRAS* status.¹¹⁸

NRAS mutations are seen in 11-24% of mucosal melanoma other than CoM.^{128,129,134} *NRAS* mutations have not been reported in posterior UM,^{131,137} although (similar to what is seen for *BRAF*) they may be encountered in iris melanoma (reported by one study in 3/10 cases¹³²).

NF1 mutations

In cutaneous melanoma, *NF1* mutations are common (occurring in 12-14%) being the third most frequent mutation after *BRAF* and *NRAS*; often *NF1* co-occurs with either of these.^{117,133} *NF1* mutations in the skin are associated with UV exposure¹³³ and *NF1*-mutated cutaneous melanoma have a high mutational load (compared to *BRAF/RAS*/triple-WT),¹³⁸ which may imply that they are more sensitive to immunotherapy (as was shown for anti-PD-1 therapy in cutaneous melanoma patients¹³⁹). In cutaneous melanoma, patients with *NF1*-mutated lesions have a worse survival than those with *BRAF/RAS* mutations.¹³⁸ All of this makes *NF1* an interesting gene.

NF1 mutations are indeed frequently observed in CoM and occur in 33%,¹¹⁸ however (possibly due to the smaller numbers studied) they are not associated with clinical characteristics or prognosis.¹¹⁸ To our knowledge, *NF1* status is unreported in precursors of CoM.

In mucosal melanoma, *NF1* mutations are readily observed with a frequency of 18-37%.^{129,140} *NF1* mutations are commonly absent in UM, but deletion of the *NF1* locus was reported in one tumour in a study on 38 cases of UM (3%).¹⁴¹

KIT mutations

KIT mutations are rare in cutaneous melanoma: the incidence varies for anatomic location however, as total absence is reported in non-*chronic sun-damaged* (CSD) melanoma, and up to 28% in CSD melanoma.¹⁴² Commonly, *KIT* mutations are mutually-exclusive with *BRAF* and *NRAS*.

In a composite of four studies on CoM, only 1 in 68 cases (1%) demonstrated a *KIT* mutation,^{17,134,143,144} demonstrating similar rarity. Immunohistochemical staining of *KIT* occurs in about 50% of CoM, but this showed no correlation to mutational status.¹⁴³ *KIT* mutations are seen more often in CoM in Asians (11%) compared to Caucasians,⁴² which concurs with the finding of a lower *BRAF* frequency. *KIT* mutations are absent in conjunctival nevi, and rarely seen in PAM with atypia,¹⁴³ however little data are available.

KIT mutations are more common in acral and mucosal melanoma (23 and 16%, resp.,¹³⁴ and some studies even report up to 40%. While they were initially unreported in UM,¹³⁴ later reports found up to 9% in choroidal lesions,¹⁴⁴ and 7% in iris melanoma.¹³²

PTEN mutations (PI3K/AKT)

An important mediator of the PI3K/AKT/mTOR pathway is *PTEN*, that acts as an inhibitor. *PTEN*'s function is partially determined by its location in the cell: nuclear (instead of cytoplasmatic) *PTEN* has a tumour suppressive role; loss of *PTEN* therefore stimulates tumour formation. *PTEN* loss is often mutually exclusive with *NRAS* mutations (and thus concurrent with *BRAF* mutations).

PTEN loss is commonly observed in cutaneous melanoma (65%),¹⁴⁵ and recent work showed that it was associated with worse survival, possibly by helping immune evasion.¹⁴⁶ *PTEN* loss has been described in CoM as well, but apart from a relation with more pigmentation, it was not related to other characteristics or prognosis in 70 lesions.¹⁴⁷ Several other mTOR-related proteins in CoM were associated with a high mitotic rate and thicker lesions, however.¹⁴⁸ Nuclear *PTEN* loss was observed more frequently in CoM than in conjunctival nevi,¹⁴⁷ suggesting an important role in melanoma development. This was similarly seen in cutaneous melanoma versus nevi.¹⁴⁵

A comparative study between CoM and UM showed that mTOR effectors were higher in CoM, and that UM showed a higher *PTEN* expression.¹⁴⁸ *PTEN* loss in UM (of unreported anatomical location) was reported in 12/75 cases (16%),¹⁴⁹ and *PTEN* mutations were observed in 3 out of 30 (10%) iris melanoma.¹³²

TERT promotor mutations

TERT promotor mutations (further referred to as '*TERT* mutations') are found in approximately 30% of primary cutaneous melanoma lesions.^{150,151} *TERT* mutations are reported more often in older patients, and their frequency may vary based on tumour location, resulting in studies

reporting up to 68% in primary cutaneous melanoma.¹⁵² Increased *TERT* activity relates to worse prognosis in cutaneous melanoma, but the role of either *TERT* mutations or *TERT* expression (not necessarily coinciding) is unclear.¹⁵²

Very similar to the observed rates in the skin, *TERT* mutations are present in 32-43% of primary CoM.^{18,116,153} In two studies, analysing 38 and 39 CoM lesions, no relation was found between *TERT* mutation status and clinical parameters or outcome (including patient age, tumour size, location, recurrences, survival).^{18,153} The absence of a relation between *TERT* mutations and tumour location is remarkable, as *TERT* mutations in CoM show C>T or CC>TT nucleotide changes,¹⁵³ demonstrating a UV signature,⁸⁰ and one would expect these to occur mainly in bulbar conjunctiva. A recent, and larger, study with data of 47 CoM showed that presence of a *TERT* mutation correlated with metastatic disease, emphasizing an importance for therapeutic decision making.¹¹⁶

TERT mutations are not found in conjunctival nevi or PAM without atypia.¹⁵³ A small number, 2/25 (8%), of PAM *with* atypia carried the mutation, however.¹⁵³ This may indicate an important distinction between benign and malignant lesions, equally to what is observed in cutaneous lesions where melanoma and melanoma in situ have *TERT* mutations, but benign precursors not. A change in *TERT* may be an important early step in melanoma transformation, occurring, however later than the *BRAF* mutation.¹⁵⁴

TERT mutations are rare in mucosal melanoma, with a range of 6-8%.^{129,155} Presumably, this relates to the tumour locations, often lacking exposure to UV. Similarly it may be no surprise that *TERT* mutations are very rare in UM: several studies report on a total absence,^{18,151} but they are found sporadically (in 1/102 cases,¹⁵³ and 1/50 cases¹⁵⁶).

Other mutations: GNAQ/11 and BAP1

An analysis of genetics of CoM shows its distinction from the most common ocular melanoma: UM. Important genes in UM biology are *GNAQ/11*, *BAP1*, *SF3B1* and *EIF1AX* (extensively reviewed by Smit et al.¹⁰). Knowledge of these genes is relevant for CoM to understand a link with possible melanoma predisposition syndromes, and to identify the origin of unknown (secondary) conjunctival lesions based on tumour genetics, e.g. differentiating CoM from intraocular melanoma that has perforated the sclera.

GNAQ/11 signalling activates several pathways in cancer including MAPK,¹³¹ and YAP1.^{157,158} The *GNAQ/11* gene is mutated in nearly all UM,^{131,159} and mutations are seen already in uveal nevi.¹⁶⁰ *GNAQ/11* mutations are absent in CoM,¹⁷ other mucosal melanoma,⁹⁰ conjunctival nevi and PAM.^{125,161} A *GNAQ* mutation was reported in two cases of conjunctival blue nevi, however,

indicating a different cellular origin than the common epithelial nevus.¹²⁵ *GNAQ/11* mutations are absent in cutaneous melanoma, but individual cases have been reported in chronically sun-damaged skin.¹⁵⁹

Other mutations in UM that are important for tumour progression occur mainly in three genes: *BAP1*, *SF3B1*, and *EIF1AX*, which were reported in 43%, 26%, and 21% of primary UM, respectively.¹⁰ Mutations in *BAP1* are related to the worst prognosis and these tumours often metastasize within a few years.¹⁶² BAP1 immunohistochemistry (IHC) is nowadays commonly applied to assess *BAP1*, as a prognostic factor in UM.¹⁶³

BAP1 (located on chromosome 3) was discovered a decade ago,¹⁶⁴ with the BAP1 protein as a deubiquitinating hydrolase with several functions such as protein deubiquitination, cell cycle regulation, DNA damage repair, and regulation of gene expression;¹⁶⁵ loss of BAP1 expression has been linked to increased inflammation¹⁶⁶ and angiogenesis in UM,¹⁶⁷ but much about its function remains to be unveiled.

Different from what is seen in posterior UM, *BAP1* mutations sporadically occur in iris melanoma (3%)¹³² and are uncommon in melanomas other than UM. They are practically absent in acral, mucosal, and cutaneous melanoma, though they may occur in cutaneous melanoma lacking chronic solar damage.¹⁶⁸ Remarkably however, only few studies exist on sporadic *BAP1* mutations in cutaneous melanoma. Recent work on TCGA data showed that the prognostic effect of BAP1 mRNA expression was opposite for UM and cutaneous melanoma, suggesting differential roles.¹⁶⁹ Even so, BAP1 loss is not observed in conjunctival lesions, and its status is not commonly assessed.⁸⁸

Chromosomal aberrations

Chromosomal copy number alterations (CNAs) are relevant to tumourigenesis as they influence the function of locally encoded genes.

Though few reports exist on the topic, a plethora of CNAs has been reported for CoM, indicating complex karyotypes. Gains have been reported in chromosomes 1q, 3p, 6p, 7, 8q, 10q, 11p, 11q, 12p, 13q, 14p, and 17q, and losses in chromosomes 1p, 3q, 4q, 6p, 6q, 8p, 9, 10, 11q, 12q, 13, 15p, 16, 17p, 19, and 22.^{17,88,89,171-173} The most frequently reported CNA is 6p amplification, which has been reported in up to 61% of CoM.¹⁷¹

Table 3. Prevalence of genetic mutations in various melanoma types.

	Conjunctival Melanoma	Cutaneous Melanoma	UM (posterior, unless otherwise noted)	Mucosal melanoma (other than CoM)
BRAF	4/15 (27%) ¹³⁴ 3/21 (14%) ¹³⁰ 23/78 (29%) ¹⁷ 2/5 (40%) ¹²⁴ 5/22 (23%) ¹²⁷ 11/31 (35%) ¹²³ 10/39 (26%) ¹²¹ 39/111 (35%) ³⁰ 4/53 (8%) ^{42 *1}	16/44 (36%) ¹³⁷ 398/774 (51%) ¹¹⁹ 115/253 (46%) ¹²⁰ 166/318 (52%) ¹¹⁷ 87/217 (40%) ¹³⁶ 82/213 (38%) ¹³³ 3/10 (10%) without CSD ⁹³ 22/40 (59%) with CSD ⁹³	0/88 (0%) ¹³⁰ 0/62 (0%) ¹³⁷ 0/48 (0%) ¹³¹ 0/23 (0%) ¹¹⁹ 1/30 (3%) iris ¹³²	0/26 (0%) ¹¹⁹ 1/6 (17%) ¹²⁰ 2/56 (4%) ¹²⁸ 0/45 (0%) ¹³⁴ 6/71 (8%) ¹²⁹
NRAS	0/11 (0%) ¹³⁴ 14/78 (18%) ¹⁷	7/60 (12%) ¹³⁴ 1/27 (4%) ¹³⁷ 20/114 (18%) ¹³⁵ 53/217 (24%) ¹³⁶ 58/213 (27%) ¹³³	0/47 (0%) ¹³⁷ 0/48 (0%) ¹³¹ 3/30 (10%) iris ¹³²	8/56 (14%) ¹²⁸ 9/37 (24%) ¹³⁴ 8/71 (11%) ^{129 *2}
KIT	1/13 (8%) ¹³⁴ 0/5 (0%) ¹⁴⁴ 0/42 (0%) ¹⁷ 0/8 (0%) ¹⁴³ 6/53 (11%) ^{42 *1}	1/58 (2%) ¹³⁴ 0/18 (0%) without CSD ^{142 *3} 5/18 (28%) with CSD ^{142 *3}	0/60 (0%) ¹³⁴ 6/64 (9%) chor+CB ¹⁴⁴ 2/6 (33%) iris ¹⁴⁴ 2/30 (7%) iris ¹³²	15/38 (39%) ¹⁴² 2/56 (4%) ¹²⁸ 7/45 (16%) ¹³⁴ 9/19 (47%) ¹⁴⁰ 5/71 (7%) ¹²⁹
TERT	12/38 (32%) ¹⁸ 16/39 (41%) ¹⁵³ 20/47 (43%) ¹¹⁶	16/56 (29%) ¹⁵¹ 27/77 (33%) ¹⁵⁰ 131/194 (68%) ¹⁵²	0/47 (0%) ¹⁸ 0/25 (0%) ¹⁵¹ 1/50 (2%) ¹⁵⁶ 1/102 (1%) ¹⁵³	4/71 (6%) ¹²⁹ 4/49 (8%) ¹⁵⁵
NFI	21/63 (33%) ¹¹⁸	26/213 (12%) ¹³³ 45/318 (14%) ¹¹⁷	1/38 (3%) ¹⁴¹ 0/24 (0%) ¹⁷⁰	13/71 (18%) ¹²⁹ 7/19 (37%) ¹⁴⁰
GNAQ	0/39 (0%) ¹⁷ 0/4 (0%) ¹⁶¹ 0/11 (0%) ¹³¹ 0/9 (0%) ¹⁵⁹ 0/12 (0%) ⁹⁰	0/15 (0%) without CSD ¹³¹ 1/27 (4%) with CSD ¹³¹ 1/74 (0%) with CSD ¹⁵⁹ 0/90 (0%) without CSD ¹⁵⁹	12/27 (44%) ¹⁶¹ 22/48 (46%) ¹³¹ 48% ^{159 *4}	0/14 (0%) ¹³¹ 0/28 (%) ⁹⁰
GNAI1	0/39 (0%) ¹⁷ 0/9 (0%) ¹⁵⁹ 0/12 (0%) ⁹⁰	0/74 (0%) with CSD ¹⁵⁹ 0/90 (0%) without CSD ¹⁵⁹	34% ^{159 *5}	0/28 (0%) ⁹⁰
BAP1	0/5 (0%) ⁸⁸	0/15 (0%) with CSD ¹⁶⁸ 2/15 (13%) without CSD ¹⁶⁸	13/33 (39%) ¹⁶⁸ 35/74 (47%) ¹⁶³ 1/30 (3%) iris ^{132 *6}	0/15 (0%) ¹⁶⁸

Abbreviations: CSD, chronic solar damage; Chor, choroidal; CB, ciliary body.

*1 In contrast to many other studies, this work includes an Asian population.⁴²

*2 Any mutation in RAS genes was observed in 12/71 (17%) of cases: NRAS mutations comprised 8/71 (11%), KRAS mutations 4/71 (6%).¹²⁹

*3 Note the marked difference in KIT frequency between CSD and non-CSD lesions.¹⁴²

*4 Any GNAQ mutation in 48%, consisting of Q209 in 73/163 (44.8%), and R183 in 4/145 (2.8%).¹⁵⁹

*5 Any GNAI1 mutation in 34%, consisting of Q209 in 52/163 (31.9%), and R183 in 3/145 (2.1%).¹⁵⁹

*6 BAP1 immunohistochemistry loss in 9/30 (30%) cases.¹³²

Table 4. Prevalence of genetic mutations in precursor lesions of CoM.

	Conjunctival Nevus Cases (%)	PAM without atypia Cases (%)	PAM with atypia Cases (%)
BRAF	14/28 (50%) ¹²⁴ 13/23 (56%) ¹²⁵ 15/35 (43%) ¹²³ 7/37 (19%) ¹²¹ 9/12 (75%) ^{30 *1}	0/11 (0%) ¹²⁴ 0/17 (0%) ¹²¹	0/4 (0%) ¹²⁴ 0/13 (0%) ¹²¹ 2/8 (25%) ^{30 *1}
NRAS	9/23 (39%) ¹²⁵	N.A.	N.A.
KIT	0/5 (0%) ¹⁴³	N.A.	1/3 (33%) ¹⁴³
TERT	0/56 (0%) ¹⁵³	0/14 (0%) ¹⁵³	2/25 (8%) ¹⁵³
NF1	N.A.	N.A.	N.A.
GNAQ	0/29 (0%) ¹⁶¹ 0/23 (0%) ^{125 *3}	0/7 (0%) ^{161 *2}	0/7 (0%) ^{161 *2}
GNAI1	N.A.	N.A.	N.A.
BAP1	N.A.	N.A.	N.A.

Abbreviation: N.A., not applicable.

*1 Lesions were selected on later development of CoM (paired lesions), possibly introducing bias to malignancy.

*2 Unknown status of atypia.

*3 A GNAQ mutation was reported in 2/2 (100%) of blue nevi of the conjunctiva.¹²⁵

The observed CNAs in CoM resemble those in cutaneous melanoma.⁹³ And similar to what is seen in cutaneous melanoma,¹⁷⁴ CNAs in CoM were observed more frequently in *BRAF*/*NRAS*-wildtype tumours.¹⁷ CNAs in CoM are distinct from observations in UM¹⁷ where loss of chromosome 3 (which includes the *BAP1* gene) occurs frequently and is related to the development of metastases.¹⁶⁴ Other alterations that are frequently observed occur in chromosomes 8q and 6 (reviewed by Jager et al., 2020.⁹).

Most of the reported CNAs in CoM have no relation with clinical parameters or prognosis. A recent study identified that deletion of chromosome 10q was related to the presence of *BRAF* mutations, increased tumour thickness, metastasis development, and lymph invasion.¹⁷¹ Genes encoded by the 10q region are *SUFU*, *NEURL1*, *PDCD4*, and *C10orf90* (all of which are tumour suppressor genes). The work by Kenawy et al, studying 59 lesions, shows the relevance of CNAs in CoM when assessing a relatively large cohort;¹⁷¹ multicentre projects such as these are therefore essential to obtain sufficient numbers.

Predisposition syndromes

Several genetic disorders or syndromes exist that predispose to the development of cancer and melanoma, e.g. the *Familial atypical multiple mole melanoma* (FAMMM) syndrome and the *BAP1*-tumour predisposition syndrome. To our knowledge, no such syndromes have been identified for CoM, possibly due to the rarity of this disease. It is likely however, from melanocyte biology, that

certain syndromes that are associated with cutaneous melanoma, relate to development of CoM as well. Further studies are warranted, to identify patients at risk of CoM, and to optimize guidelines for screening.

A well-known melanoma syndrome is the FAMMM syndrome, also known as ‘dysplastic nevus syndrome’, which is associated with an increased risk for dysplastic nevi and cutaneous melanoma.¹⁷⁵ Despite suggestions from earlier reports,¹⁷⁶ more recent insights show that individuals with FAMMM do not have an increased risk for developing conjunctival pigmented disease (including melanoma) compared to others in the population.¹⁷⁷ Small numbers limit the strength of conclusions however.

Neurofibromatosis type I (Von Recklinghausen disease) is a genetic disorder, caused by a loss-of-function mutation in the (tumour suppressor) *NF1* gene.¹⁷⁸ Abnormal function of the neurofibromin protein increases RAS activity, resulting in development of several benign and malignant tumours. From this biology, it is no surprise that an increased risk for cutaneous melanoma has been reported,¹⁷⁸ although others state that the risk is not above chance,¹⁷⁹ and that sampling bias is a major concern. Several reports exist of CoM in patients with NF1-disease, but rarity of both conditions limits a conclusion on chance.¹⁸⁰⁻¹⁸³ It remains controversial whether NF1-disease predisposes to UM.¹⁸⁴

The nevus of Ota (oculodermal melanocytosis) is a congenital pigmented condition of the periocular area, which includes the skin, sclera, uvea and orbit. It was estimated that the lifetime risk for UM development in this condition was 1:400,¹⁸⁵ which is clearly above chance alone, and once UM develops, these patients have an increased risk for metastases.¹⁸⁶ Several cases of cutaneous melanoma have been reported in relation to a nevus of Ota,¹⁸⁷ including detrimental melanoma with orbital invasion,¹⁸⁸ but it has not been concluded that there is a true increased risk. Despite an apparent involvement of the ocular surface, we are not aware of reports on CoM associated with an ocular nevus of Ota. Pigmentation in nevi of Ota is not conjunctival however, and mutations in *GNAQ* can be found,¹³¹ explaining a relation to blue nevi and UM rather than to cutaneous melanoma or CoM.

The *BAP1* tumour predisposition syndrome leads to increased risks for several malignancies, including UM¹⁸⁹ and to a lesser extent cutaneous melanoma.¹⁶⁸ There has been a report on a patient with the *BAP1* tumour predisposition syndrome and a conjunctival melanoma, who later developed a cutaneous melanoma; unfortunately no molecular testing or BAP1 staining was performed on the conjunctival lesion, questioning its true origin as primary conjunctival or metastatic lesion.¹⁹⁰

miRNA

Micro-RNA (miRNA) is a class of small non-coding RNA that can regulate gene expression post-transcriptionally.¹⁹¹ Conceptually, they can affect the expression of proto-oncogenes and tumour suppressor genes, and can serve as potentially diagnostic and prognostic markers, and as targets

for therapy. Often, miRNA have multiple targets, making it difficult to study individual effects. A plethora of miRNA have been identified in cutaneous melanoma, emerging as a new field in oncology.¹⁹¹

The first analysis of miRNA in CoM was presented by Larsen et al.¹⁹² Studying 37 lesions, they found 25 miRNA that were differentially expressed between CoM and normal conjunctiva; several were concordant with miRNA known from cutaneous melanoma, while none were observed that had previously been associated with UM. Clustering based on seven miRNA showed that low, intermediate and high expression related in ascending order to increased CoM tumour thickness, but a true prognostic value was limited as only two miRNA related to recurrences, and none to development of metastasis.

Later work from the same group by Mikkelsen et al. analysed 13 CoM with paired metastatic lesions, and 25 CoM lesions that did not develop metastasis during a follow up of at least 5 years.¹⁹³ MiRNA were identified that showed a differential expression between non-metastatic and metastatic CoM, between CoM and its coupled metastasis, and between CoM and normal conjunctiva. Interestingly, pathway analysis of the involved miRNA showed that the hippo pathway and p53 were involved in the differentiation of normal conjunctiva to CoM. Unfortunately, there was a poor correlation between the array data and qPCR validation, implying that results need to be confirmed.

Ipenburg et al. studied 20 CoM and 6 conjunctival nevi, and validated the results in 19 CoM and 13 conjunctival nevi from another institution.¹⁹⁴ They identified five miRNA's (out of the 377 studied) that showed increased levels in CoM versus conjunctival nevi, and found that the homeobox gene clusters constituted a possibly shared pathway. No differences were found between lesions with or without metastases and no relation with clinical characteristics was reported. As an advantage of miRNA analysis, Ipenburg noted that miRNA testing may be used in cases with only very little available tissue, making it into a potential classifier to differentiate between a nevus and a melanoma.

Conclusions (Genetics)

Genetic mutations in CoM follow the same pattern as cutaneous and other mucosal melanoma. Frequently-observed mutations are those in *BRAF*, *NRAS*, *NF1*, and *TERT*. *KIT* mutations are rare but may relate to subgroups in non-Caucasians. Mutations in CoM combine the patterns from skin lesion with chronic sun damage (CSD) as well as non-CSD, being most like intermittently sun-exposed cutaneous melanoma. Although both are ocular tumours, CoM are genetically very different from UM, with mutations usually occurring in *GNAQ11* or *BAP1*.

Little is known about tumour predisposition syndromes and CoM development. It may be expected that the FAMMM syndrome and Neurofibromatosis type 1 are related to CoM development, but the rarity of both limits the chance of finding a CoM in a patient with NF.

The diagnostic value of tumour genetics is currently confined to specific cases. The mutational profile may differentiate primary CoM or cutaneous melanoma from UM (and its metastases); differentiating a primary CoM from a lesion with a cutaneous origin is genetically difficult. Recent studies on miRNA show that expression profiles may help to differentiate benign from malignant conjunctival lesions, but this requires further validation.

The value of genetics for prognostic purposes in CoM is currently limited. Recently, *TERT* mutation presence was identified as an important factor,¹¹⁶ as was loss of chromosome 10q,¹⁷¹ both being related to metastasis development. Together with miRNA expression profiles, this shows that there may be prognostic use in the future. Genetic characterization of CoM may additionally be used to identify the most appropriate targeted therapy e.g. with *BRAF* inhibitor therapy, for selected patients. Although *BRAF* status is currently not predictive of outcome in CoM, it may become a prognostic factor in the future now that patients with *BRAF* mutations can receive targeted treatment.

4. IMMUNOLOGY

Tumour immunology

The human immune system is of paramount importance for tumour growth and control. Inflammation is therefore considered one of the hallmarks of cancer.^{109,195} The immune system may inhibit tumour growth by killing tumour cells, but may also provide cytokines and chemokines that stimulate growth and tumour spreading. Tumours, on their turn, may use mechanisms to prevent attack by the immune system.

An important part of the specific immune response against tumour cells is played by Cytotoxic T cells (CTLs, also referred to as 'effector T cells'). These cells kill tumour cells when they recognize a specific antigen, presented via HLA Class I on the tumour cell membrane.¹⁹⁶ Tumours may learn to escape the killing effects of these T cells by loss of expression of HLA molecules, causing decreased recognition. Another escape mechanism acts via immune checkpoints, i.e. molecules that naturally help to diminish the activity of CTLs, preventing auto-immunity. It was recently discovered that these checkpoints can be blocked, leading to a new class of drugs (i.e. immune checkpoint-inhibitors (ICIs)) with promising results in many malignancies, including metastatic cutaneous melanoma. The first checkpoint inhibitor that was approved for metastatic melanoma by the *United States Food and Drug Administration* (FDA) in 2011 was ipilimumab, an IgG monoclonal antibody that

blocks *cytotoxic T-lymphocyte-associated protein 4* (CTLA4).²² Later, in 2014, monoclonal antibodies against *Programmed cell death protein 1* (PD-1) followed, when nivolumab and pembrolizumab were approved by the FDA.^{23,197} The 2018 Nobel Prize in Physiology or Medicine was awarded to Dr James P. Allison and Dr Tasuku Honjo for their discovery of CTLA4 and PD-1, respectively.

In this section, we discuss the current knowledge on infiltrating immune cells and expression of immunologic markers in conjunctival melanoma (CoM), and relate it to relevant observations from cutaneous and uveal melanoma (UM). In chapter 5.3, we elaborate on newly developed checkpoint inhibitor therapies and discuss the first clinical observations of their application in CoM.

Infiltrating lymphocytes and macrophages

Cell types of tumour infiltrate

The tumour micro-environment may contain a wide range of immune cells which play a role in the innate (non-specific) and the adaptive (specific) immune responses. Two main types of cells can be identified: histiocytes (i.e. macrophages and dendritic cells) and lymphocytes (i.e. B cells, T cells, and NK cells).

Macrophages are monocytes that originate in the bone marrow and circulate in the blood, until they are recruited to specific sites by chemokines. They have a role in protection against infections and in wound healing, through the production of various growth factors and cytokines.¹⁹⁸ Macrophages can be of an M1 or M2 subtype (though this should be considered as a spectrum), with different receptors, effector functions and chemokines.¹⁹⁹ M1-type macrophages target infectious diseases and can kill bacteria; they express high levels of pro-inflammatory cytokines, such as IL-12 and tumour necrosis factor (TNF). M2-type macrophages have an anti-inflammatory, pro-angiogenic, tissue remodelling role (and produce IL-10). Differentiation follows in response to microbial agents or exposure to cytokines such as interferon (IFN)-gamma.¹⁹⁹ While the total number of macrophages can be determined through marker CD68, the M2 type macrophages are commonly identified by double staining with monoclonal antibodies against CD68 as well as CD163. Tumour-associated macrophages (TAM's) are mainly of the M2 type,¹⁹⁹ which was demonstrated in cutaneous melanoma, CoM,²⁰⁰ as well as in UM.²⁰¹ M2 type macrophages are notably linked to increased tumour angiogenesis in several melanomas,²⁰¹⁻²⁰³ where vessels serve to supply nutrients as well as provide a dissemination route for metastases.

Similar to macrophages, *dendritic cells* (DCs) are part of the antigen-presenting cell family; they play a major role in the initiation and regulation of immunological processes. They induce an antitumour response by cross-presenting antigens to both CD8+ and CD4+ cells, and can activate NK cells. DCs go through a maturation process,¹⁹⁶ but this will not be further discussed in this chapter.

Lymphocytes comprise a group of cells with various functions. *B cells* are known to produce antibodies, which help protect against infections.²⁰⁴ Antibodies have a great target specificity, and B cells may support immune responses of other cells, such as T cells. Several different *T cell* types can be identified, such as the already mentioned CTLs, which express marker CD8. These *CD8+ T cells* can kill tumour cells and inhibit tumour growth by releasing IFN-gamma and TNF-alfa. Another important group of T cells is made up of *T helper cells* (Th) that express CD4. This group consists of several subtypes: *Th1* and *Th2* help the anti-tumour response by stimulating CD8+ T cells via the production of IFN-gamma, Transforming Growth Factor (TGF)-beta and IL-2.²⁰⁵ *Th1* cells can activate macrophages and help with the maturation of dendritic cells.¹⁹⁶ *T regulatory cells* (Tregs) are a specific subclass of Th cells that suppress immune responses. They express the protein *forkhead box P3* (FoxP3). Normally they have a role in maintaining immunologic self-tolerance and preventing autoimmune disease. In cancer, they may inhibit the anti-tumour action of other T cells.²⁰⁶ A cell type with a broader reactivity is the *Natural Killer* (NK) cell. NK cells are able to kill cells that lack HLA Class I expression, or that express NK-activating ligands.²⁰⁷ They are the effector cells of the innate immune system, but also interact with adaptive responses of the T and B cells.¹⁹⁶

Tumour infiltrate in CoM

The first reports on inflammation in CoM date back to the second half of the 20th century.^{208,209} Using regular histopathological examination, the presence of inflammatory cells was studied. Inflammatory cells could be divided into lymphocytes (small cells with a large nucleus) and macrophages, which have a large nucleus and ample cytoplasm, and often contain pigment (i.e. melanophages). It was soon recognized that infiltrate could be analysed for its prognostic value, analogous to findings from cutaneous melanoma,²¹⁰ and some studies likewise identified a significant association between the presence of infiltrate and better survival in CoM (Table 5).^{209,211}

The amount of infiltrate demonstrated quite some variability. In early studies, absence of infiltrate was reported in 0 to 51%, and several subjective grading scales were used to describe the presence of infiltrating cells. Jay reported on a considerable set of 73 cases of CoM, noting *no infiltrate* in 13 cases (18%), *few cells* in 17 cases (23%), *moderate numbers* in 26 cases (36%) and *numerous cells* in 17 cases (23%).²⁰⁸

Several studies found no relation between the presence of infiltrate in CoM and prognosis.^{208,212,213} Others did find such an association,²⁰⁹ including Folberg, who studied the *thickest* known lesion of each patient (which could include later biopsies or recurrences as well) in a large set of 98 CoM associated with PAM; he found that lack of inflammation was associated with worse survival.²¹¹ Another study found that inflammation was not related to survival in a univariate regression model, but that it did relate to better survival in a multivariate model which included other histological parameters.²¹⁴

In the 1980s, monoclonal antibodies were created that helped to identify subtypes of the lymphocyte family. This allowed the identification of specific cell types, each with a specific cell surface marker and function: CD3 is a general T cell marker, CD8 is associated with cellular toxicity (CTLs) and CD4 is associated with the T-helper (Th) function. Anastassiou et al. observed variable amounts of infiltrating CD3+ cells in 26/32 (81%) of evaluable CoM.²¹⁵ CD68+ cells (macrophages) were present in almost all cases (in 33/34 (97%) of samples). There was no relation between the number of CD3+ or CD68+ cells and tumour-related mortality. A similar study in the same era used immunohistochemical staining to study the presence of lymphocytes and CD68+ macrophages in 60 specimens of CoM.²¹⁶ Lymphocytes were seen more frequently in limbal lesions, and numbers were inversely related to tumour thickness; they were not associated with the development of recurrences or survival. The number of macrophages was not associated with tumour location, thickness, or prognosis.

A decade later, Cao et al. continued on this work and studied the infiltrate of T cells and macrophages in 27 CoM using immunofluorescence (Figure 7).²⁰⁰ All samples demonstrated infiltrate, again in varying amounts. Epibulbar (or cT1) lesions showed higher numbers of CD3+CD8- (i.e. Th) cells compared to non-bulbar (or cT2) CoM. The number of infiltrating cells was not related to gender, age, recurrences, metastasis or survival. There was an inverse relation between lesion thickness and numbers of CTLs (CD3+CD8+) and M2 macrophages (CD68+CD163+), which corresponded to the findings of Tuomaala. Cao additionally observed an inverse relation between largest basal diameter (LBD) and all types of lymphocytes. It was hypothesized that in the absence of infiltrate, including CTLs, CoM can grow unrestrained.

Cao noted that, using the same antibodies and techniques as in prior studies on UM, CoM contains higher densities of CD4+ overall, CD4+ Th, and CD4+ Treg cells than UM, but densities of CD8+ T cells and macrophages were lower; the cause of this is unknown. Just as in UM and cutaneous melanoma, the majority of macrophages in CoM were of the M2 type.²⁰⁰

Some studies report on the importance of ratios between infiltrating cells: a high CTL/Treg and M1/M2 ratio was related to improved survival in cervical cancer and cutaneous melanoma.^{217,218} In Cao's work on CoM, however, these ratios did not relate significantly to survival or recurrences.

Major historical work on infiltrate in cutaneous melanoma was performed by Clark et al.,²¹⁰ who introduced the term tumour-infiltrating lymphocytes (TILs) and proposed the traditional classification system of TILs as being 'absent' (absent or not infiltrating), 'non-brisk' (i.e. focal presence) or 'brisk' (i.e. present at the base of lesions, or diffuse intratumourally).²²⁰ [An illustrative review on the history of TIL research was presented by Mihm et al.²²¹].

Clark noted that the absence of TILs was related to a worse survival in cutaneous melanoma.²²⁰ Later studies were contradictory: immune infiltrate was often related to unfavourable tumour characteristics, but not uniformly to worse survival.^{222,223}

Possibly, these discrepancies can be explained by characteristics of study groups. It has long been reported that TILs have impact in the vertical growth phase, but not in the (earlier) radial growth phase,²²⁰ limiting conclusions in studies that include smaller, radial growth phase, lesions. Even so, the impact of TILs may be most clear in thicker lesions, despite thicker lesions having lower TIL numbers in general.²²³

When looking at Tregs specifically, it was observed that a high FoxP3 expression was associated with worse survival in 185 primary cutaneous melanoma patients, independent of lesion thickness. This points towards a suppressive action of Tregs in this malignancy.²²⁴

The role of macrophages in cutaneous melanoma is less well understood. In 202 samples, high counts of CD68+ cells were related to unfavourable features such as a greater Breslow thickness, ulceration, a higher mitotic rate and a high microvascular density (of both blood vessels and lymphatic vessels).²⁰³ However, in this study no relation with relapse-free or overall survival was noticed. The finding that both blood vessels and lymphatic vessels were increased in lesions with many macrophages is interesting, as cutaneous melanoma (as well as CoM!) is known to disseminate via both routes, with an especially important role for the lymphatic route.

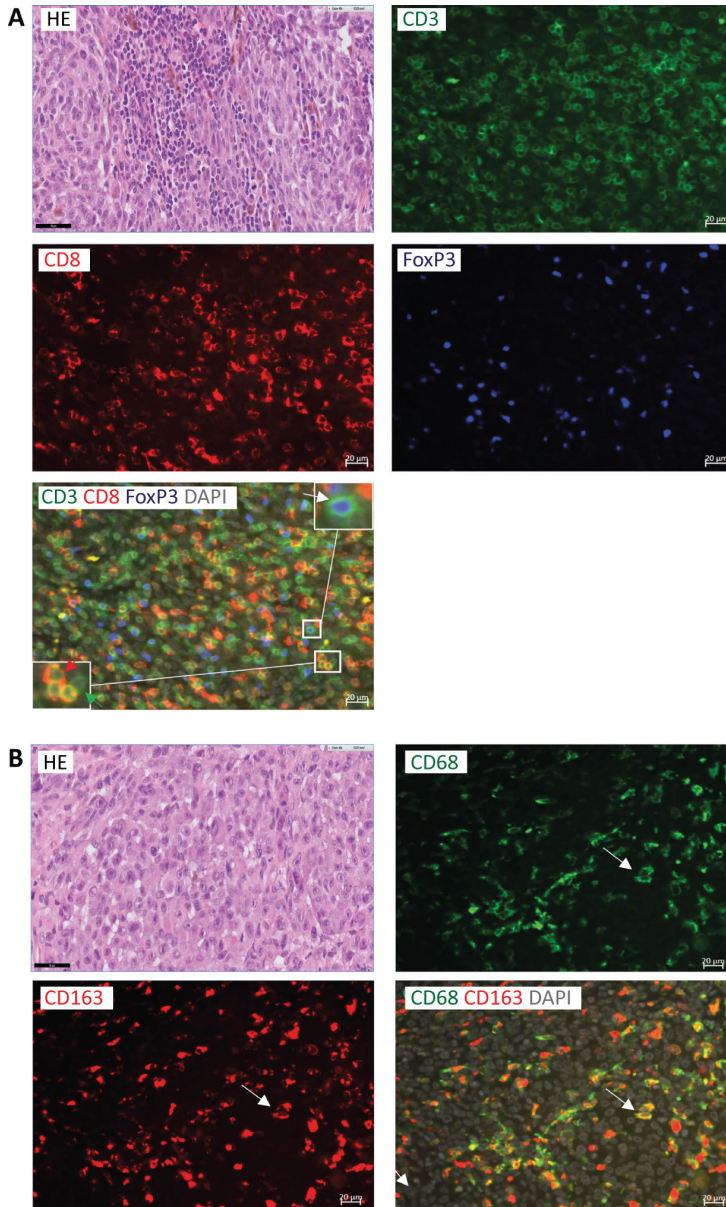


Figure 7. Tumour infiltrate in CoM. (A) CoM tissue was stained using H&E, CD3 (green, membrane), CD8 (red, membrane) and FoxP3 (blue, nucleus). The merged images allows for identification of individual cell types: the combination of nuclear blue Foxp3 and surface green CD3 staining (white arrow) indicates the presence of CD3+CD8-Foxp3+ T cells. The green arrow indicates a CD3+CD8-Foxp3- T cell, and the red arrow points at CD3+CD8+ T cells. (B) Staining with H&E, CD68 (green, cytoplasm/membrane), and CD163 (red, cytoplasm/membrane). The merged image shows double-positive M2 type macrophages cells. The scale bar of immunofluorescence images is 20 μm, and of H&E images is 50 μm. [Figure re-used from Cao et al. 2017 with permission.²⁰⁰]

Table 5. Studies reporting on immune infiltrate in CoM. The separation between early and later work is based on the development of immunohistochemical staining techniques.

Study	n=	Infiltrate (cell type and technique)	Presence of infiltrate	Relation with Characteristics	Relation with Survival
“early work”					
Jay, 1965. ²⁰⁸	73	(no methods or cell types specified)	none n=13 (18%) few n=17 (23%) moderate n=26 (36%) numerous n=17 (23%)	No data reported	No sign. relation with survival
Crawford, 1980. ²⁰⁹	19	(H&E) “most were lymphocytes”	no cells at base: 0 (0%) few at base: 6 (32%) moderate at part of base: 4 (21%) moderate at whole base: 6 (32%) intense at whole base: 3 (16%)	No data reported	Presence has better survival.
McGhee, 1982. ²¹²	28	(no methods or cell types specified)	mild: 19 (68%) moderate: 7 (25%) severe: 2 (7%)	No data reported	No relation with survival
Folberg, 1985. ²¹¹	98	(no methods or cell types specified)	[First lesion] lack=47%, present=53%	No data reported	No sign. relation with survival
Jeffrey, 1986. ²¹³	37	(no methods or cell types specified)	[Thickest lesion] lack=51%, present=49%	No data reported	Presence has better survival.
Bobic-Radovanovic, 1998. ²¹⁴	61	(H&E) (no methods or cell types specified)	Not reported	No data reported	No relation with survival
“later work”					
Anastassiou, 2004. ²¹⁵	32	(IHC) CD3+	No: 6 (19%), few: 14 (44%) mod: 10 (31%), strong: 2 (6%)	No data reported	Univariate: no relation with survival. Multivariate: presence has better survival
				No data reported	No relation with survival

Table 5. Continued

Study	n=	Infiltrate (cell type and technique)	Presence of infiltrate	Relation with Characteristics	Relation with Survival
Tuomaala, 2007. ²¹⁶	34	(IHC) CD68+	No: 1 (3%), few: 12 (35%) mod: 15 (44%), strong: 6 (18%)	No data reported	No relation with survival
	60	(H&E) Lymphocytes	Few: 27 (45%) Moderate: 17 (28%) Many: 16 (27%)	More in limbal, thinner lesions.	No relation with rec/survival.
	58	CD68+ macrophages	Few: 7 (12%) Moderate: 24 (41%) Many: 27 (47%)	No relation with location/thickness	No relation with rec/survival.
Cao, 2017. ²⁰⁰	27	Immuno-fluorescence, (median [range])	CD3: 151 [71–637] CD3+CD8+: 68 [26–335] CD3+CD8-: 70 [26–314] CD3+CD8-Foxp3-: 44 [13–202] CD3+CD8-Foxp3+: 30 [10–124]	CD8+: more in thinner and smaller LBD CD8- (mainly Foxp3-) more in epibulbar lesions	No relation with rec/survival.
		Immuno-fluorescence, (median [range])	CD68: 59 [19–248] CD68CD163: 39 [8–220]	M2: more in thinner lesions	No relation with rec/survival.
	60	IHC CD8+	No: 6 (10%) <5%: 20 (33%), 5–50%: 26 (43%) >50%: 8 (13%)	More in non-epithelioid cell types, non-bulbar location	No relation with rec/survival.

Abbreviations: Rec, recurrences; M2, M2-type macrophages; H&E, haematoxylin and eosin; IHC, immunohistochemistry.

UV radiation was found to influence the immune infiltrate in cutaneous melanoma lesions, by recruiting macrophages and neutrophils.^{81,82} This is relevant for CoM as parts of the conjunctiva are sun-exposed. Macrophages have pro-angiogenic effects that are relevant for tumour dissemination. These effects include an increased IFN-gamma signalling, with upregulation of CCL8.⁸² Even so, neutrophils stimulate angiogenesis and promote migration of melanoma cells towards blood vessels.⁸¹

Many concepts from tumour immunology have been contrasted between extraocular melanoma and intraocular UM. In the latter, the presence of inflammatory cells is known to be unfavourable for many decades.^{225,226} By this remarkable position, UM provides an example of immunologic *failure* to destroy tumour cells, elucidating mechanisms of tumour escape that are relevant to other tumours as well. As such, alterations in HLA and PD-L1 expression are very relevant to CoM and are discussed in sections 4.3 and 4.4.

Similarities between extra-ocular melanoma and UM are seen in the role of (pro-inflammatory) macrophages and (immune suppressing) Tregs. The presence of both CD68+ and CD163+ cells is related to unfavourable features in UM as monosomy 3, ciliary body involvement, greater LBD, and worse survival.²²⁷⁻²²⁹ Even so TAMs are related to a higher vascular density.^{167,201,228} FoxP3+ cells (Tregs) were identified relatively recently in UM,²²⁷ and while the prognostic significance needs further study, the presence of intratumoural Tregs has been related to a poorer clinical outcome²³⁰ similar to what is seen in cutaneous melanoma.

Recent work shows that UM with increased numbers of CD8+ cells, increasingly express immune checkpoint inhibitors ‘Indolamine 2,3-Dioxygenase 1’ (IDO1) and ‘T cell immunoreceptor with Ig and ITIM domains’ (TIGIT) (which limit the efficacy of CTLs to kill tumour cells).²³¹ This may explain the opposite effects of TIL presence on survival in UM and other melanomas. The expression of these checkpoints in CoM is unknown, but may be relevant in cases that fail to respond against immunotherapy. Even so, Fas Ligand is a suppressor of immune activity and being expressed in conjunctival epithelium,²³² may contribute to CoM resistance against new therapies.

Discussing the infiltrate in CoM

Despite a limited number of studies on CoM, several conclusions can be drawn regarding the role of the immune infiltrate (Table 6). As such, immune cells appear to be favourable: in early reports on CoM an association was established between the presence of immune cells and a favourable prognosis.^{209,211,214} The specific roles for subtypes of the infiltrate need to be elucidated however, as later studies, examining the specific presence of T cells or macrophages, failed to confirm an association with survival.^{200,215,216} Two recent works (by Tuomaala and Cao) identified an inverse relation between lymphocytes and tumour thickness (a known unfavourable factor), but did not see the same relation with macrophages. These findings in CoM largely mirror those of cutaneous

melanoma where infiltrating lymphocytes correspond with a favourable prognosis (and thin lesions). Interestingly, an immune infiltrate in cutaneous melanoma has especially prognostic significance in vertical growth phase and thick lesions. CoM apparently behaves more as a vertical-growth phase cutaneous melanoma than as a radial growth phase tumour. Though little data is available for other mucosal melanoma, findings are similar to those in CoM, as in oral mucosal melanoma the absence of TILs was related to more metastases (but not to worse survival).²³³

The role of TILs in CoM, and cutaneous melanoma, is clearly different from the role in intraocular UM, where their presence is associated with a worse prognosis. Interestingly, the presence of TILs is often positively correlated with the presence of TAMs: this has been reported for CoM,²⁰⁰ cutaneous melanoma,²⁰³ and UM.²²⁷ In UM, the presence of both cell types is prognostically unfavourable, while in cutaneous melanoma they appear to have opposing effects. The mild or absent relations between infiltrate and prognosis in cutaneous melanoma and CoM may therefore be explained, as the different cell types counteract each other. Another important issue may be that inhibitory forces (from Tregs or immune checkpoints) are more pronounced in intra-ocular than extra-ocular melanoma, explaining why the prognostic role of infiltrate is less in cutaneous melanoma and CoM.

Future projects could analyse the infiltrate in CoM with larger sample sizes, to compare findings from cutaneous melanoma with more statistical power. The role of macrophages should be unveiled, together with its influence on angiogenesis and development of lymphatic vessels (which is of major importance for metastases). Translating important lessons from UM research, the inhibitory aspects of checkpoint inhibition, expression of mechanisms as IDO and TIGIT, and the role of Tregs deserve attention. The presence of immune cells cannot be considered a binary event, with varying and even opposing roles for various cell types, and understanding this is a requirement before clinical steps may be undertaken, as to select patients for T cell-based immunotherapies.

Table 6. Overview of the effects of infiltrate in different types of melanoma.

Presence of:	Conjunctival Melanoma		Cutaneous Melanoma		Uveal Melanoma	
	Clinical characteristics	Prognosis	Clinical characteristics	Prognosis	Clinical characteristics	Prognosis
TILs	Good	Good/NS	Good	Good	Bad	Bad/NS
TAMs	NS	NS	Bad	Bad	Bad	Bad

Abbreviations: TIL, tumour infiltrating lymphocyte; TAM, tumour associated macrophage; NS, not significant.

HLA expression

The HLA system

The *human leukocyte antigen* system (HLA, the human counterpart of the *major histocompatibility complex* (MHC)) comprises a class of molecules with a major role in immunology. Two main types of HLA are identified: HLA Class I and HLA Class II. The *HLA Class I molecule* consists of two polypeptide chains: the non-polymorphic light b2-microglobulin (B2M) chain (encoded on chromosome 15), and the highly polymorphic heavy alpha chain (encoded by the *HLA* gene on chromosome 6p21).²³⁴ Different types of the HLA Class I molecules are HLA-A, HLA-B and HLA-C. HLA Class I proteins are expressed on (almost) all nucleated cells. Their function is to present peptides from intracellular proteins and invasive viruses to the T cell receptor of CD8+ killer T cells. Also, HLA Class I inhibits NK cell activity.²³⁵ *HLA Class II molecules* have a different structure with an alpha and beta chain (both encoded on chromosome 6). Major variants are HLA-DM/DO/DP/DQ/DR. HLA Class II is mainly present on immune cells, such as B cells, some T cells, and antigen-presenting cells (APCs). It can be upregulated on other cells during inflammation. Its function is to present peptides from outside the cell, and to interact with CD4+ T helper cells.²³⁵ Some HLA alleles, as HLA-B27, are strongly related to the development of specific diseases.²³⁶

In cancer research, HLA Class I has received much attention for its role in mediating interactions between tumour cells and T cells. Loss of HLA Class I (which can either be reversible (“soft”) or irreversible (“hard”))²³⁷ is a mechanism to escape immune surveillance, and has been associated with worse survival in many malignancies, including cutaneous melanoma.²³⁸ IFN-gamma can upregulate HLA Class I expression and therefore may restore the susceptibility of tumour cells to be lysed by T cells. However, counterbalancing mechanisms exist as NK cells on their turn are being activated by the absence of HLA Class I.²⁰⁷

Expression of HLA Class I on CoM

HLA Class I expression in CoM was studied by Cao et al. in 23 samples using immunofluorescence.²³⁹ A marked positive expression was observed for HLA-A, HLA-B/C or B2M in a third of lesions, which is less than seen in cutaneous melanoma.²⁴⁰

The level of expression of HLA Class I in CoM was not related to the tumour’s basal diameter, development of recurrences or metastases, or survival. There was a correlation with prognostic factors however, as epibulbar/T1 CoM had a higher HLA Class I expression, and thicker CoM had a lower expression of HLA Class I. An increased expression of HLA Class I was associated with a higher number of CD68+CD163+ macrophages, and tended to be so with CD8+ lymphocytes, even in this small series; this suggests that macrophages and T cells play a role in stimulating HLA Class I expression in CoM, similar to the situation in UM.²⁴¹ In vitro work on three CoM cell

lines demonstrated that addition of IFN gamma indeed caused upregulation of HLA Class I and of its transcriptional regulators CIITA, IRF1, NLRC5, and the *Transporters associated with Antigen Processing* TAP1 and TAP2.²³⁹

It is not yet known whether the low expression of HLA Class I in CoM is due to mutations, loss of heterozygosity (LOH) in chromosomes 6 or 15, epigenetic downregulation, or prior immune selection that led to outgrowth of HLA Class I negative tumour cells.²⁴² A study of CoM-derived cell lines showed that at least two of three cells lines contained a hard loss of HLA antigens: cell line CRMM1 had lost its HLA-A2 cell surface expression and cell line CRMM2 its HLA-B44 expression.²³⁹

The relatively recent findings on HLA expression in CoM are in line with earlier studies from cutaneous melanoma. Expression of HLA Class I and TAP1 and 2 is associated with decreased lesion thickness,^{238,243,244} and a longer time to disease progression and longer survival.²³⁸ Even so, metastases have a lower HLA Class I expression compared to primary lesions,^{238,244,245} and HLA expression is favorably associated with tumour regression.²⁴⁶

HLA expression may be of a different phenotype in metastases compared to primary lesions due to selective outgrowth, and the expression on metastases may become resistant to upregulation due to mutations in the IFN pathway.²⁴⁷

Recent work shows that HLA I expression relates to survival after checkpoint inhibition in advanced melanoma patients: homozygosity of at least one allele and LOH are related to worse survival (with less variation to present tumour antigens), and while the presence of an HLA-B44 allele was related to significantly better survival after checkpoint inhibition, presence of an HLA-B62 allele was related to reduced survival. This can have implications for the design of future trials,²⁴⁸ including for potential studies on CoM.

The role of tumour infiltrate on HLA expression can be demonstrated by a comparison between CoM and UM. In UM, a high HLA Class I expression is similarly associated with the presence of both leukocytes and macrophages (CD3, CD4, CD8, CD11B, CD68),^{231,241,249} but it is associated with *unfavourable* tumour characteristics, such as monosomy 3 and *decreased* prognosis.^{229,249} This led to the hypothesis that NK cells are specifically important for tumour surveillance in UM,^{250,251} while CTLs are more important in cutaneous and conjunctival melanoma. This is supported by findings that NK cells are more effective in killing HLA-negative cells in the blood compared to in lymphoid vessels,²⁴⁷ which fits the metastasis pattern of both UM and CoM.

Discussing HLA expression in CoM

Few projects focused on HLA expression in CoM, but the available data suggest that HLA expression is associated with favourable tumour traits, and that expression is increased in the presence of an immune infiltrate. These concepts are in line with work from cutaneous melanoma, and to some extent with UM. Understanding HLA expression in CoM is important as it underlies the efficacy of T cell-mediated therapy. Downregulation of HLA Class I can limit this efficacy, and HLA expression may therefore be a selection criterion for therapy in patients, as advised by Cao.²³⁹ Even so, upregulation of HLA may be a part of future therapies to enhance the efficacy of immunotherapy. It should be studied whether subtypes of HLA respond differently to T cell-mediated therapy in CoM, as can be expected from work on cutaneous melanoma.

PD-1/PD-L1 expression

Immune checkpoints

The interaction between CTLs and tumour cells is influenced by various stimuli, including the checkpoint pathways. One of the major checkpoint pathways acts via PD-1/PD-L1. PD-1 is a glycoprotein that is expressed on activated T cells and that can bind to its ligand PD-L1 on the surface of tumour cells and macrophages. The PD1/PD-L1 interaction results in several inhibitory events within T cells, including inhibition of cytokine and enzyme production, and inducing stagnation of cell cycle or even apoptosis. This prevents T cells from targeting the tumour cell (Figure 8).²⁵² Other ligands for PD-1 exist, such as PD-L2, but these are beyond the scope of this manuscript.

Blockade of the PD-1/PD-L1 interaction is the underlying mechanism of anti-PD-1/PD-L1 therapies. In advanced cutaneous melanoma, anti PD-1 therapy has proven successful, with nivolumab treatment showing a better overall survival (OS) and progression-free survival (PFS) than chemotherapy (dacarbazine).²³ Both nivolumab²⁵³ and pembrolizumab¹⁹⁷ monotherapy (both against PD-1) provide a better response (with a higher overall survival and progression-free survival) compared to the original checkpoint inhibitor ipilimumab (anti-CTLA4).

The CTLA-4 protein is another negative regulator of T cells (Figure 8).²⁵⁴ Its function is based on the fact that T cells require more than one stimulatory signal to be activated. CTLA4 is expressed on T cells, and competes with CD28 molecules to bind B7, which is their shared ligand. CTLA4 has a greater affinity for the ligand however, and while a CD28-to-B7 binding would lead to *increased* T cell activity, a CTLA4-to-B7 binding does not cause activation and may even cause *inhibition* of the cell. The full mechanism is not fully understood however, and is likely more complex, involving for instance CTLA4-mediated activation of Tregs. To explain checkpoint inhibition in CoM, we will focus on the PD-1/PD-L1 interaction.

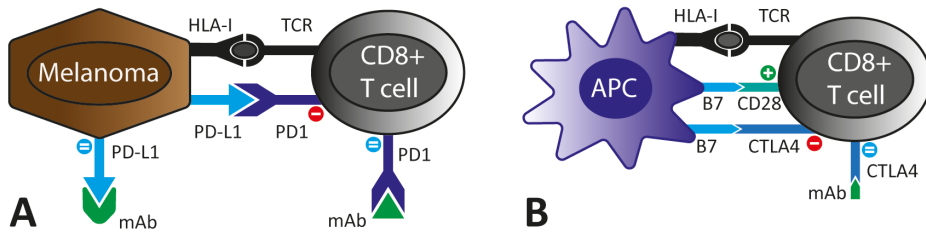


Figure 8. Checkpoints in tumour immunology. (A) CTLs interact with melanoma cells via HLA Class I molecules. Downregulation of HLA Class I causes CTL failure to recognize melanoma cells. Binding of PD-1 to its ligand PD-L1 causes inactivation of T cells, reducing the tumour killing capacity. Monoclonal antibodies against PD-1 or PD-L1 prevents the inactivating signal, resulting in undisturbed T cell activation. (B) T cells require co-stimulation apart from signalling via the TCR. Binding of B7 to CD28 causes activation, while binding of B7 to CTLA4 causes reduced signalling or even inactivation of T cells. Monoclonal antibodies against CTLA4 prevent the inactivating signal, resulting in undisturbed T cell activation. Abbreviations: TCR, T cell receptor; HLA-I, HLA Class I molecule; mAb, monoclonal antibody; APC, antigen presenting cell.

Predictive value of PD-L1 expression for treatment response

Tumour cells can express variable levels of PD-L1. One would expect that the level of PD-L1 expression on tumour cells is a marker to predict response against specific (invasive) checkpoint inhibitors, since it is the blocking of this exact PD-1/PD-L1 axis that underlies the mechanism of action. Various studies on advanced cutaneous melanoma indeed observed better response rates to checkpoint inhibitors and longer survival in patients with PD-L1 positive tumour cells compared to PD-L1 negative lesions: this was reported for pembrolizumab (anti-PD1),²⁵⁵ nivolumab (anti-PD1),²³ ipilimumab (anti-CTLA4) and nivolumab + ipilimumab combination therapy.²⁵⁶ Even so, a melanoma type with little PD-L1 expression (UM)^{257,258} shows little response to checkpoint inhibitor therapy.²⁴ However, in cutaneous melanoma many patients with PD-L1 negative lesions also responded favourably to these treatments: while 53% of patients with PD-L1 positive lesions had an objective response to nivolumab treatment, also 33% of patients with PD-L1 negative lesions had a response, providing survival benefit compared to chemotherapy for PD-L1 negative lesions as well.²³ It has been suggested that not only PD-L1 expression on tumour cells is relevant to therapy, but also the expression on cells in the tumour microenvironment.²⁵⁹

Adding to the debate on the usefulness of PD-L1 assessment, there is concern about the sensitivity of specific immunohistochemistry staining tests for PD-L1, with different scoring systems and cut-off levels being used between studies.²⁶⁰ In addition, PD-L1 expression may vary over time.²⁶¹

PD-L1 expression in CoM

Only a few studies analysed expression of checkpoint inhibitors in CoM. An early study on PD-L1 expression in mucosal melanoma of the head and neck, observed positive PD-L1 in 3/23 cases; the three cases of CoM that were included in this set were all negative.²⁶² All nine samples of cutaneous melanoma that were used as control were PD-L1 positive.

Cao et al. studied PD-L1 expression in 27 cases of CoM (Figure 9).²⁰⁰ Using a cut-off of 5% of cells, PD-L1 was expressed on tumour cells in 5 (19%) cases, and on stromal cells in 16 (59%) cases (Fig 4.3). Stromal expression mainly involved M2 macrophages. PD-L1 expression on tumour cells was associated with more metastasis and disease-specific death, studied in a cohort with a median follow up time of 46 months.

Recent work on 65 CoM confirmed that PD-L1 is expressed more often on immune cells (58%) than on tumour cells (10%), and that PD-L1 expression on CoM is associated with worse survival at a median follow-up time of 29 months; however, expression results varied between two applied IHC antibodies.²¹⁹

The reported PD-L1 expression on CoM tumour cells (up to 19%)^{200,219} is somewhat less than reported in cutaneous melanoma (30-35%)^{23,263}. A large study on cutaneous melanoma reported a somewhat lower level of PD-L1 positive expression (24% of cases), but in a fairly large number of cases (11%), no status could be determined so the actual expression may be different.²⁵³ PD-L1 expression on CoM is higher than on other mucosal melanoma (sino-nasal, vaginal, rectal).⁹⁰ Importantly, the cut-off for deeming a sample positive has a major influence on the reported numbers: one study reported PD-L1 expression in 76% of cutaneous melanoma samples, but used a cut-off of only 1% of cells expressing PD-L1.²⁵⁵

PD-1 expression has not been identified on CoM tumour cells, but is expressed on T cells in 17 (63%)²⁰⁰ and 15 (23%)²¹⁹ of cases. In both studies, no significant relation with patient outcome was established, but Cao reported a trend between PD-1 expression and a higher number of recurrences.

IFN-gamma is known to enhance PD-L1/PD-1 expression in cutaneous melanoma.²⁶³ In vitro analysis by flow cytometry of three CoM cell lines (CRMM1, CRMM2, CM2005.1) demonstrated no background expression of PD-L1, while one cell line (CRMM2) showed expression of PD-1. Addition of IFN-gamma induced upregulation of HLA Class I (used as control) in all three cell lines, with two lines showing an increase in the expression of PD-L1 (CRMM2 and CM2005.1), and one of PD-1 (CRMM2). These findings show that IFN-gamma, produced by tumour-infiltrating lymphocytes (TILs), may be responsible for enhancing PD-L1 expression in CoM.²⁰⁰ This is similar to findings in cutaneous melanoma.²⁶³

A limitation to studies regarding PD-L1 in CoM is the small size of tissue samples. PD-L1 expression can be quite heterogenous within samples²⁶⁴ and it is not known how a small sample represents the PD-L1 status of the tumour as a whole.²⁰⁰ Since cutaneous melanoma patients with PD-L1 positive as well as negative tumours may respond favourably to anti-PD-1 treatment, this may not so much be a selection criteria for treatment per se (and negative staining should not prohibit CoM patients from entering trials).²⁵⁵ Even so, combining checkpoint inhibition with other interventions such as radiotherapy may be beneficial, as is currently attempted in UM.⁹

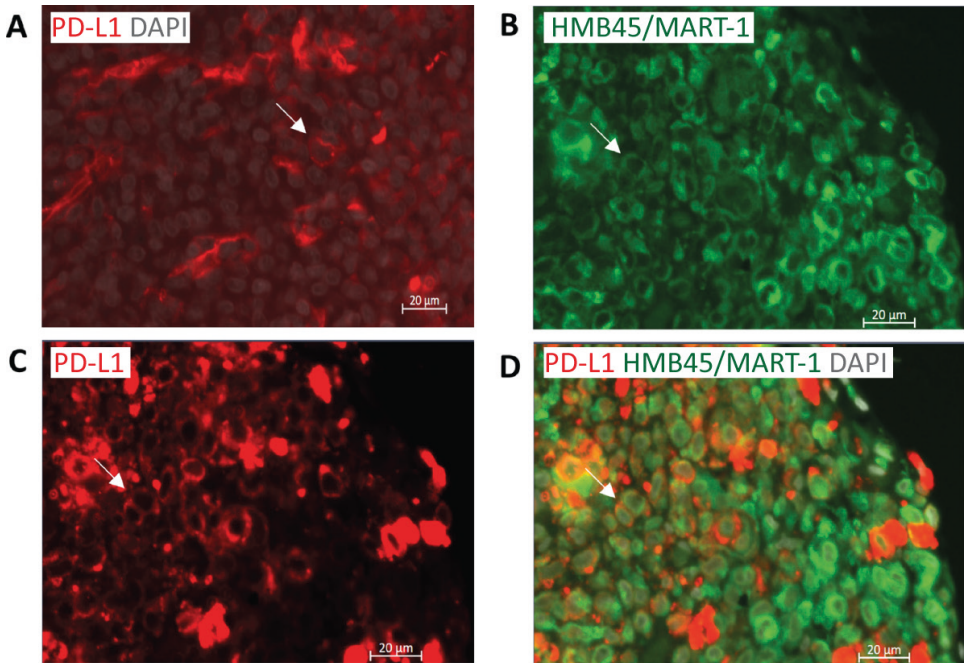


Figure 9. PD-L1 expression in CoM. (A) Membranous PD-L1 staining (red) is demonstrated in a positive control (human tonsil tissue). (B) Staining with HMB45/MART-1 (green, cytoplasmic/membranous) allows for identification of tumour cells. (C) PD-L1 (red, membranous) is expressed in the studied tissue. (D) Double staining shows that PD-L1 is expressed on CoM tumour cells. [Figure re-used from Cao et al., 2017 with permission.²⁰⁰]

Discussing PD-L1 expression in CoM

The expression of PD-L1 in CoM seems to mirror the findings of cutaneous melanoma, though somewhat lower percentages of expression have been reported. Whether PD-L1 expression has prognostic value or is a therapeutic indicator in CoM has not yet been established, but it can be expected that larger studies would identify this, as is seen in cutaneous melanoma. The relevance of this predictive effect may be similarly limited however, as PD-L1 negative CoM may still respond

to immunotherapy. Prior to initiation of larger translational studies in CoM, it may be needed to overcome technical issues such as the difficulty of obtaining representative PD-L1 expression results from small tissue samples.

Novel approaches regarding checkpoint inhibition in cutaneous melanoma include the combination with radiotherapy²⁶⁵ or photodynamic therapy in preclinical models.²⁶⁶ As radiotherapy has been well-established in CoM, this may be readily-transferable to CoM as well.

Conclusions (Immunology)

Similar to the situation in cutaneous melanoma, the presence of an immune infiltrate is favourable in CoM. Questions remain on the exact role of all cell types of the immune infiltrate, and regarding the role of inhibitory forces such as those of T-regs. It is as yet unknown how infiltrating macrophages relate to (lymph and blood) vessel development in CoM, which may provide a possible dissemination route for metastases. Even more, expression of IDO and TIGIT (relevant in *intra* ocular melanoma as an escape from the immune system) may have relevance in the conjunctiva, but this is currently unknown.

HLA expression is common in CoM, and as expected by its role in interaction with immune cells, has a relation with favourable traits. Downregulation may limit the efficacy of T cell-based therapies, while upregulation may enhance the susceptibility to immunological clearance. Screening for HLA expression, or the presence of specific alleles, may become part of patient workup prior to immunotherapy in CoM.

Like cutaneous melanoma, CoM is known to frequently express PD-L1. The prognostic and predictive value is limited however, as in cutaneous melanoma even negative lesions could respond to anti-PD-L1 therapy. It is a promising target for therapy, possibly in combination with other treatments such as photodynamic therapy or radiotherapy.

5. TARGETED THERAPY AND CHECKPOINT INHIBITOR THERAPY IN COM

Current therapy of localised and metastatic CoM

Tumour location and extent are currently the main determinants for therapy in CoM. Localised disease is commonly treated by surgical excision using a 'no touch technique' and adjuvant therapy.¹¹ Adjuvant therapy includes cryotherapy (with a "double freeze-thaw" technique²⁶⁷), topical chemotherapy (such as mitomycin-c drops²⁶⁸ or interferon-alfa²⁶⁹), and / or radiotherapy. For radiotherapy several techniques exist of external radiotherapy or brachytherapy using plaque or handheld applicators).^{270,271} Treatment of palpebral lesions may be more complex, as this site is

more difficult to approach, and adjuvant brachytherapy can only be delivered via adapted ‘outward’ applicators.²⁷² Widespread lesions require a more extensive approach, with extensive surgery, radiotherapy,²⁷³ and ultimately even orbital exenteration.¹¹

The approach to adjuvant therapy varies between institutions, as no data supports superiority of either, and availability differs.^{11,274,275} Most authors include cryotherapy to conjunctival margins by default, as part of the surgical procedure presented by Shields et al.;²⁶⁷ for corneal involvement, alcohol epitheliectomy is performed. Additional adjuvant therapy may be reserved for cases with incomplete resection, with concurrent PAM, or for recurrences, but others advise to use it for all.²⁷⁶ Radiotherapy is well-accepted for incomplete margins, topical chemotherapy can be applied for concurrent (and widespread) PAM.^{13,275}

Importantly, patients have a better prognosis if initial treatment is delivered in a centre with expertise in ocular oncology (preventing delay and possible inappropriate or incomplete resection), calling for general ophthalmologists to swiftly refer patients with a suspicious conjunctival lesion.^{13,71}

CoM may disseminate to lymph nodes (regional) as well as systemic sites (distant). Most often dissemination involves the parotid (pre-auricular), cervical and submandibular lymph nodes, and the lungs, liver, brain and skin, respectively.^{12,277} In CoM, the lymph nodes are often believed to be the first site of metastasis; systemic metastases may develop independently as well.^{13,277} While lymph metastases are important in CoM and cutaneous melanoma, this differs from UM since the uvea lacks lymphatic drainage. The sentinel lymph node biopsy (SLNB) is a technique to detect micro-metastases in the first node(s) that are theoretically reached by disseminating tumour cells. If detected, adjuvant therapy can be administered, and a lymphadenectomy can be performed, preventing further spread.²⁷⁸ There is debate on the position of SLNB in CoM management,²⁷⁹ however with new therapeutic options for metastatic disease, the clinical relevance is rising and the SLNB is performed more frequently.

There is currently no standard therapy for metastatic CoM.²⁸⁰ Most often, guidelines from cutaneous melanoma are followed, e.g. as presented in the European guideline for melanoma.²⁸¹ In patients with few metastases, or those at specific accessible locations, selective surgical metastatectomy or radiotherapy can be applied. In widely disseminated disease, systemic agents are required. Up till a few years ago, cytotoxic chemotherapy was the only available option, which was associated with poor response rates and survival benefit. In the management of cutaneous melanoma, newly-developed immunotherapy and targeted therapy (as will be discussed later on) have substituted these agents as first line therapy, leaving conventional chemotherapy as a last-resort option after failure, or in those cases where (more expensive) other treatments are not available.

Targeted therapy in CoM

‘Targeted therapy’ or ‘small molecule inhibitors’ involves drugs that target genetic mutations and (upregulated) pathways that are related to malignancies, and that are absent in healthy tissues. It has been suggested to use cutaneous melanoma therapies for CoM.

Since 2013, the use of targeted therapies has been reported for a small number of CoM. We are not aware of clinical trials or large cohorts that are formally studying these drugs in CoM, and therefore resort to small series, single case reports and preliminary in vitro work. The aim for systemic targeted therapy in CoM is mainly 1) to treat widespread local disease, that is too large for excision, or as an alternative for orbital exenteration, or 2) to treat regional and distant metastases.

In this section, we will discuss patient-related outcomes of several MAPK-pathway-inhibitors in CoM, and present several new drugs with a suggested potential based on preclinical work or similarities to cutaneous melanoma.

BRAF and MEK inhibitors

The MAPK pathway can be inhibited by drugs targeting *BRAF* and MEK. Inhibitors of *BRAF* are the most well-established targeted therapy drug type in cutaneous melanoma (Table 7). First reports on single-agent therapy demonstrated survival benefit for vemurafenib (trade name: Zelboraf, Hoffmann-La Roche²⁸³) versus dacarbazine chemotherapy in metastatic disease in previously untreated cutaneous melanoma patients with a *BRAF* V600E mutation.¹⁹ Later, other inhibitors were introduced as well (i.e. dabrafenib (trade name: Tafinlar, Novartis²⁸²), and recently encorafenib (trade name: Braftovi, Array Biopharma²⁸⁴).

Table 7. BRAF and MEK inhibitors.

	Generic name	Brand name	Dosing and route*	FDA reference
<i>BRAF</i> inhibitors	dabrafenib	Tafinlar	150 mg, twice daily oral	FDA, 2013. ²⁸²
	vemurafenib	Zelboraf	960 mg, twice daily oral	FDA, 2011. ²⁸³
	encorafenib	Braftovi	450 mg, once daily oral**	FDA, 2018. ²⁸⁴
MEK inhibitors	trametinib	Mekinist	2 mg, once daily oral	FDA, 2013. ²⁸⁵
	cobimetinib	Cotellic	60 mg, once daily oral	FDA, 2020. ²⁸⁶
	binimetinib	Mektovi	45 mg, twice daily oral**	FDA, 2018. ²⁸⁷

Abbreviations: FDA, Food and Drug Administration.

* Dosing as indicated for (metastatic) melanoma, for other indications please read product information.

** Encorafenib and binimetinib are indicated for combined use.

A common issue with *BRAF* inhibition is the development of treatment resistance, that often occurs within a year. This can be due to several mechanisms such as upregulation of *NRAS*,²⁸⁸ NF1,²⁸⁹ or ERK,²⁹⁰ and downregulation of PTEN;²⁹¹ it has recently been linked to upregulation of other pathways such as YAP1^{292,293} and even PD-L1,²⁹⁴ providing escape from immune cells. Combining *BRAF* and MEK inhibition is a solution to overcome this resistance, and combination therapy demonstrated prolonged survival over *BRAF* monotherapy in cutaneous melanoma: vemurafenib (*BRAF* inhibitor) and cobimetinib (MEK inhibitor; trade name: Cotellic, Genentech²⁸⁶) were superior versus vemurafenib alone.²⁹⁵ A similar result was reported when dabrafenib (*BRAF* inhibitor) and trametinib (MEK inhibitor; trade name: Mekinist, Novartis²⁸⁵) were combined versus dabrafenib alone²⁹⁶ or versus vemurafenib alone²⁹⁷. Recently, binimetinib (trade name: Mektovi, Array Biopharma²⁸⁷) has been introduced as well.

While clinical outcomes regarding *BRAF* and MEK inhibition in CoM have been reported [section 5.2.2.], preclinical work continues to optimize treatment by e.g. analysing combined pathway inhibition. In vitro work on three CoM cell lines by Cao et al tested the mutation-specific effect of two *BRAF* inhibitors and a MEK inhibitor.¹²¹ The two *BRAF* inhibitors (vemurafenib and dabrafenib) inhibited growth of cell lines CRMM1 and CM2005 (both harbouring a *BRAF* mutation) although not with the same sensitivity. This could not be explained by *PTEN* loss (which was not found in any of the cell lines). Both drugs caused paradoxical activation of the MAPK pathway in a third cell line, CRMM2 (with an *NRAS* mutation, no *BRAF* mutation); an effect that was later confirmed by El Zaoui et al.¹²³ Cao et al showed that MEK inhibition had an inhibitory effect on all three cell lines, and that combined inhibition of MEK and AKT even showed synergistic effects.¹²¹

Reported cases of targeted therapy in CoM.

BRAF and MEK inhibitors are (similar to the situation in cutaneous melanoma) the most well-studied small molecule inhibitors in CoM. We have been able to find the reports on seven CoM patients who received treatment with a *BRAF* inhibitor (Table 8). It is likely that more patients have been treated, however, but not reported. In one case, treatment of the conjunctival lesion was the sole aim, in a patient who was free of lymph- or distant metastases.²⁹⁸ This patient had a recurrence of CoM that would otherwise have been treated with orbital exenteration. There was a good response to vemurafenib, with tumour decrease and a stable situation for 3 years. The six other reported patients with targeted therapy had metastatic disease. As a response to the landmark paper by Griewank et al on the prevalence of *BRAF* mutations in CoM,¹⁷ Weber et al. noticed that a patient with a *BRAF*V600E mutation and several distant metastases, developed only a mild response to vemurafenib monotherapy (with progression after 2 months).²⁹⁹ They suggested that *BRAF* inhibitors may not be successful in CoM as in cutaneous melanoma, possibly due to frequent *PTEN* loss in CoM, which may have contributed to treatment resistance. Griewank replied by reporting a patient who was treated with dabrafenib for metastases and who had a partial response with a significant 62% tumour reduction; this patient, however, developed new lesions after 6

months.³⁰⁰ A similar case was reported by Maleka et al.: a patient with metastatic CoM was treated with vemurafenib, and initially had a good response with metastases reduction; however, after 4 months, re-appearance of lesions occurred, with death shortly thereafter.³⁰¹ Notably, this patient had been treated with several other therapies, including a gene trial with AdCD40L, cyclophosphamide and radiotherapy for brain metastases, questioning the role of each individual therapy to the overall response. A promising report was delivered by Pinto Torres et al. on a CoM patient with metastatic lesions who received vemurafenib and had a complete response during the 3 years of follow up.³⁰²

Similar to what is seen in cutaneous melanoma, *BRAF* inhibitors have been combined with MEK inhibitors to overcome the issue of resistance in CoM. Two cases of treatment for lymph node metastases have been reported; however, there are limitations to the interpretation. One patient received dabrafenib and trametinib with a good response, being alive 1 year later.³⁰³ Though promising, a longer follow up time would be preferred to assess the effect of the combination therapy. The other patient received dabrafenib and trametinib, with good response, but needed a switch to vemurafenib monotherapy after 1.5 months due to the development of adverse events (i.e. nausea and vomiting).³⁰⁴ Progressive disease then caused a further switch to pembrolizumab (an immune checkpoint inhibitor), and vemurafenib again. Addition of cobimetinib to the vemurafenib was required to obtain a good response. Though this case resulted eventually in disease control, therapy was complex and adverse events were a major issue.

Table 8. Overview of reported cases on targeted therapy in CoM.

Study	Patient	Type of CoM	Type and Dosage Immunotherapy	Other Treatments	Clinical Outcome	Adverse Events
Indicated for primary CoM						
Pahlitzsch, 2014. ²⁹⁸	F, 80y Recurrence. No metastases. <i>BRAF</i> muta (exon 15).	Indic: pr preferred non-exenteration R: vemurafenib [no dose]	Prior: exc (incomplete), Plaque brachytherapy Later: resection	Good response, tumour decrease, stable for 3yr. Then deterioration general health. [death not mentioned]	Weight loss, nausea, vomiting, headache	
Indicated for metastatic disease						
Weber, 2013. ²⁹⁹	M, 45y Merastatic CoM (subcutaneous, lung, bone) <i>BRAF</i> muta (v600e).	Indic: unresectable metastases R: vemurafenib 960mg 2dd	Prior CoM: resection Prior Mets: none	Initially good response, but after 2months progression. [death not mentioned]	Not reported	
Griewank, 2013. ³⁰⁰	M, 43y Merastatic CoM (intramuscular, lung, brain) <i>BRAF</i> not reported.	Indic/ unresectable metastases R: dabrafenib [no dose]	Prior CoM: resection, Ruth, PBI. Prior Mets: dacarbazine chemo	PR. 62% tumour reduction. After 6months new lesions. [death not mentioned]	Not reported	
Maleka, 2016. ³⁰¹	F, 53y Merastatic CoM (orbit, parotid gland, lung, brain) <i>BRAF</i> muta (v600e)	Indic: unresectable metastases R: vemurafenib 960mg 2dd; later 720mg 2dd due to AE.	Prior CoM: resection, cryo, mmc, enucleation. Prior Mets: Temozolomide, AdCD40L with cyclophosphamide, Brain radiotherapy.	Initially good response, reduction of metastases. After 4 months, re-appearance of metastases and death.	Skin rash	
Pinto Torres, 2017. ³⁰²	F, 59y Merastatic CoM (Oropharyngeal wall) <i>BRAF</i> muta (v600e)	Indic: unresectable metastases R: vemurafenib 960mg 2dd; later 480mg 2dd due to AE.	Prior CoM: exc, Prior Mets: radiotherapy 20Gy/5fractions	CR. No recur in 3yr later. Developed breast cancer.	Arthralgia, diarrhea, skin rash	

Table 8. Continued.

Study	Patient	Type of CoM	Type and Dosage Immunotherapy	Other Treatments	Clinical Outcome	Adverse Events
Combined therapy						
Dagi Glass, 2017. ³⁰⁴	F, 61y	Recurrent CoM Lymph metastases. <i>BRAF</i> muta (v600e)	Indic: alternative to extensive surgery R: 1) dabrafenib + trametinib; 2) vemurafenib (due to AE); 3) pembrolizumab (due to progression); 4) vemurafenib + cobimetinib (due to progression)	Prior CoM: excision, cryo Prior Lymph mets: parotidectomy and neck dissection	1: good for 1.5 months, then AE. 2-3: mixed, not complete. 4: eventually good response. [death not mentioned]	1: nausea, vomiting 2-4: not reported.
Rossi, 2019. ³⁰³	M, 70y	Metastatic CoM (lymph) <i>BRAF</i> muta (v600e)	Indic: unresectable metastases R: dabrafenib 150mg 2dd + trametinib 2mg 1dd	Prior CoM: excision Prior lymph mets: parotidectomy and neck dissection	Good response, 1yr later. Alive.	Fever, Hyper- transaminasemia

Abbreviations: CoM,conjunctival melanoma; pembro, pembrolizumab; ipi,ipilimumab; nivo,nivolumab; PR, partial response; CR, complete response; exc, complete response; exc, orbital exenteration; exc,excision; cryo,cryotherapy; mmc,mytomycin-g; SLNB,sentinel lymph node biopsy; M,male; F,female; AE, adverse event; met, metastasis; muta, mutation; Indic., indication; R,drug prescription.

Adverse events following BRAF/MEK inhibitors

Adverse events (AE) following targeted therapy proved common in cutaneous melanoma patients, with the occurrence of any AE in over 90% of all patients following *BRAF* inhibitors or combined *BRAF*/MEK inhibitor treatment (Table 9).²⁹⁶

AEs of *BRAF* inhibition in cutaneous melanoma patients were most commonly arthralgia, rash, photosensitivity, alopecia, fatigue, or diarrhoea.^{19,20,296} AEs are comparable between vemurafenib and dabrafenib monotherapy, but some differences are observed, as photosensitivity is seen more often with vemurafenib but pyrexia and chills are more common with dabrafenib. Addition of a MEK inhibitor to a *BRAF* inhibitor slightly alters the AE profile. Most AEs occur more frequently following combination therapy.

A noticeable AE that raised concern following introduction of vemurafenib was the development of cutaneous squamous cell carcinoma (SCC).¹⁹ The proposed mechanism is that *BRAF* inhibitors accelerate the progression of subclinical cancerous lesions; addition of a MEK inhibitor reduces this effect, resulting in the observation that patients with combined BRAF/MEK inhibitor treatment less often develop SCC than those on *BRAF* inhibitor alone.

The management of most adverse events requires dose reduction or switch to another drug type, however, in advanced grades additional topical or systemic therapy is needed.³⁰⁵

An ocular complication that may occur following *BRAF*/MEK inhibition is serous retinopathy (including edema and retinal detachment). This has been reported in 4% of vemurafenib, and 27% of vemurafenib + cobimetinib combination patients.²⁰

The observed AEs in the sparse reports of targeted therapy in CoM are in line with the earlier reports on cutaneous melanoma. Development of rash urged a vemurafenib dose reduction in one patient,³⁰¹ and development of arthralgia, diarrhoea, and rash caused vemurafenib dose reduction in another.³⁰² One patient who was on dabrafenib plus trametinib developed nausea and vomiting that urged a switch to another drug.³⁰⁴ Of note, since similar dosing schemes of targeted therapy are used for CoM as well for cutaneous melanoma, similar adverse events would be expected.

Table 9. Occurrence of most common adverse events (AE) following targeted therapy in cutaneous melanoma patients. (Note: less-frequently occurring AEs were omitted in this table, see the original reports for full details)

	Vemurafenib*		Vemurafenib + Cobimetinib*		Dabrafenib**		Dabrafenib + Trametinib**	
	Any grade (%)	≥ 3 (%)	Any grade (%)	≥ 3 (%)	Any grade (%)	≥ 3 (%)	Any grade (%)	≥ 3 (%)
Any AE					96	34	95	32
Diarrhea	33	1	61	7	14	1	24	1
Fatigue	33	3	37	5	35	1	35	2
Rash	68	16	73	17	22	1	23	0
Nausea	26	1	43	1	26	1	30	0
Vomiting					14	1	20	1
Arthralgia	42	5	38	3	27	0	24	1
Pyrexia	24	0	29	1	28	2	51	6
Alopecia	33	1	17	1	26	0	7	0
Headache					29	1	30	1
Cutaneous SCC	13	13	4	4	9	4 #	2	2 #
Keratoacanthoma	9	9	2	1				

Abbreviations: AE, Adverse event. SCC, squamous cell carcinoma.

*Phase III trial, study group vemurafenib n=246, cobimetinib+vemurafenib n=247.²⁰

**Phase III trial, study group dabrafenib n=211, dabrafenib+trametinib n=209.²⁹⁶

#SCC and keratoacanthoma combined.

Preclinical targets

Apart from the relatively well-established *BRAF* and MEK inhibitors that have been implemented already on a small scale in the treatment of CoM, several newer drugs can be suggested for CoM, based on small scale patient-related studies from cutaneous melanoma, and preclinical assessment using CoM models. Development of these drugs is important to overcome the issue of treatment resistance, and to properly target all tumours regardless of their mutational background.

Preclinical assessment of drugs is often performed using cell lines. To our knowledge, only a few CoM cell lines exist, harbouring either a *BRAF* or *NRAS* mutation (Table 10). To examine the potential efficacy of drugs in the full range of CoM, it would be interesting to develop cell lines with various combinations of *BRAF/NRAS* and other mutations such as *PTEN*, *NF1*, and *TERT*.

Table 10. Conjunctival melanoma cell lines.

Cell line	BRAF mutation	NRAS mutation	Other mutations	Reference
CRMM1	V600E	WT		Nareyeck, 2005. ³⁰⁶
CRMM2	WT	Q61L		Nareyeck, 2005. ³⁰⁶
CM2005.1	V600E	WT		Keijser, 2007. ³⁰⁷
T1527A	G466E	WT	HRAS Q61R	El Zaoui, 2019. ¹²³

Abbreviations: WT, wildtype.

c-KIT inhibition

KIT inhibition is currently best known for the treatment of gastrointestinal stromal tumours (GIST) using imatinib. In GIST, about 75% of lesions harbour a *KIT* mutation, explaining the sensitivity to this drug.³⁰⁸ The c-KIT inhibitor imatinib showed a response rate of 16 to 29% in phase II studies of metastatic (cutaneous) melanoma harbouring *KIT* alterations.³⁰⁹⁻³¹¹ The alteration type is very relevant, as metastatic patients with a *KIT* mutation showed a response rate of 54%, while those with *KIT* amplification showed a response rate of 0%.³¹¹ Since *KIT* mutations are rare in CoM, imatinib (or other drugs with *KIT* inhibitory effects, such as sunitinib, dasatinib and nilotinib that are currently all in phase 2 studies in melanoma)¹¹⁴ will likely not be suitable for large scale use in CoM. With proper screening for mutation status, however, they can be part of a successful personalized treatment.

ERK1/2 inhibition

ERK (consisting of the kinases ERK1 and ERK2) is a distal actor in the MAPK pathway (Figure 6). Reactivation can be seen with resistance of upstream MAPK inhibition, and as such it is an important target to overcome *BRAF*-inhibitor resistance.²⁹⁰ ERK1/2 can be inhibited by ulixertinib (BVD-523), a drug that showed potential in preclinical melanoma models,³¹² and that showed an acceptable safety profile and evidence of activity in solid tumours including melanoma in a phase I study.³¹³ In that work, 9/19 patients with *BRAF*-mutated melanoma had a partial response (PR) or stable disease following failed *BRAF* inhibition; 9/17 patients with *NRAS*-mutated melanoma had PR or stable disease as well. To our knowledge, ulixertinib has not yet been evaluated in CoM. It is promising however that both *BRAF* and *NRAS* mutated lesions seem to be responding, suggesting a role as rescue medication.

PI3K/AKT/mTOR

The PI3K/AKT/mTOR pathway has an important role in melanoma, acting alongside MAPK (Figure 6). Targeting can be of specific use in *BRAF*-WT or *BRAF*-inhibitor-resistant melanoma. The therapeutic potential follows increased activity in CoM compared to conjunctival nevi,¹²³ and also in CoM compared to UM.¹⁴⁸

Cell proliferation of three CoM cell lines (harbouring either a *BRAF* or *NRAS* mutation) could be inhibited by AKT inhibition using MK2206.¹²¹ Using cell lines, the most promising effect was obtained by combining MK2206 (AKT inhibitor) with MEK162 (MEK inhibitor), that caused a stronger cell cycle arrest compared to single-agent treatment.

Another study using the same cell lines found that the PI3K inhibitor pictilisib was more effective than the dual PI3K/mTOR inhibitor dactolisib.¹²³ The genetic background of the cell lines was important however: both pictilisib and dactolisib were active against CRMM1 (*BRAF* mutation), pictilisib alone was effective against CRMM2 (*NRAS* mutation), and none were effective against T1527A, a cell line lacking *BRAF*V600E and *NRAS* mutations (Table 10).

The results from CoM are in line with earlier reports on cutaneous melanoma cell lines: in *NRAS*-mutated cell lines, cells were more sensitive to MEK inhibition than to PI3K/mTOR inhibition alone, but combined inhibition was superior.³¹⁴ However, there are some troublesome reports of PI3K/AKT inhibition in melanoma patients: trametinib (MEK inhibitor) and GSK2141795 (AKT inhibitor) combined had no response in 10 *NRAS*-mutated and 10 *BRAF*-WT + *NRAS*-WT melanoma (including 3 UM) and therapy was not well tolerated.³¹⁵

TERT

The abundance of *TERT* mutations in CoM poses an opportunity for treatment. Reverse transcriptase inhibitors, e.g. azidothymidine, can target *TERT* mutated tumours by targeting reverse transcriptase activity.³¹⁶ Other approaches include a telomerase inhibitor, such as Imetelstat (GRN163L).¹¹⁵ These drugs have not been studied in CoM however, but show promising results in vitro and in early stage patient studies of several cancers.

EZH2

Epigenetics concern processes that alter gene expression and regulation, without involving changes in the DNA sequence itself. The polycomb repressive complex 2 (PRC2) is involved in many of these epigenetic processes, with the ‘enhancer of zeste homolog 2’ (EZH2) as a core subunit of PRC2, that is overexpressed in several cancers.³¹⁷ EZH2 causes silencing of (tumour suppressor) genes, and is frequently overexpressed in cutaneous melanoma, but not in cutaneous nevi, indicating a role in tumour progression with potential as a therapeutic target.³¹⁸

Cao et al. found that EZH2 is not expressed on melanocytes of normal conjunctiva or PAM, but was expressed in 13/26 CoM lesions and 7/8 lymph node metastases.³¹⁹ Just as in skin lesions, this implies a role for EZH2 in malignant transformation. EZH2 expression in CoM was significantly related to older age, larger tumour thickness and worse overall survival. Using two EZH2 inhibitors,

GSK503 and UNC1999, cell growth of three CoM cell lines (CRMM1, CRMM2 and CM2005.1) as well as tumour growth in a zebrafish model could be repressed. While, as far as we know, EZH2 inhibition has not been studied in CoM patients, there may be a potential therapeutic benefit.

Checkpoint inhibitor therapy in CoM

Checkpoint inhibitors

The first approved checkpoint inhibitor for advanced cutaneous melanoma was ipilimumab, a monoclonal antibody against CTLA4 (Yervoy, Bristol-Myers Squibb) (Table 11). Ipilimumab showed improved survival compared to treatment with gp100 in unresectable stage III or IV cutaneous melanoma patients (overall survival 10.1 months versus 6.4 months).²² Ipilimumab, combined with dacarbazine chemotherapy, also provided a significantly longer overall survival compared to dacarbazine with placebo (overall survival 11.2 months versus 9.1 months).³²⁰ In 2014, two new drugs targeting PD1 were introduced: nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck Sharp & Dohme).

Pembrolizumab (administered at both two or three week intervals) gave a better survival compared to ipilimumab, with a 6-month PFS of approximately 47% for pembrolizumab, and 27% for ipilimumab.¹⁹⁷ In advanced cutaneous melanoma patients, who had progressed after ipilimumab or ipilimumab plus a *BRAF*-inhibitor, nivolumab demonstrated a better response than chemotherapy (32% versus 11%).³²¹ Nivolumab had also a better overall survival and progression-free survival than dacarbazine in untreated advanced melanoma patients lacking a *BRAF* mutation; with nivolumab, the 1-year overall survival went from 42.1 to 72.9%, the median progression-free survival from 2.2 months to 5.1 months.²³

Since CTLA4 and PD-1 act on T cells via different mechanisms (Figure 8), there is a rationale to combine blockade therapy. Indeed, combining nivolumab and ipilimumab led to better survival (progression-free survival of 11.5 months) than either of the therapies alone (6.9 months for nivolumab, 2.9 months for ipilimumab) in advanced cutaneous melanoma patients.²⁵³

Checkpoint inhibitors have been used to treat a limited number of patients with locally advanced or metastatic CoM. In the absence of formal trials, the current literature consists of single-case reports and small case-series (Table 12). First treatments were administered around 2013,^{26,322} but the first reports on checkpoint inhibitors in CoM appeared in the literature from 2017 onwards.^{302,323,324} The use of checkpoint inhibitors in CoM follows the same basic principles and similar dosing schemes as in locally-advanced or metastatic cutaneous melanoma, as described in the FDA reports on ipilimumab (trade name: Yervoy),³²⁵ pembrolizumab (trade name: Keytruda),³²⁶ and nivolumab (trade name: Opdivo).³²⁷ Drugs targeting PD-1 (pembrolizumab, nivolumab) as well as

drugs targeting CTLA4 (ipilimumab) have been used in CoM, as single-agent as well as in various combinations. Due to different aims of therapy, the findings for patients who were treated for a primary CoM or for metastatic disease, will be discussed separately.

Table 11. Checkpoint inhibitors.

	Generic name	Brand name	Dosing and route*	FDA reference
Anti-CTLA4	ipilimumab	Yervoy	3 mg/kg iv, every 3 weeks	FDA, 2020. ³²⁵
Anti-PD-1	nivolumab	Opdivo	240 mg iv, every 2 weeks (or 480 mg every 4 weeks)	FDA, 2020. ³²⁷
	pembrolizumab	Keytruda	200 mg iv, every 3 weeks	FDA, 2020. ³²⁶

Abbreviation: FDA, Food and Drug Administration; iv, intravenously.

**dosing as indicated for (metastatic) melanoma, for other indications please read product information.*

Primary CoM

In several cases, immune checkpoint inhibitors have been used as treatment for primary CoM (Table 12, cases 1-5). In four cases, immunotherapy was offered as alternative to orbital exenteration for patients who refused eye-removing therapy; in one case, treatment was for an extensive lesion with an insufficient response to prior local therapy.

The first reported patient (M, 60) received pembrolizumab single-agent therapy and had an immediate good response, with flattening of a nodular recurrence at 6 months.³²³ A similar favourable response to pembrolizumab single therapy was seen for an extensive in-situ lesion (F, 53).²⁷

The third patient (F, 94), reported initial progression with pembrolizumab single-agent therapy, but had a partial response with pembrolizumab + ipilimumab combination therapy. This patient died 5 months later from an unrelated cause.³²²

In two patients, a successful response was reported for the third attempted scheme of therapy, which included addition of topical IFN- α . One of these patients (M, 76) had no response to ipilimumab single-agent therapy, a minimal response to pembrolizumab single-agent therapy, but a complete response following pembrolizumab + topical IFN- α drops.³²² The second patient (F, 84) had minimal success after pembrolizumab single-agent therapy, showed progression with pembrolizumab + ipilimumab, but stable disease with pembrolizumab + ipilimumab + IFN- α topical treatment.³²²

Finger noted that local IFN- α seemed to synergize the effect of PD-L1 inhibition.³²² IFN- α on itself has been used longer to treat malignancies (with a small survival benefit in cutaneous

melanoma patients),³²⁸ and also ocular malignancies, including ocular surface squamous neoplasia (OSSN)^{329,330} and CoM^{331,332}. IFN- α is known to stimulate immune reactions, with upregulation of HLA molecules, promotion of NK cell activity and activation of CD8+ T cells, making it a candidate for co-treatment with anti-PD-1 and anti-CTLA4 in cancer.³³³⁻³³⁵ In a one-armed phase 1b/2 study on pegylated-IFN- α and pembrolizumab in 43 mucosal and cutaneous melanoma, an improved response rate compared to the expected rate was found for pembrolizumab alone;³³⁶ however, this study needs further evaluation. As Cao showed, PD-L1 and PD1 could be upregulated on CoM cell lines through IFN- γ .²⁰⁰ It may well be that the same happens under the influence of IFN- α , providing a good target for checkpoint inhibition.

Metastatic CoM

In twelve reported cases, immunotherapy has been administered to CoM patients with metastatic disease (Table 12, cases 6-17). Eight patients had systemic metastases, one patient had regional (lymph node) metastases, and three patients had both. In three patients, treatment of metastases as well as local tumour control was attempted at the same time.

Five CoM patients received nivolumab single-agent treatment.^{26,337} All had a good response, although in one case (M, 71; systemic metastases; *BRAF* V600E mutated) this treatment was supplemented with dabrafenib, trametinib and radiotherapy, hampering the conclusions on the individual effect of nivolumab.³³⁷ One patient, with systemic and lymph node metastases, received pembrolizumab single-agent therapy, with a near to complete resolution.³⁰² Another patient, with multifocal CoM and lymph node metastases, received ipilimumab as a single therapy [after tumour debulking and brachytherapy, and lymph node dissection] and had excellent local control and no new lymphatic or systemic metastases.³³⁸

Four patients received more than one type of checkpoint inhibitor.^{26,27,322,339} One patient, with systemic and lymph node metastases, received both ipilimumab and nivolumab. There was a reduction of the systemic tumour burden, and the patient survived at least 3 years.³²²

In three patients, there was a switch to other drugs due to treatment failure, or development of adverse events. In the first patient with systemic metastases, ipilimumab + nivolumab combination therapy induced hepatitis, causing a switch to nivolumab alone. This treatment with nivolumab, however, induced an infusion reaction, necessitating a switch to pembrolizumab, which was followed by a favourable response with stable disease for 2 years.³³⁹ Interestingly, the response to this anti-PD-1 drug was favourable, while PD-L1 expression of the primary CoM was negative.

The second patient, who initially had stable disease for 6 months with pembrolizumab, eventually showed progression. Ipilimumab (with dacarbazine) caused a partial response, but had to be discontinued due to the development of hepatotoxicity.²⁶

In the third patient, who was initially treated with ipilimumab single-agent therapy, a new lymph node metastasis developed. After another round of ipilimumab, the patient later developed skin metastases. A third treatment with pembrolizumab was started, and no new developments were reported in the 2 years thereafter.³²²

Discussing the cases on checkpoint inhibitor therapy in CoM

All of the 17 currently-reported cases (both with primary as well as metastatic CoM) eventually developed a favourable response to checkpoint inhibition. Unfortunately, little is known about the reasons for initial treatment failure in some of the cases. In only one patient, the PD-L1 status of the tumour was known, which was negative, and this patient experienced a good response to PD-1 blockade.³³⁹ More studies into the patient characteristics are needed to learn about these mechanisms and the predictive value of checkpoint expression. With the small numbers, and various different treatment regimens, it is impossible to conclude on the superiority of any of the checkpoint inhibitors in CoM. It may be expected however, as in cutaneous melanoma, that PD-1 blockade is more effective than blockade of CTLA4, and that a combined blockade of PD-1 and CTLA4 may yield even better results.^{253,256}

While the current reports are promising, it may be that other (unsuccessful) cases did not end up in the literature, skewing the results for CoM to a favourable outcome. We concur with the statement of Sagiv et al. of the group of Dr. Esmaeli that “our observations are so far cautiously optimistic”.²⁶ Immunotherapy can be considered promising for CoM patients who need additional treatment to local therapy, or who develop metastatic disease, and is well-justified in those needy cases.

Adverse events following checkpoint inhibitor therapy

Checkpoint inhibitors allow T cells to respond against tumour cells, but can equally cause an increased T cell-response against normal tissues. These unwanted events are known as immune-related adverse effects (irAEs) and can be severe. Part of the pathophysiology can be an increased number of activated CD4+ and CD8+ T cells, as was detected in peripheral blood of cutaneous melanoma patients following ipilimumab treatment.³⁴⁰

Table 12. Overview of reported cases on checkpoint inhibitor therapy in CoM.

Study	Patient	Type of CoM	Type and Dosage of Immunotherapy *1	Other Treatments	Clinical Outcome	Adverse Events
Indicated for primary CoM						
Kini, 2017, ^{3,23}	M, 60s	Recurrence. Fornix, anterior orbit, limbus.	Indic: Preferred non-exenteration (other eye low vision). R: pembro 150mg iv every 3wks, 1y.	Prior: exc and cryo Later: exc and cryo	Good response. At 6m, nodule was flat. [survival not reported]	No AE.
Finger, 2019, ^{3,22}	F, 94	First. Bulbar to eyelid. No metastasis.	Indic: Age, comorbidity, refused exent. R: First: pembro. Second: pembro+ipi *2.	None	Pembro: progression Pembro+ipi: PR. Died after 5m, unrelated. *3	No related AE.
Finger, 2019, ^{3,22}	M, 76	Recurrence. Cornea to eyelid. No metastasis. <i>BRAF</i> , <i>KIT</i> , <i>NRAS</i> wt.	Indic: Progression despite chemo R: First: ipi. Second: pembro. Third: pembro+IFN-alfa drops *4	Prior: multiple local treatments incl IFN-alfa drops.	Ipi: no response Pembro: minimal response, but complete with IFN-alfa. After 36m still CR.	Ipi: adrenal insuff > ipi stop + steroids. Pembro: dermatitis > steroids, antihist
Finger, 2019, ^{3,22}	F, 84	Recurrence. Cornea to eyelids. No metastasis.	Indic: Preferred non-exenteration R: First: pembro. Second: pembro+ipi *2. Third: pembro+ipi+IFN-alfa *5	Prior: multiple local therapies incl exc, cryo, mmc, brachytherapy	Pembro: minimal success Pembro+ipi: progression Alive 1.5yr after first pembro	No AE reported
Hong, 2020, ²⁷	F, 53	First.*6 Bulbar to tarsal. No metastasis.	Indic: Preferred non-exenteration R: pembro	Prior: none Later: mmc	CR, after 12m near complete reduction of pigment. Disease free 12m of follow up.	Cutaneous pruritus

Table 12. Continued.

Study	Patient	Type of CoM	Type and Dosage of Immunotherapy *1	Other Treatments	Clinical Outcome	Adverse Events
Indicated for metastatic disease						
Sagiv, 2018. ²⁶	F, 68	Recurrent CoM. 2yr after last: syst mets to lung; <i>BRAF^{wt}</i>	Indic: systemic metastases R: First: pembro. Second: ipi+dacarb *7	Prior CoMs: exc, mmc, exent, 30Gy orbit radiotherapy, SLNB, parotidectomy.	Pembro: Stable at 6m, then progression. Ipi+dacarb: PR Alive at time of writing.	Pembro: No reported. Ipi+dacarb: hepatotox > stop.
Pinto Torres, 2017. ³⁰²	M, 51	Recurrent CoM. <i>BRAF^{wt}</i> . mets to lymph+skin	Indic: systemic and lymph node mets R: pembro	Prior CoMs: exc. Lymphadenectomy <i>Antiviral therapy for HIV.</i>	Near to CR after 3 rd cycle. Survival at least 2yr after diagnosis of mets.	No AE: "good tolerance"
Sagiv, 2018. ²⁶	F, 58	CoM recurrence to orbit Mets to lung, liver	Indic: orbital and systemic metastases R: nivo	Prior CoMs: excisions, parotidectomy, exent	CR of orbital and meta lesions. Alive at time of writing	Elevated liver enzymes at 3m > nivo stopped.
Sagiv, 2018. ^{26*} 11	F, 28	CoM recurrence After 5yr: mets in breast, lung, bone	Indic: systemic metastases R:nivo	Prior CoM: Exc+cryo+mmc	PR of systemic mets, later CR. Disease free 3yrs after nivo.	No AE reported.
Sagiv, 2018. ²⁶	F, 47	Recurrent CoM 6,5yr after last CoM: mets to lung	Indic: systemic metastases R: nivo	Prior CoMs: exc, cryo, plaque, parotidectomy, lymphadenectomy, adjuv topical IFN, mmc	Resolution of lung lesions. 7m after nivo stop free from disease	Diarrhea (AI colitis) > nivo stopped, prednisol, infliximab

Table 12. Continued.

Study	Patient	Type of CoM	Type and Dosage of Immunotherapy *1	Other Treatments	Clinical Outcome	Adverse Events
Sagiv, 2018, ²⁶	M, 74	Recurrent CoM 2m after last: lung mets.	Indic: systemic metastases R: nivo	Prior CoMs: excisions. (declined extent)	Decrease in tumour size Disease free 1m after nivo stop	After 11m: ir colitis > nivo stopped, prednisone
Kiyohara, 2020, ³³⁷	M, 71	Recurrent CoM <i>BRAF</i> v600e pos mets bone + liver	Indic: systemic metastases R: nivo	Prior CoMs: exc, cryo, enucl, vemurafenib Currently: dabrafenib, trametinib, radiotherapy	Died 24m after combi therapy	No AE reported
Chaves, 2018, ³³⁸	M, 72	First CoM. bulbar to tarsal Lymph nodes pos.	Indic: multifocal CoM + lymph mets R: ipi	This CoM: debulking, brachytherapy, (refused extent). SLNB > dissection	Response, excellent local tumour control. Okay 16m post radioth.	Mild fatigue, ceased after last cycle ipi. *8
Finger, 2019, ³²²	F, 72	CoM: Epibulbar <i>BRAF</i> v600k mut 9y later: metastases in lung, liver, bone, skin, lymph nodes	Indic: Systemic and lymph metastases R: ipi + nivo *9	CoM: exc + topical chemo. Meras: no other tx	Resolution of the subcutaneous nodules. Reduction of systemic tumour burden. 3yrs survival at least.	Hepatotoxicity > treatment delay Colitis > fluids, steroids, infliximab Pneumonitis > steroids, inhalers
Chang, 2019, ³³⁹	F, 60	CoM recurrence <i>NRAS</i> mut, <i>BRAF</i> wt, KITwt, PDL1-. Liver metastases	Indic: primary CoM+ syst metastasis. R: First: ipi + nivo *9. Second: nivo *10. Third: pembro	Prior CoMs: Excisions, cryo, SLNBneg (declined orbital radiotherapy)	Ipi/nivo: unknown Pembro: CoM and mets response, stable at 2yr.	ipi/nivo: hepatitis. > switched to nivo. Nivo: infusion reaction > pembro

Table 12. Continued.

Study	Patient	Type of CoM	Type and Dosage of Immunotherapy *1	Other Treatments	Clinical Outcome	Adverse Events
Finger, 2019. ³²²	F, 76	First CoM. <i>NR4Smut</i> Mult. Lymph mets Skin metastasis	Indic: Lymph and systemic metastasis R: First: ipi. Second: ipi. Third: pembro	CoM: exc, cryo, mmc LN: Parotidectomy, surgery, radiotherapy. Skin Mets: exc, radiation	Ipi: (1.5yr) New lymph mets Ipi: (3yr) skin meta Pembro: alive after 2yr	Ipi: no AE No other AE reported
Hong, 2020. ²⁷	M, 66	First CoM. Fornix and orbit. Syst mets in lung and liver.	Indic: primary CoM+ syst metastasis. R:ipi+nivo	None reported	Resolution of the conj lesion, nice response of syst mets.	Pituitary failure > replacement hydroxycortisone and thyroxine.

Abbreviations: CoM, conjunctival melanoma; pembro, pembrolizumab; ipi, ipilimumab; nivo, nivolumab; PR, partial response; CR, complete response; exc, orbital exenteration; exc, excision; cryo, cryotherapy; mmc, mytomycin-c; SLNB, sentinel lymph node biopsy; M, male; F, female; AE, adverse event; met, metastasis; Indic, indication; R, drug prescription.

Footnotes:

*1 Unless otherwise reported, standard regimens for immunotherapy were:

Pembrolizumab: 2mg/kg every 3wks, up to 200mg.

Ipilimumab: 3mg/kg every 3wks (for unresectable/metastatic melanoma)

Nivolumab: 240mg every 2wks (which equals 3mg/kg)

*2 Added dose of ipilimumab: 1mg/kg.

*3 The patient died due to congestive heart failure.

*4 Dose of IFN-alfa drops: 1million units/cc, 4 times daily.

*5 Intradermal IFN-alfi, 3 million units per eyelid, monthly interval.

*6 The reported diagnosis was 'conjunctival melanoma in situ'.

*7 Dose of dacarbazine: 800-1000mg/m2, every 3wks

*8 Other (likely unrelated) AE were: partial lash loss, dry eye, peripheral corneal vascularization, secondary glaucoma and radiation-induced posterior cataract.

*9 Dose of nivolumab: 1mg/kg every 3wks

*10 Dose of nivolumab: 240 mg every 2 weeks for 2 cycles and 480 mg every 4 weeks for 1 cycle

*11 This case was presented earlier as well in a report by Ford et al., 2017.³²⁴

Adverse events due to checkpoint inhibitors can affect any organ, but commonly are directed against the skin (pruritus, rash) or gastrointestinal system (diarrhea, colitis), and less frequently the liver or endocrine system (including general fatigue).^{22,197,256,321,341} The profile and frequency of irAEs is similar for nivolumab and pembrolizumab (both being anti-PD-1 drugs), while somewhat more common in ipilimumab (as an anti-CTLA4 drug), particularly when combined with nivolumab.³⁴¹ Grade 1-2 adverse events of diarrhea, fatigue, pruritus and rash have each been reported in about 20-40% of patients receiving checkpoint inhibition, demonstrating their relatively common occurrence (Table 13). Adverse events of grade 3 or higher were reported in 10% of pembrolizumab-treated patients, 22% of nivolumab, 28% of ipilimumab, and 59% of nivolumab + ipilimumab treated cases with cutaneous melanoma.^{197,256}

Some of the adverse events resemble manifestations of auto-immune disease. Checkpoint inhibitors were reported to cause a decrease in pigmentation of the skin (i.e. vitiligo)³⁴² and even of choroidal nevi in the fundus.³⁴³

Table 13. Occurrence of most common adverse events (AE) following immunotherapy in (cutaneous) melanoma.

	Nivolumab*	Ipilimumab*	Nivo+Ipi*	Pembrolizumab**
Any 1-2 grade AE	64 %	58 %	37 %	63 %
Any 3-4 grade AE	22 %	28 %	59 %	10 %***
	Grade 1-2 / 3-4	Grade 1-2 / 3-4	Grade 1-2 / 3-4	Any grade / 3-5
Diarrhea	19 % / 3 %	28 % / 6 %	36 % / 9 %	14 % / 1.1 %
Fatigue	36 % / 1 %	28 % / 1 %	34 % / 4 %	19 % / 0.4 %
Pruritus	22 % / <1 %	36 % / <1 %	34 % / 2 %	14 % / 0 %
Rash	23 % / <1 %	21 % / 2 %	27 % / 3 %	13 % / 0 %
Nausea	13 % / 0 %	16 % / 1 %	26 % / 2 %	11 % / 0.4 %

Abbreviations: AE, adverse event.

**Phase III trial, CheckMate 067 report. Study group nivolumab n=316, ipilimumab n=315, nivo+ipi n=314; Hodi et al., 2018.²⁵⁶*

***Phase III trial, KEYNOTE 006 report (with an every 3-week dosing scheme). Study group pembrolizumab n=277; Robert et al., 2015.¹⁹⁷*

****Any grade 3-5 adverse event.*

As there is only a limited number of reports on checkpoint inhibition in CoM, there are very few reports on AE's in these patients (Table 12). In 6 out of 17 reports, no AE's were reported at all. In the CoM patients who did develop AE's, similar events were noted as in patients receiving immunotherapy for cutaneous melanoma.^{197,256} For ipilimumab single-agent therapy, adrenal insufficiency,³²² hepatotoxicity,²⁶ and fatigue³³⁸ have been reported. With nivolumab single-agent therapy, elevated liver enzymes,²⁶ diarrhoea,²⁶ colitis,²⁶ and an infusion reaction³³⁹ have been

reported. Pembrolizumab single agent therapy may have been the best-tolerated drug, with only one out of 8 patients reporting dermatitis.³²² The combination of ipilimumab + nivolumab led to hepatotoxicity, colitis and pneumonitis,³²² and pituitary failure.²⁷ The development of reported AE's in CoM patients required discontinuation of therapy, switch to another type of immunotherapy, or additional specific treatments, e.g. with corticosteroids or antihistamines.

Of specific note are the 'ocular irAEs', i.e. adverse events in the ocular region after checkpoint inhibitor treatment for melanoma at any site. Such ocular irAEs have been reported after anti-CTLA4 treatment in 1.3% of patients³⁴⁴ and after anti-PD1 treatment in 1.6% of patients³⁴⁵. Ocular irAEs mostly include uveitis, orbital inflammation, dry eyes, and blurred vision,³⁴⁴⁻³⁴⁶ as recently reviewed by Dalvin et al., 2018.³⁴⁷ Rare events include Vogt-Koyanagi-Harada (VKH) syndrome with serous retinal detachment,³⁴⁸ or ocular rosacea.³⁴⁹ Most ocular irAEs can be treated with topical corticosteroids, and only rarely systemic therapy is required.³⁵⁰ Notably, immune checkpoint inhibition may be associated with site-specific metastases, as remarkable cases of vitreous metastases of cutaneous melanoma were reported.^{351,352} Not only ocular oncologists, but also general ophthalmologists should be aware of these events as immunotherapy is increasingly being applied, and it becomes more common for these irAEs to present in an ophthalmological practice.

Conclusions (Targeted therapy and checkpoint inhibitor therapy in CoM)

Conventional therapy of localised CoM relies on surgical excision with adjuvant therapy including cryotherapy, topical chemotherapy and / or radiotherapy. Treatment of extensive disease can be more complex, depending on individual cases. Treatment options for disseminated disease are very limited, and no consensus exists on the optimal approach. It is mainly in extensive and disseminated disease that new therapies are urgently needed. Targeted therapy and immunotherapy have been recently introduced successfully for the treatment of (advanced) cutaneous melanoma, and these therapies can be beneficial to CoM patients as well. Inhibitors of BRAF and MEK act in CoM due to the presence of *BRAF* mutations and activation of the MAPK pathway; a notable benefit is seen in the combination of these two drugs.

Several new targets for therapy are under investigation in CoM, e.g. c-KIT, ERK1/2, PI3K/AKT/mTOR, TERT and EZH2. While the value of such targeted therapies has yet to be determined, these may perhaps not be a cure for all, but should be seen as part of a personalized approach after genetic screening. This can e.g. be beneficial for patients with no *BRAF* mutation (limiting response to current BRAF inhibitors) or when a (rare) *KIT* mutation is present.

Checkpoint inhibitors emphasize similarities in the tumour micro-environment of CoM and that of cutaneous melanoma, while differing greatly from UM. Promising results from anti-CTLA4 and anti-PD-1/PD-L1 antibodies in small series of CoM show that these have a first-line position in

metastatic disease. While testing for expression of checkpoint molecules such as PD-1/PD-L1 could theoretically add to a personalized approach, current predictive values for treatment response are limited and we urge that negative-expressing patients are not excluded.

A secondary effect of the introduction of aforementioned therapies is the renewed interest in the SLNB in CoM. Findings of SLNB can now be followed by a curative intent, and early detection of metastases may improve the benefit of new treatments.

While results of both targeted and checkpoint inhibitor therapy are promising, clinicians should be aware of the specific adverse events and not forget that these can include all organ tracts and can be severe. As in any clinical approach, this should be weighted in the decision for certain treatment.

6. FUTURE DIRECTIONS AND CONCLUSIONS

Future directions

Recent developments in cancer research show that tumour genetics and immunology are promising fields that not only lead to a better understanding of CoM, but also provide new targets for therapy. Future projects are numerous, and illustrate that genetics and immunology are intertwined. A recurrent theme for CoM is to translate knowledge from studies on more abundant cutaneous melanoma. While this may be beneficial to the treatment of CoM (as a rare disease), it should be stressed however that the eye has several unique features and that conjunctiva-specific characteristics must not be overlooked.

Important new work is to further investigate the genetic background of CoM, and to evaluate the impact of genetics on tumour behaviour. This is facilitated by rapidly-developing sequencing techniques. Important questions that need answering are how to differentiate benign from malignant lesions, how to identify the most ominous lesions (with a risk for recurrence or metastasis, warranting extensive treatment and intensive follow-up) and how to select the most suitable therapy for individual patients. The mutational status (of genes such as *BRAF*, *NRAS*, and *TERT*) has proved to be important, and warrants studies into less common genes such as *KIT*. Chromosome status and expression of miRNA showed promising results to differentiate and prognosticate lesions, but require confirmation for further use. Genetics suggest that subgroups of CoM exist with distinct driver mutations and pathway activation.⁸⁹ While this parallels the principles learned from cutaneous melanoma it is important to look for CoM-specific groups. Following up on the questions on the development of CoM, differences between e.g. sun-exposed and non-exposed lesions need further evaluation, as well as the role of precursor lesions, melanin pigments and the immune system in melanocyte transformation.

The clinical revolution of recent years was the introduction of targeted therapies (BRAF/MEK inhibitors) and checkpoint inhibitors (anti CTLA4/PD-L1) as treatment of locally-advanced or disseminated CoM. This parallels guidelines from cutaneous melanoma and is likely to be implemented even more as drugs become more available and clinicians learn about their use. A first question is to identify patients who may benefit most from these therapies, or reversely, to match a patient to the optimal therapy. Apart from earlier mentioned tumour genetics, immune parameters as expression of HLA and PD-L1, or presence of immune cells, should be evaluated as biomarkers for a therapeutic response. Current studies show that expression of theoretically-important markers (such as PD-L1) is not a prerequisite for a therapeutic response, however, and that much is to be learned. A second question is how to overcome treatment resistance, which is unfortunately a common event in patients who respond well initially. We advocate to study combinations of *BRAF* inhibitors with not only MEK inhibitors, but also drugs targeting the AKT pathway, and possibly even YAP1.³⁵³ Furthermore, the combination of PD-1/PDL-1 and CTLA4 inhibition should be studied. The addition of immune-stimulating agents such as IFN- α may be interesting as promising reports have emerged in the CoM literature; topical IFN- α drops are already used for topical treatment of malignant ocular surface disease, and are readily available. Even so, radiotherapy or photodynamic therapy may be added to immunotherapy as an enhancer of the immune system. This may facilitate use of aforementioned drugs in not only metastasized CoM patients, but also local disease.

In addition to earlier mentioned developments, several new drugs and druggable targets are under investigation in preclinical studies for CoM or in cutaneous melanoma. New drugs target cKIT, ERK1/2, PI3K-AKT, TERT, and EZH2. Some rely on specific (rare) mutations, suggesting that these are suitable for individualized therapy, or as last-resort, but with unknown value for the majority of patients. By the rarity of CoM it is not feasible to evaluate all of these targets in CoM itself, so data may need extrapolation from other tumour types. Screening in CoM models is warranted prior to introduction in clinical studies, however, to prevent the pursuit of inappropriate targets. The plethora of new drugs poses an additional question, however, to determine which combination of (targeted and immuno-) therapy is optimal, and whether simultaneous or sequential treatment should be applied.

For targeted therapy and checkpoint inhibitor therapy, evidence on their potential benefit is solid enough to advise inclusion of (metastatic) CoM patients in trials. The earlier-mentioned lack of proper biomarkers would argue for liberal inclusion criteria. The rarity of CoM calls for international collaboration to obtain sufficient numbers, and to include CoM patients in trials of cutaneous (and when applicable, mucosal) melanoma. A separate registry should be advised however, to prevent loss of CoM specific data.

A concurrent development with the introduction of therapies for metastatic CoM, is the increased use of screening methods for (lymph node) metastases such as with the SLNB. There is debate on its position in CoM,²⁷⁹ but the SLNB is being implemented more frequently, and has been suggested as an addition to the current AJCC staging system for its prognostic value.¹⁴ It is likely that advanced staging becomes routine practice for CoM, in which not only tumours are better characterized, but (lymph node) metastases as well.

Conclusions

From an ophthalmological perspective, CoM is a remarkable melanoma: it is very different from the far more prevalent UM (often referred to as ‘ocular melanoma’), and much more resembles cutaneous and mucosal melanoma in its biology. The genetic background of CoM is characterized by mutations in *BRAF*, *NRAS*, *NF1*, and *TERT* and it has a complex karyotype with various aberrations. Genetic studies confirm that UV radiation contributes to CoM development, suggesting differences between sun-exposed and non-exposed conjunctiva, but little is yet known about the relation between clinical (tumour) characteristics and genetic profile. The relation between CoM and the tumour micro-environment is being unravelled, with a favourable role for the presence of immune cells and expression of HLA molecules, and an unfavourable role for expression of checkpoint molecules such as PD-L1.

New therapies that became available for cutaneous melanoma in recent years, show promising results in CoM. Targeted therapy (such as BRAF and MEK inhibitors) and checkpoint inhibitors (such as anti-CTLA4 and anti-PDL1 drugs) are an extension to the toolbox of clinicians who currently rely on excision and topical adjuvant therapy for localized disease, while options for treatment of disseminated disease are limited. While the major advancement of new therapies may be in the treatment of metastatic CoM, new therapies can be used to prevent mutilating extensive surgery for advanced primary CoM as well. As a side effect, better staging and screening methods (such as with a SLNB) are gaining popularity, now that they can be followed by therapeutic measures. Treatment resistance remains a major issue of the new drugs, however, so the search continues for combinations of drugs targeting separate (parts of) pathways. It is to be determined what the optimal sequence or combination of targeted and checkpoint inhibition should be.

It is expected that genetic and immunologic typing becomes regular practice in the management of CoM, for prognostication, and identification of patients for specific therapies. We strongly advocate international collaborations to study this rare disease, and the inclusion of CoM patients in cutaneous melanoma trials, with proper registries to allow for a separate evaluation of data.

Competing interests: No conflicting relationship exists for any author.

Funding: This work was supported by the following foundations: Leiden University Medical Center (MD/PhD program grant, NJB), and European Commission (Horizon 2020 grant number 667787, NJB, MJJ). The funding organizations had no role in the design or conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Financial disclosures: No financial disclosure exists for any author.

Acknowledgements: The authors like to thank Dr. Demetrios G. Vavvas (Massachusetts Eye and Ear Infirmary, Boston, USA) for his contribution to the section on melanoma incidence and tissue size (section 2.4).

REFERENCES

1. Tuomaala S, Eskelin S, Tarkkanen A, Kivela T. Population-based assessment of clinical characteristics predicting outcome of conjunctival melanoma in whites. *Invest Ophthalmol Vis Sci.* 2002;43(11):3399-3408.
2. Yu GP, Hu DN, McCormick S, Finger PT. Conjunctival melanoma: is it increasing in the United States? *Am J Ophthalmol.* 2003;135(6):800-806.
3. Triay E, Bergman L, Nilsson B, All-Ericsson C, Seregard S. Time trends in the incidence of conjunctival melanoma in Sweden. *Br J Ophthalmol.* 2009;93(11):1524-1528.
4. Isager P, Osterlind A, Engholm G, et al. Uveal and conjunctival malignant melanoma in Denmark, 1943-97: incidence and validation study. *Ophthalmic Epidemiol.* 2005;12(4):223-232.
5. McLaughlin CC, Wu XC, Jemal A, et al. Incidence of noncutaneous melanomas in the U.S. *Cancer.* 2005;103(5):1000-1007.
6. Shields CL, Markowitz JS, Belinsky I, et al. Conjunctival melanoma: outcomes based on tumor origin in 382 consecutive cases. *Ophthalmology.* 2011;118(2):389-395 e381-382.
7. Rodrigues M, Koning L, Coupland SE, et al. So Close, yet so Far: Discrepancies between Uveal and Other Melanomas. A Position Paper from UM Cure 2020. *Cancers (Basel).* 2019;11(7).
8. Mikkelsen LH, Larsen AC, von Buchwald C, et al. Mucosal malignant melanoma - a clinical, oncological, pathological and genetic survey. *Apmis.* 2016;124(6):475-486.
9. Jager MJ, Shields CL, Cebulla CM, et al. Uveal melanoma. *Nat Rev Dis Primers.* 2020;6(1):24.
10. Smit KN, Jager MJ, de Klein A, Kili E. Uveal melanoma: Towards a molecular understanding. *Prog Retin Eye Res.* 2019;100800.
11. Wong JR, Nanji AA, Galor A, Karp CL. Management of conjunctival malignant melanoma: a review and update. *Expert Rev Ophthalmol.* 2014;9(3):185-204.
12. Missotten GS, Keijser S, De Keizer RJ, De Wolff-Rouendaal D. Conjunctival melanoma in the Netherlands: a nationwide study. *Invest Ophthalmol Vis Sci.* 2005;46(1):75-82.
13. Brouwer NJ, Marinkovic M, van Duinen SG, et al. Treatment of conjunctival melanoma in a Dutch referral centre. *Br J Ophthalmol.* 2018;102(9):1277-1282.
14. Esmaeli B, Rubin ML, Xu S, et al. Greater Tumor Thickness, Ulceration, and Positive Sentinel Lymph Node Are Associated With Worse Prognosis in Patients With Conjunctival Melanoma: Implications for Future AJCC Classifications. *Am J Surg Pathol.* 2019;43(12):1701-1710.
15. Brouwer NJ, Marinkovic M, Luyten GPM, Shields CL, Jager MJ. Lack of tumour pigmentation in conjunctival melanoma is associated with light iris colour and worse prognosis. *Br J Ophthalmol.* 2019;103(3):332-337.
16. Larsen AC, Dahmcke CM, Dahl C, et al. A Retrospective Review of Conjunctival Melanoma Presentation, Treatment, and Outcome and an Investigation of Features Associated With BRAF Mutations. *JAMA Ophthalmol.* 2015;133(11):1295-1303.
17. Griewank KG, Westekemper H, Murali R, et al. Conjunctival melanomas harbor BRAF and NRAS mutations and copy number changes similar to cutaneous and mucosal melanomas. *Clin Cancer Res.* 2013;19(12):3143-3152.
18. Griewank KG, Murali R, Schilling B, et al. TERT promoter mutations in ocular melanoma distinguish between conjunctival and uveal tumours. *Br J Cancer.* 2013;109(2):497-501.
19. Chapman PB, Hauschild A, Robert C, et al. Improved survival with vemurafenib in melanoma with BRAF V600E mutation. *N Engl J Med.* 2011;364(26):2507-2516.
20. Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAF(V600)-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol.* 2016;17(9):1248-1260.

21. Esmali B, Sagiv O. Targeted Biological Drugs and Immune Check Point Inhibitors for Locally Advanced or Metastatic Cancers of the Conjunctiva, Eyelid, and Orbit. *International Ophthalmology Clinics*. 2019;59(2):13-26.
22. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363(8):711-723.
23. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. 2015;372(4):320-330.
24. Heppt MV, Steeb T, Schlager JG, et al. Immune checkpoint blockade for unresectable or metastatic uveal melanoma: A systematic review. *Cancer Treat Rev*. 2017;60:44-52.
25. Niederhorn JY. Immune escape mechanisms of intraocular tumors. *Prog Retin Eye Res*. 2009;28(5):329-347.
26. Sagiv O, Thakar SD, Kandl TJ, et al. Immunotherapy With Programmed Cell Death 1 Inhibitors for 5 Patients With Conjunctival Melanoma. *JAMA Ophthalmol*. 2018;136(11):1236-1241.
27. Hong BY-B, Ford JR, Glitza IC, et al. Immune Checkpoint Inhibitor Therapy as an Eye-Preserving Treatment for Locally Advanced Conjunctival Melanoma. *Ophthalmic Plastic & Reconstructive Surgery*. 2020;Publish Ahead of Print.
28. Virgili G, Parravano M, Gatta G, et al. Incidence and Survival of Patients With Conjunctival Melanoma in Europe. *JAMA Ophthalmol*. 2020.
29. Shields CL, Chien JL, Surakiatchanukul T, et al. Conjunctival Tumors: Review of Clinical Features, Risks, Biomarkers, and Outcomes--The 2017 J. Donald M. Gass Lecture. *Asia Pac J Ophthalmol (Phila)*. 2017;6(2):109-120.
30. Larsen AC, Dahl C, Dahmcke CM, et al. BRAF mutations in conjunctival melanoma: investigation of incidence, clinicopathological features, prognosis and paired premalignant lesions. *Acta Ophthalmol*. 2016;94(5):463-470.
31. Balzer BWR, Cherepanoff S, Joshua AM, et al. Conjunctival Melanoma in Childhood and Adolescence: A Systematic Review. *Ocul Oncol Pathol*. 2019;5(6):387-395.
32. Ghazawi FM, Darwich R, Le M, et al. Incidence trends of conjunctival malignant melanoma in Canada. *Br J Ophthalmol*. 2019.
33. Statista. European Union: total population from 2010 to 2020. 2020; Available at: <https://www.statista.com/statistics/253372/total-population-of-the-european-union-eu>. Accessed 09-02-2021.
34. World-Factbook C. CIA World Factbook USA. 2021; Available at: <https://www.cia.gov/the-world-factbook/countries/united-states/>. Accessed 09-02-2021.
35. Hu DN, Yu G, McCormick SA, Finger PT. Population-based incidence of conjunctival melanoma in various races and ethnic groups and comparison with other melanomas. *Am J Ophthalmol*. 2008;145(3):418-423.
36. Yu GP, Hu DN, McCormick SA. Latitude and incidence of ocular melanoma. *Photochem Photobiol*. 2006;82(6):1621-1626.
37. Lommatzsch PK, Lommatzsch RE, Kirsch I, Fuhrmann P. Therapeutic outcome of patients suffering from malignant melanomas of the conjunctiva. *Br J Ophthalmol*. 1990;74(10):615-619.
38. Park SJ, Oh CM, Kim BW, et al. Nationwide Incidence of Ocular Melanoma in South Korea by Using the National Cancer Registry Database (1999-2011). *Invest Ophthalmol Vis Sci*. 2015;56(8):4719-4724.
39. Vajdic CM, Krickler A, Giblin M, et al. Incidence of ocular melanoma in Australia from 1990 to 1998. *Int J Cancer*. 2003;105(1):117-122.
40. 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(4):612-617.
41. Virgili G, Gatta G, Ciccolallo L, et al. Incidence of uveal melanoma in Europe. *Ophthalmology*. 2007;114(12):2309-2315.

42. Sheng X, Li S, Chi Z, et al. Prognostic factors for conjunctival melanoma: a study in ethnic Chinese patients. *Br J Ophthalmol*. 2015;99(7):990-996.
43. Zhou C, Wang Y, Jia R, Fan X. Conjunctival Melanoma in Chinese Patients: Local Recurrence, Metastasis, Mortality, and Comparisons With Caucasian Patients. *Invest Ophthalmol Vis Sci*. 2017;58(12):5452-5459.
44. Chen PY, Liao YL, Chu YC, Tsai YJ. Conjunctival melanoma: A 20-year survey in a comprehensive medical center. *J Formos Med Assoc*. 2020.
45. Kikuchi I, Kase S, Ishijima K, Ishida S. Long-term follow-up of conjunctival melanoma treated with topical interferon alpha-2b eye drops as adjunctive therapy following surgical resection. *Graefes Arch Clin Exp Ophthalmol*. 2017;255(11):2271-2276.
46. Lee YJ, Lee C, Kim MK, Khwarg SI, Oh JY. Conjunctival pigmented lesion: Clinicopathological analysis of 85 cases in Korean population. *Sci Rep*. 2019;9(1):18204.
47. Fasina O, Oluwasola OA. Conjunctival melanoma in Southwestern Nigeria: a case series and review of literature. *Int Ophthalmol*. 2018;38(4):1747-1752.
48. Salcedo-Hernandez RA, Luna-Ortiz K, Lino-Silva LS, et al. Conjunctival melanoma: survival analysis in twenty-two Mexican patients. *Arq Bras Oftalmol*. 2014;77(3):155-158.
49. Seregard S, Kock E. Conjunctival malignant melanoma in Sweden 1969-91. *Acta Ophthalmol (Copenh)*. 1992;70(3):289-296.
50. Norregaard JC, Gerner N, Jensen OA, Prause JU. Malignant melanoma of the conjunctiva: occurrence and survival following surgery and radiotherapy in a Danish population. *Graefes Arch Clin Exp Ophthalmol*. 1996;234(9):569-572.
51. Mansson-Brahme E, Johansson H, Larsson O, Rutqvist LE, Ringborg U. Trends in incidence of cutaneous malignant melanoma in a Swedish population 1976-1994. *Acta Oncol*. 2002;41(2):138-146.
52. de Vries E, Bray FI, Coebergh JW, Parkin DM. Changing epidemiology of malignant cutaneous melanoma in Europe 1953-1997: rising trends in incidence and mortality but recent stabilizations in western Europe and decreases in Scandinavia. *Int J Cancer*. 2003;107(1):119-126.
53. Bianconi F, Crocetti E, Grisci C, Primieri C, Stracci F. What has changed in the epidemiology of skin melanoma in central Italy during the past 20 years? *Melanoma Res*. 2020;30(4):396-401.
54. Aronow ME, Topham AK, Singh AD. Uveal Melanoma: 5-Year Update on Incidence, Treatment, and Survival (SEER 1973-2013). *Ocul Oncol Pathol*. 2018;4(3):145-151.
55. Ghazawi FM, Darwich R, Le M, et al. Uveal melanoma incidence trends in Canada: a national comprehensive population-based study. *Br J Ophthalmol*. 2019;103(12):1872-1876.
56. Marks R, Motley R. *Common Skin Diseases 18th edition*. CRC Press; 2019.
57. Watsky MA, Jablonski MM, Edelhauser HF. Comparison of conjunctival and corneal surface areas in rabbit and human. *Curr Eye Res*. 1988;7(5):483-486.
58. Algaed AH, Kozak I. *Clinical Atlas of Ophthalmic Ultrasound*. Springer International Publishing; 2018.
59. Jakobiec FA. Conjunctival Primary Acquired Melanosis: Is It Time for a New Terminology? *Am J Ophthalmol*. 2016;162:3-19 e11.
60. Dalvin LA, Salomão DR, Patel SV. Population-based incidence of conjunctival tumours in Olmsted County, Minnesota. *British Journal of Ophthalmology*. 2018;102(12):1728-1734.
61. Paridaens AD, Minassian DC, McCartney AC, Hungerford JL. Prognostic factors in primary malignant melanoma of the conjunctiva: a clinicopathological study of 256 cases. *Br J Ophthalmol*. 1994;78(4):252-259.
62. Shields JA, Shields CL, Mashayekhi A, et al. Primary acquired melanosis of the conjunctiva: risks for progression to melanoma in 311 eyes. The 2006 Lorenz E. Zimmerman lecture. *Ophthalmology*. 2008;115(3):511-519 e512.

63. Folberg R, McLean IW, Zimmerman LE. Primary acquired melanosis of the conjunctiva. *Hum Pathol.* 1985;16(2):129-135.
64. Damato B, Coupland SE. Conjunctival melanoma and melanosis: a reappraisal of terminology, classification and staging. *Clin Exp Ophthalmol.* 2008;36(8):786-795.
65. Grossniklaus HE, Eberhart CG, Kivela M, TTe, World Health Organization, International Agency for Research on Cancer. *WHO classification of tumours of the eye.* 4th edition. ed: Lyon : International Agency for Research on Cancer; 2018.
66. Milman T, Eiger-Moscovich M, Henry RK, et al. Validation of the Newly Proposed World Health Organization Classification System for Conjunctival Melanocytic Intraepithelial Lesions: A Comparison with the C-MIN and PAM Classification Schemes. *Am J Ophthalmol.* 2020;223:60-74.
67. Anastassiou G, Heiligenhaus A, Bechrakis N, et al. Prognostic value of clinical and histopathological parameters in conjunctival melanomas: a retrospective study. *Br J Ophthalmol.* 2002;86(2):163-167.
68. Shields CL, Alset AE, Boal NS, et al. Conjunctival Tumors in 5002 Cases. Comparative Analysis of Benign versus Malignant Counterparts. The 2016 James D. Allen Lecture. *Am J Ophthalmol.* 2016.
69. Shields CL, Fasiuddin AF, Mashayekhi A, Shields JA. Conjunctival nevi: clinical features and natural course in 410 consecutive patients. *Arch Ophthalmol.* 2004;122(2):167-175.
70. Shields CL, Shields JA. Tumors of the conjunctiva and cornea. *Indian J Ophthalmol.* 2019;67(12):1930-1948.
71. Damato B, Coupland SE. An audit of conjunctival melanoma treatment in Liverpool. *Eye (Lond).* 2009;23(4):801-809.
72. De Wolff-Rouendaal D. Conjunctival melanoma in the Netherlands: a clinico-pathological and follow-up study. (Thesis). 1990.
73. De Potter P, Shields CL, Shields JA, Menduke H. Clinical predictive factors for development of recurrence and metastasis in conjunctival melanoma: a review of 68 cases. *Br J Ophthalmol.* 1993;77(10):624-630.
74. Kastelan S, Gverovic Antunica A, Beketic Oreskovic L, et al. Conjunctival Melanoma - Epidemiological Trends and Features. *Pathol Oncol Res.* 2018;24(4):787-796.
75. Shah P, He YY. Molecular regulation of UV-induced DNA repair. *Photochem Photobiol.* 2015;91(2):254-264.
76. Budden T, Bowden NA. The role of altered nucleotide excision repair and UVB-induced DNA damage in melanomagenesis. *Int J Mol Sci.* 2013;14(1):1132-1151.
77. de Gruijl FR. Photocarcinogenesis: UVA vs. UVB radiation. *Skin Pharmacol Appl Skin Physiol.* 2002;15(5):316-320.
78. Premi S, Wallisch S, Mano CM, et al. Photochemistry. Chemiexcitation of melanin derivatives induces DNA photoproducts long after UV exposure. *Science.* 2015;347(6224):842-847.
79. Sliney DH. How light reaches the eye and its components. *Int J Toxicol.* 2002;21(6):501-509.
80. Pleasance ED, Cheetham RK, Stephens PJ, et al. A comprehensive catalogue of somatic mutations from a human cancer genome. *Nature.* 2010;463(7278):191-196.
81. Bald T, Quast T, Landsberg J, et al. Ultraviolet-radiation-induced inflammation promotes angiogenesis and metastasis in melanoma. *Nature.* 2014;507(7490):109-113.
82. Zaidi MR, Davis S, Noonan FP, et al. Interferon- γ links ultraviolet radiation to melanomagenesis in mice. *Nature.* 2011;469(7331):548-553.
83. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer.* 2014;14(5):342-357.
84. Vajdic CM, Krickler A, Giblin M, et al. Sun exposure predicts risk of ocular melanoma in Australia. *Int J Cancer.* 2002;101(2):175-182.
85. Tucker MA, Goldstein AM. Melanoma etiology: where are we? *Oncogene.* 2003;22(20):3042-3052.

86. El-Shabrawi Y, Radner H, Muellner K, Langmann G, Hoefler G. The role of UV-radiation in the development of conjunctival malignant melanoma. *Acta Ophthalmol Scand.* 1999;77(1):31-32.
87. Rivolta C, Royer-Bertrand B, Rimoldi D, et al. UV light signature in conjunctival melanoma; not only skin should be protected from solar radiation. *J Hum Genet.* 2016;61(4):361-362.
88. Swaminathan SS, Field MG, Sant D, et al. Molecular Characteristics of Conjunctival Melanoma Using Whole-Exome Sequencing. *JAMA Ophthalmol.* 2017;135(12):1434-1437.
89. Cisarova K, Folcher M, El Zaoui I, et al. Genomic and transcriptomic landscape of conjunctival melanoma. *PLoS Genet.* 2020;16(12):e1009201.
90. Mikkelsen LH, Maag E, Andersen MK, et al. The molecular profile of mucosal melanoma. *Melanoma Res.* 2020;30(6):533-542.
91. Johansson PA, Brooks K, Newell F, et al. Whole genome landscapes of uveal melanoma show an ultraviolet radiation signature in iris tumours. *Nat Commun.* 2020;11(1):2408.
92. Craig S, Earnshaw CH, Virós A. Ultraviolet light and melanoma. *The Journal of Pathology.* 2018;244(5):578-585.
93. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med.* 2005;353(20):2135-2147.
94. Brouwer NJ, Marinkovic M, Luyten GPM, Shields CL, Jager MJ. Pigmentation of conjunctival melanoma recurrences and outcome. *Graefes Arch Clin Exp Ophthalmol.* 2019;257(8):1783-1788.
95. De Leeuw SM, Smit NP, Van Veldhoven M, et al. Melanin content of cultured human melanocytes and UV-induced cytotoxicity. *J Photochem Photobiol B.* 2001;61(3):106-113.
96. Wakamatsu K, Hu DN, McCormick SA, Ito S. Characterization of melanin in human iridal and choroidal melanocytes from eyes with various colored irides. *Pigment Cell Melanoma Res.* 2008;21(1):97-105.
97. Brenner M, Hearing VJ. The protective role of melanin against UV damage in human skin. *Photochem Photobiol.* 2008;84(3):539-549.
98. Mitra D, Luo X, Morgan A, et al. An ultraviolet-radiation-independent pathway to melanoma carcinogenesis in the red hair/fair skin background. *Nature.* 2012;491(7424):449-453.
99. Kusmartsev S, Nefedova Y, Yoder D, Gabrilovich DI. Antigen-specific inhibition of CD8+ T cell response by immature myeloid cells in cancer is mediated by reactive oxygen species. *J Immunol.* 2004;172(2):989-999.
100. Zhang Y, Choksi S, Chen K, et al. ROS play a critical role in the differentiation of alternatively activated macrophages and the occurrence of tumor-associated macrophages. *Cell Res.* 2013;23(7):898-914.
101. Hu DN, McCormick SA, Seedor JA, Ritterband DC, Shah MK. Isolation, purification and cultivation of conjunctival melanocytes. *Exp Eye Res.* 2007;84(4):655-662.
102. Iwamoto S, Burrows RC, Grossniklaus HE, et al. Immunophenotype of conjunctival melanomas: comparisons with uveal and cutaneous melanomas. *Arch Ophthalmol.* 2002;120(12):1625-1629.
103. Olsen CM, Carroll HJ, Whiteman DC. Estimating the attributable fraction for melanoma: a meta-analysis of pigmentary characteristics and freckling. *Int J Cancer.* 2010;127(10):2430-2445.
104. Vajdic CM, Krickler A, Giblin M, et al. Eye color and cutaneous nevi predict risk of ocular melanoma in Australia. *Int J Cancer.* 2001;92(6):906-912.
105. 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(1):54-60.
106. Schmidt-Pokrzywniak A, Jockel KH, Bornfeld N, Sauerwein W, Stang A. Positive interaction between light iris color and ultraviolet radiation in relation to the risk of uveal melanoma: a case-control study. *Ophthalmology.* 2009;116(2):340-348.
107. Houtzagers LE, Wierenga APA, Ruys AAM, Luyten GPM, Jager MJ. Iris Colour and the Risk of Developing Uveal Melanoma. *Int J Mol Sci.* 2020;21(19).

108. Shields CL, Yaghy A, Dalvin LA, et al. Conjunctival Melanoma: Features and Outcomes Based on the Fitzpatrick Skin Type in 540 Patients at a Single Ocular Oncology Center. *Ophthalmic Plast Reconstr Surg*. 2020.
109. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011;144(5):646-674.
110. McCain J. The MAPK (ERK) Pathway: Investigational Combinations for the Treatment Of BRAF-Mutated Metastatic Melanoma. *P t*. 2013;38(2):96-108.
111. Garnett MJ, Marais R. Guilty as charged: B-RAF is a human oncogene. *Cancer Cell*. 2004;6(4):313-319.
112. Davies MA. The role of the PI3K-AKT pathway in melanoma. *Cancer J*. 2012;18(2):142-147.
113. Kiuru M, Busam KJ. The NF1 gene in tumor syndromes and melanoma. *Lab Invest*. 2017;97(2):146-157.
114. Meng D, Carvajal RD. KIT as an Oncogenic Driver in Melanoma: An Update on Clinical Development. *Am J Clin Dermatol*. 2019;20(3):315-323.
115. Sugarman ET, Zhang G, Shay JW. In perspective: An update on telomere targeting in cancer. *Mol Carcinog*. 2019;58(9):1581-1588.
116. van Ipenburg JA, Naus NC, Dubbink HJ, et al. Prognostic value of TERT promoter mutations in conjunctival melanomas in addition to clinicopathological features. *Br J Ophthalmol*. 2020:[Online ahead of print].
117. Cancer-Genome-Atlas-Network. Genomic Classification of Cutaneous Melanoma. *Cell*. 2015;161(7):1681-1696.
118. Scholz SL, Cosgarea I, Susskind D, et al. NF1 mutations in conjunctival melanoma. *Br J Cancer*. 2018;118(9):1243-1247.
119. Greaves WO, Verma S, Patel KP, et al. Frequency and spectrum of BRAF mutations in a retrospective, single-institution study of 1112 cases of melanoma. *J Mol Diagn*. 2013;15(2):220-226.
120. Menzies AM, Haydu LE, Visintin L, et al. Distinguishing clinicopathologic features of patients with V600E and V600K BRAF-mutant metastatic melanoma. *Clin Cancer Res*. 2012;18(12):3242-3249.
121. Cao J, Heijkants RC, Jochemsen AG, et al. Targeting of the MAPK and AKT pathways in conjunctival melanoma shows potential synergy. *Oncotarget*. 2016.
122. Kim SY, Kim SN, Hahn HJ, et al. Metaanalysis of BRAF mutations and clinicopathologic characteristics in primary melanoma. *J Am Acad Dermatol*. 2015;72(6):1036-1046 e1032.
123. El Zaoui I, Bucher M, Rimoldi D, et al. Conjunctival Melanoma Targeted Therapy: MAPK and PI3K/mTOR Pathways Inhibition. *Invest Ophthalmol Vis Sci*. 2019;60(7):2764-2772.
124. Goldenberg-Cohen N, Cohen Y, Rosenbaum E, et al. T1799A BRAF mutations in conjunctival melanocytic lesions. *Invest Ophthalmol Vis Sci*. 2005;46(9):3027-3030.
125. Francis JH, Grossniklaus HE, Habib LA, et al. BRAF, NRAS, and GNAQ Mutations in Conjunctival Melanocytic Nevi. *Invest Ophthalmol Vis Sci*. 2018;59(1):117-121.
126. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. *Nat Genet*. 2003;33(1):19-20.
127. Gear H, Williams H, Kemp EG, Roberts F. BRAF mutations in conjunctival melanoma. *Invest Ophthalmol Vis Sci*. 2004;45(8):2484-2488.
128. Zebary A, Jangard M, Omholt K, Ragnarsson-Olding B, Hansson J. KIT, NRAS and BRAF mutations in sinonasal mucosal melanoma: a study of 56 cases. *Br J Cancer*. 2013;109(3):559-564.
129. Cosgarea I, Ugurel S, Sucker A, et al. Targeted next generation sequencing of mucosal melanomas identifies frequent NF1 and RAS mutations. *Oncotarget*. 2017;8(25):40683-40692.
130. Spendlove HE, Damato BE, Humphreys J, et al. BRAF mutations are detectable in conjunctival but not uveal melanomas. *Melanoma Res*. 2004;14(6):449-452.
131. Van Raamsdonk CD, Bezrookove V, Green G, et al. Frequent somatic mutations of GNAQ in uveal melanoma and blue naevi. *Nature*. 2009;457(7229):599-602.

132. van Poppelen NM, Vaarwater J, Mudhar HS, et al. Genetic Background of Iris Melanomas and Iris Melanocytic Tumors of Uncertain Malignant Potential. *Ophthalmology*. 2018;125(6):904-912.
133. Krauthammer M, Kong Y, Bacchiocchi A, et al. Exome sequencing identifies recurrent mutations in NF1 and RASopathy genes in sun-exposed melanomas. *Nat Genet*. 2015;47(9):996-1002.
134. Beadling C, Jacobson-Dunlop E, Hodi FS, et al. KIT gene mutations and copy number in melanoma subtypes. *Clin Cancer Res*. 2008;14(21):6821-6828.
135. Houben R, Becker JC, Kappel A, et al. Constitutive activation of the Ras-Raf signaling pathway in metastatic melanoma is associated with poor prognosis. *J Carcinog*. 2004;3:6.
136. Heppt MV, Siepmann T, Engel J, et al. Prognostic significance of BRAF and NRAS mutations in melanoma: a German study from routine care. *BMC Cancer*. 2017;17(1):536.
137. Cruz F, 3rd, Rubin BP, Wilson D, et al. Absence of BRAF and NRAS mutations in uveal melanoma. *Cancer Res*. 2003;63(18):5761-5766.
138. Cirenajwis H, Lauss M, Ekedahl H, et al. NF1-mutated melanoma tumors harbor distinct clinical and biological characteristics. *Mol Oncol*. 2017;11(4):438-451.
139. Johnson DB, Frampton GM, Rioth MJ, et al. Targeted Next Generation Sequencing Identifies Markers of Response to PD-1 Blockade. *Cancer Immunol Res*. 2016;4(11):959-967.
140. Hintzsche JD, Gorden NT, Amato CM, et al. Whole-exome sequencing identifies recurrent SF3B1 R625 mutation and comutation of NF1 and KIT in mucosal melanoma. *Melanoma Res*. 2017;27(3):189-199.
141. Foster WJ, Fuller CE, Perry A, Harbour JW. Status of the NF1 Tumor Suppressor Locus in Uveal Melanoma. *Archives of Ophthalmology*. 2003;121(9):1311-1315.
142. Curtin JA, Busam K, Pinkel D, Bastian BC. Somatic Activation of KIT in Distinct Subtypes of Melanoma. *Journal of Clinical Oncology*. 2006;24(26):4340-4346.
143. Alessandrini L, Parrozzani R, Bertorelle R, et al. C-Kit SCF receptor (CD117) expression and KIT gene mutation in conjunctival pigmented lesions. *Acta Ophthalmol*. 2013;91(8):e641-645.
144. Wallander ML, Layfield LJ, Emerson LL, et al. KIT mutations in ocular melanoma: frequency and anatomic distribution. *Mod Pathol*. 2011;24(8):1031-1035.
145. Tsao H, Mihm MC, Sheehan C. PTEN expression in normal skin, acquired melanocytic nevi, and cutaneous melanoma. *Journal of the American Academy of Dermatology*. 2003;49(5):865-872.
146. Cabrita R, Mitra S, Sanna A, et al. The Role of PTEN Loss in Immune Escape, Melanoma Prognosis and Therapy Response. *Cancers (Basel)*. 2020;12(3).
147. Westekemper H, Karimi S, Susskind D, et al. Expression of HSP 90, PTEN and Bcl-2 in conjunctival melanoma. *Br J Ophthalmol*. 2011;95(6):853-858.
148. Populo H, Soares P, Rocha AS, Silva P, Lopes JM. Evaluation of the mTOR pathway in ocular (uvea and conjunctiva) melanoma. *Melanoma Res*. 2010;20(2):107-117.
149. Abdel-Rahman MH, Yang Y, Zhou XP, et al. High frequency of submicroscopic hemizygous deletion is a major mechanism of loss of expression of PTEN in uveal melanoma. *J Clin Oncol*. 2006;24(2):288-295.
150. Horn S, Figl A, Rachakonda PS, et al. TERT promoter mutations in familial and sporadic melanoma. *Science*. 2013;339(6122):959-961.
151. Vinagre J, Almeida A, Pópulo H, et al. Frequency of TERT promoter mutations in human cancers. *Nat Commun*. 2013;4:2185.
152. Hugdahl E, Kalvenes MB, Mannelqvist M, Ladstein RG, Akslen LA. Prognostic impact and concordance of TERT promoter mutation and protein expression in matched primary and metastatic cutaneous melanoma. *Br J Cancer*. 2018;118(1):98-105.

153. Koopmans AE, Ober K, Dubbink HJ, et al. Prevalence and implications of TERT promoter mutation in uveal and conjunctival melanoma and in benign and premalignant conjunctival melanocytic lesions. *Invest Ophthalmol Vis Sci*. 2014;55(9):6024-6030.
154. Shain AH, Yeh I, Kovalyshyn I, et al. The Genetic Evolution of Melanoma from Precursor Lesions. *New England Journal of Medicine*. 2015;373(20):1926-1936.
155. Jangard M, Zebary A, Ragnarsson-Olding B, Hansson J. TERT promoter mutations in sinonasal malignant melanoma: a study of 49 cases. *Melanoma Res*. 2015;25(3):185-188.
156. Dono M, Angelini G, Cecconi M, et al. Mutation frequencies of GNAQ, GNA11, BAP1, SF3B1, EIF1AX and TERT in uveal melanoma: detection of an activating mutation in the TERT gene promoter in a single case of uveal melanoma. *Br J Cancer*. 2014;110(4):1058-1065.
157. Feng X, Degese MS, Iglesias-Bartolome R, et al. Hippo-independent activation of YAP by the GNAQ uveal melanoma oncogene through a trio-regulated rho GTPase signaling circuitry. *Cancer Cell*. 2014;25(6):831-845.
158. Yu FX, Luo J, Mo JS, et al. Mutant Gq/11 promote uveal melanoma tumorigenesis by activating YAP. *Cancer Cell*. 2014;25(6):822-830.
159. Van Raamsdonk CD, Griewank KG, Crosby MB, et al. Mutations in GNA11 in uveal melanoma. *N Engl J Med*. 2010;363(23):2191-2199.
160. Vader MJC, Madigan MC, Versluis M, et al. GNAQ and GNA11 mutations and downstream YAP activation in choroidal nevi. *Br J Cancer*. 2017;117(6):884-887.
161. Dravtman-Storobinsky O, Cohen Y, Frenkel S, Pe'er J, Goldenberg-Cohen N. Lack of oncogenic GNAQ mutations in melanocytic lesions of the conjunctiva as compared to uveal melanoma. *Invest Ophthalmol Vis Sci*. 2010;51(12):6180-6182.
162. Yavuzvigitoglu S, Koopmans AE, Verdijk RM, et al. Uveal Melanomas with SF3B1 Mutations: A Distinct Subclass Associated with Late-Onset Metastases. *Ophthalmology*. 2016;123(5):1118-1128.
163. Koopmans AE, Verdijk RM, Brouwer RW, et al. Clinical significance of immunohistochemistry for detection of BAP1 mutations in uveal melanoma. *Mod Pathol*. 2014;27(10):1321-1330.
164. Harbour JW, Onken MD, Roberson ED, et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science*. 2010;330(6009):1410-1413.
165. Carbone M, Yang H, Pass HI, et al. BAP1 and cancer. *Nat Rev Cancer*. 2013;13(3):153-159.
166. Gezgin G, Dogrusöz M, van Essen TH, et al. Genetic evolution of uveal melanoma guides the development of an inflammatory microenvironment. *Cancer Immunol Immunother*. 2017;66(7):903-912.
167. Brouwer NJ, Gezgin G, Wierenga APA, et al. Tumour Angiogenesis in Uveal Melanoma Is Related to Genetic Evolution. *Cancers (Basel)*. 2019;11(7).
168. Wiesner T, Obenaus AC, Murali R, et al. Germline mutations in BAP1 predispose to melanocytic tumors. *Nature Genetics*. 2011;43(10):1018-1021.
169. Liu-Smith F, Lu Y. Opposite Roles of BAP1 in Overall Survival of Uveal Melanoma and Cutaneous Melanoma. *J Clin Med*. 2020;9(2).
170. Royer-Bertrand B, Torsello M, Rimoldi D, et al. Comprehensive Genetic Landscape of Uveal Melanoma by Whole-Genome Sequencing. *Am J Hum Genet*. 2016;99(5):1190-1198.
171. Kenawy N, Kalirai H, Sacco JJ, et al. Conjunctival melanoma copy number alterations and correlation with mutation status, tumor features, and clinical outcome. *Pigment Cell Melanoma Res*. 2019;32(4):564-575.
172. Vajdic CM, Hutchins AM, Kricker A, et al. Chromosomal gains and losses in ocular melanoma detected by comparative genomic hybridization in an Australian population-based study. *Cancer Genet Cytogenet*. 2003;144(1):12-17.

173. Lake SL, Jmor F, Dopierala J, et al. Multiplex ligation-dependent probe amplification of conjunctival melanoma reveals common BRAF V600E gene mutation and gene copy number changes. *Invest Ophthalmol Vis Sci*. 2011;52(8):5598-5604.
174. Gast A, Scherer D, Chen B, et al. Somatic alterations in the melanoma genome: a high-resolution array-based comparative genomic hybridization study. *Genes Chromosomes Cancer*. 2010;49(8):733-745.
175. Soura E, Eliades PJ, Shannon K, Stratigos AJ, Tsao H. Hereditary melanoma: Update on syndromes and management: Genetics of familial atypical multiple mole melanoma syndrome. *J Am Acad Dermatol*. 2016;74(3):395-407; quiz 408-310.
176. Bataille V, Boyle J, Hungerford JL, Newton JA. Three cases of primary acquired melanosis of the conjunctiva as a manifestation of the atypical mole syndrome. *Br J Dermatol*. 1993;128(1):86-90.
177. Seregard S, af Trampe E, Månsson-Brahme E, et al. Prevalence of primary acquired melanosis and nevi of the conjunctiva and uvea in the dysplastic nevus syndrome. A case-control study. *Ophthalmology*. 1995;102(10):1524-1529.
178. Seminog OO, Goldacre MJ. Risk of benign tumours of nervous system, and of malignant neoplasms, in people with neurofibromatosis: population-based record-linkage study. *British Journal of Cancer*. 2013;108(1):193-198.
179. Guillot B, Dalac S, Delaunay M, et al. Cutaneous malignant melanoma and neurofibromatosis type 1. *Melanoma Research*. 2004;14(2):159-163.
180. Medina Mendez CA, Singh AD. Multifocal melanoma of the conjunctiva in an African American patient with neurofibromatosis type 1. *Cornea*. 2014;33(7):750-751.
181. Rubinstein TJ, Plessec TP, Singh AD. Desmoplastic melanoma of the eyelid and conjunctival melanoma in neurofibromatosis type 1: a clinical pathological correlation. *Surv Ophthalmol*. 2015;60(1):72-77.
182. Stacy RC, Kenyon KR, Jakobiec FA, Colby KA. Conjunctival melanoma arising from primary acquired melanosis in a patient with neurofibromatosis type I. *Cornea*. 2010;29(2):232-234.
183. To KW, Rabinowitz SM, Friedman AH, Merker C, Cavanaugh CP. Neurofibromatosis and neural crest neoplasms: primary acquired melanosis and malignant melanoma of the conjunctiva. *Surv Ophthalmol*. 1989;33(5):373-379.
184. Honavar SG, Singh AD, Shields CL, Shields JA, Eagle RC, Jr. Iris melanoma in a patient with neurofibromatosis. *Surv Ophthalmol*. 2000;45(3):231-236.
185. Singh AD, De Potter P, Fijal BA, et al. Lifetime prevalence of uveal melanoma in white patients with oculo(dermal) melanocytosis. *Ophthalmology*. 1998;105(1):195-198.
186. 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 Ophthalmology*. 2013;131(8):993-1003.
187. Patel BC, Egan CA, Lucius RW, et al. Cutaneous malignant melanoma and oculodermal melanocytosis (nevus of Ota): report of a case and review of the literature. *J Am Acad Dermatol*. 1998;38(5 Pt 2):862-865.
188. Radhadevi CV, Charles KS, Lathika VK. Orbital malignant melanoma associated with nevus of Ota. *Indian J Ophthalmol*. 2013;61(6):306-309.
189. 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(12):856-859.
190. Chau C, van Doorn R, van Poppelen NM, et al. Families with BAP1-Tumor Predisposition Syndrome in The Netherlands: Path to Identification and a Proposal for Genetic Screening Guidelines. *Cancers (Basel)*. 2019;11(8).
191. Gajos-Michniewicz A, Czyz M. Role of miRNAs in Melanoma Metastasis. *Cancers (Basel)*. 2019;11(3).

192. Larsen AC, Mikkelsen LH, Borup R, et al. MicroRNA Expression Profile in Conjunctival Melanoma. *Invest Ophthalmol Vis Sci*. 2016;57(10):4205-4212.
193. Mikkelsen LH, Andersen MK, Andreassen S, et al. Global microRNA profiling of metastatic conjunctival melanoma. *Melanoma Res*. 2019;29(5):465-473.
194. van Ipenburg JA, Gillis Ing AJM, Dorssers LCJ, et al. MicroRNA Profiling in Benign and Malignant Conjunctival Melanocytic Lesions. *Ophthalmology*. 2020;127(3):432-434.
195. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: links to genetic instability. *Carcinogenesis*. 2009;30(7):1073-1081.
196. Antohe M, Nedelcu RI, Nichita L, et al. Tumor infiltrating lymphocytes: The regulator of melanoma evolution. *Oncol Lett*. 2019;17(5):4155-4161.
197. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2015;372(26):2521-2532.
198. Coussens LM, Werb Z. Inflammation and cancer. *Nature*. 2002;420(6917):860-867.
199. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol*. 2002;23(11):549-555.
200. Cao J, Brouwer NJ, Richards KE, et al. PD-L1/PD-1 expression and tumor-infiltrating lymphocytes in conjunctival melanoma. *Oncotarget*. 2017;8(33):54722-54734.
201. Bronkhorst IH, Ly LV, Jordanova ES, et al. Detection of M2-macrophages in uveal melanoma and relation with survival. *Invest Ophthalmol Vis Sci*. 2011;52(2):643-650.
202. Makitie T, Summanen P, Tarkkanen A, Kivela T. Microvascular density in predicting survival of patients with choroidal and ciliary body melanoma. *Invest Ophthalmol Vis Sci*. 1999;40(11):2471-2480.
203. Storr SJ, Safuan S, Mitra A, et al. Objective assessment of blood and lymphatic vessel invasion and association with macrophage infiltration in cutaneous melanoma. *Mod Pathol*. 2012;25(4):493-504.
204. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood*. 2008;112(5):1570-1580.
205. Zhu J, Paul WE. CD4 T cells: fates, functions, and faults. *Blood*. 2008;112(5):1557-1569.
206. Corthay A. How do regulatory T cells work? *Scand J Immunol*. 2009;70(4):326-336.
207. Ljunggren HG, Karre K. In search of the 'missing self': MHC molecules and NK cell recognition. *Immunol Today*. 1990;11(7):237-244.
208. Jay B. Naevi and Melanomata of the Conjunctiva. *Br J Ophthalmol*. 1965;49:169-204.
209. Crawford JB. Conjunctival melanomas: prognostic factors a review and an analysis of a series. *Trans Am Ophthalmol Soc*. 1980;78:467-502.
210. Clark WH, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res*. 1969;29(3):705-727.
211. Folberg R, McLean IW, Zimmerman LE. Malignant melanoma of the conjunctiva. *Hum Pathol*. 1985;16(2):136-143.
212. McGhee CN, Ni C, Albert DM, Chu FR. Conjunctival melanoma. *Int Ophthalmol Clin*. 1982;22(3):35-56.
213. Jeffrey IJ, Lucas DR, McEwan C, Lee WR. Malignant melanoma of the conjunctiva. *Histopathology*. 1986;10(4):363-378.
214. Bobic-Radovanovic A, Latkovic Z, Marinkovic J, Radovanovic Z. Predictors of survival in malignant melanoma of the conjunctiva: a clinico-pathological and follow-up study. *Eur J Ophthalmol*. 1998;8(1):4-7.
215. Anastassiou G, Esser M, Bader E, Steuhl KP, Bornfeld N. Expression of cell adhesion molecules and tumour infiltrating leucocytes in conjunctival melanoma. *Melanoma Res*. 2004;14(5):381-385.
216. Tuomaala S, Toivonen P, Al-Jamal R, Kivela T. Prognostic significance of histopathology of primary conjunctival melanoma in Caucasians. *Curr Eye Res*. 2007;32(11):939-952.

217. Jordanova ES, Gorter A, Ayachi O, et al. Human leukocyte antigen class I, MHC class I chain-related molecule A, and CD8+/regulatory T-cell ratio: which variable determines survival of cervical cancer patients? *Clin Cancer Res.* 2008;14(7):2028-2035.
218. Melief SM, Visconti VV, Visser M, et al. Long-term Survival and Clinical Benefit from Adoptive T-cell Transfer in Stage IV Melanoma Patients Is Determined by a Four-Parameter Tumor Immune Signature. *Cancer Immunol Res.* 2017;5(2):170-179.
219. Lassalle S, Nahon-Esteve S, Frouin E, et al. PD-L1 Expression in 65 Conjunctival Melanomas and Its Association with Clinical Outcome. *Int J Mol Sci.* 2020;21(23).
220. Clark WH, Jr., Elder DE, Guerry Dt, et al. Model predicting survival in stage I melanoma based on tumor progression. *J Natl Cancer Inst.* 1989;81(24):1893-1904.
221. Mihm MC, Jr., Mule JJ. Reflections on the Histopathology of Tumor-Infiltrating Lymphocytes in Melanoma and the Host Immune Response. *Cancer Immunol Res.* 2015;3(8):827-835.
222. Azimi F, Scolyer RA, Rumcheva P, et al. Tumor-infiltrating lymphocyte grade is an independent predictor of sentinel lymph node status and survival in patients with cutaneous melanoma. *J Clin Oncol.* 2012;30(21):2678-2683.
223. Taylor RC, Patel A, Panageas KS, Busam KJ, Brady MS. Tumor-infiltrating lymphocytes predict sentinel lymph node positivity in patients with cutaneous melanoma. *J Clin Oncol.* 2007;25(7):869-875.
224. Gerber AL, Munst A, Schlapbach C, et al. High expression of FOXP3 in primary melanoma is associated with tumour progression. *Br J Dermatol.* 2014;170(1):103-109.
225. Davidorf FH, Lang JR. Lymphocytic infiltration in choroidal melanoma and its prognostic significance. *Trans Ophthalmol Soc U K.* 1977;97(3):394-401.
226. de la Cruz PO, Jr., Specht CS, McLean IW. Lymphocytic infiltration in uveal malignant melanoma. *Cancer.* 1990;65(1):112-115.
227. Bronkhorst IH, Vu TH, Jordanova ES, et al. Different subsets of tumor-infiltrating lymphocytes correlate with macrophage influx and monosomy 3 in uveal melanoma. *Invest Ophthalmol Vis Sci.* 2012;53(9):5370-5378.
228. Makitie T, Summanen P, Tarkkanen A, Kivela T. Tumor-infiltrating macrophages (CD68+) cells and prognosis in malignant uveal melanoma. *Invest Ophthalmol Vis Sci.* 2001;42(7):1414-1421.
229. Maat W, Ly LV, Jordanova ES, et al. Monosomy of chromosome 3 and an inflammatory phenotype occur together in uveal melanoma. *Invest Ophthalmol Vis Sci.* 2008;49(2):505-510.
230. Mougiakakos D, Johansson CC, Trocme E, et al. Intratumoral forkhead box P3-positive regulatory T cells predict poor survival in cyclooxygenase-2-positive uveal melanoma. *Cancer.* 2010;116(9):2224-2233.
231. Robertson AG, Shih J, Yau C, et al. Integrative Analysis Identifies Four Molecular and Clinical Subsets in Uveal Melanoma. *Cancer Cell.* 2017;32(2):204-220 e215.
232. Brignole F, De Saint-Jean M, Goldschild M, et al. Expression of Fas-Fas ligand antigens and apoptotic marker APO2.7 by the human conjunctival epithelium. Positive correlation with class II HLA DR expression in inflammatory ocular surface disorders. *Exp Eye Res.* 1998;67(6):687-697.
233. Song H, Wu Y, Ren G, Guo W, Wang L. Prognostic factors of oral mucosal melanoma: histopathological analysis in a retrospective cohort of 82 cases. *Histopathology.* 2015;67(4):548-556.
234. Natarajan K, Li H, Mariuzza RA, Margulies DH. MHC class I molecules, structure and function. *Rev Immunogenet.* 1999;1(1):32-46.
235. Mosaad YM. Clinical Role of Human Leukocyte Antigen in Health and Disease. *Scand J Immunol.* 2015;82(4):283-306.
236. Howell WM. HLA and disease: guilt by association. *Int J Immunogenet.* 2014;41(1):1-12.
237. Garrido F, Cabrera T, Aptsiauri N. "Hard" and "soft" lesions underlying the HLA class I alterations in cancer cells: implications for immunotherapy. *Int J Cancer.* 2010;127(2):249-256.

238. Kageshita T, Hirai S, Ono T, Hicklin DJ, Ferrone S. Down-regulation of HLA class I antigen-processing molecules in malignant melanoma: association with disease progression. *Am J Pathol.* 1999;154(3):745-754.
239. Cao J, Brouwer NJ, Jordanova ES, et al. HLA Class I Antigen Expression in Conjunctival Melanoma Is Not Associated With PD-L1/PD-1 Status. *Invest Ophthalmol Vis Sci.* 2018;59(2):1005-1015.
240. Campoli M, Ferrone S. HLA antigen changes in malignant cells: epigenetic mechanisms and biologic significance. *Oncogene.* 2008;27(45):5869-5885.
241. de Waard-Siebinga I, Hilders CG, Hansen BE, van Delft JL, Jager MJ. HLA expression and tumor-infiltrating immune cells in uveal melanoma. *Graefes Arch Clin Exp Ophthalmol.* 1996;234(1):34-42.
242. Garrido F, Perea F, Bernal M, et al. The Escape of Cancer from T Cell-Mediated Immune Surveillance: HLA Class I Loss and Tumor Tissue Architecture. *Vaccines (Basel).* 2017;5(1).
243. Bocker EB, Suter L, Bruggen J, et al. Phenotypic dynamics of tumor progression in human malignant melanoma. *Int J Cancer.* 1985;36(1):29-35.
244. D'Alessandro G, Zardawi I, Grace J, McCarthy WH, Hersey P. Immunohistological evaluation of MHC class I and II antigen expression on nevi and melanoma: relation to biology of melanoma. *Pathology.* 1987;19(4):339-346.
245. Ferrone S, Marincola FM. Loss of HLA class I antigens by melanoma cells: molecular mechanisms, functional significance and clinical relevance. *Immunol Today.* 1995;16(10):487-494.
246. Carretero R, Romero JM, Ruiz-Cabello F, et al. Analysis of HLA class I expression in progressing and regressing metastatic melanoma lesions after immunotherapy. *Immunogenetics.* 2008;60(8):439-447.
247. Garrido F, Aptsiauri N. Cancer immune escape: MHC expression in primary tumours versus metastases. *Immunology.* 2019;158(4):255-266.
248. Chowell D, Morris LGT, Grigg CM, et al. Patient HLA class I genotype influences cancer response to checkpoint blockade immunotherapy. *Science.* 2018;359(6375):582-587.
249. van Essen TH, van Pelt SI, Bronkhorst IH, et al. Upregulation of HLA Expression in Primary Uveal Melanoma by Infiltrating Leukocytes. *PLoS One.* 2016;11(10):e0164292.
250. Blom DJ, Luyten GP, Mooy C, et al. Human leukocyte antigen class I expression. Marker of poor prognosis in uveal melanoma. *Invest Ophthalmol Vis Sci.* 1997;38(9):1865-1872.
251. Ericsson C, Seregard S, Bartolazzi A, et al. Association of HLA class I and class II antigen expression and mortality in uveal melanoma. *Invest Ophthalmol Vis Sci.* 2001;42(10):2153-2156.
252. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int Immunol.* 2007;19(7):813-824.
253. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. *N Engl J Med.* 2015;373(1):23-34.
254. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nat Immunol.* 2002;3(7):611-618.
255. Daud AI, Wolchok JD, Robert C, et al. Programmed Death-Ligand 1 Expression and Response to the Anti-Programmed Death 1 Antibody Pembrolizumab in Melanoma. *J Clin Oncol.* 2016;34(34):4102-4109.
256. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol.* 2018;19(11):1480-1492.
257. Qin Y, Petaccia de Macedo M, Reuben A, et al. Parallel profiling of immune infiltrate subsets in uveal melanoma versus cutaneous melanoma unveils similarities and differences: A pilot study. *Oncoimmunology.* 2017;6(6):e1321187.
258. Javed A, Arguello D, Johnston C, et al. PD-L1 expression in tumor metastasis is different between uveal melanoma and cutaneous melanoma. *Immunotherapy.* 2017;9(16):1323-1330.

259. Kleinovink JW, Marijt KA, Schoonderwoerd MJA, et al. PD-L1 expression on malignant cells is no prerequisite for checkpoint therapy. *Oncoimmunology*. 2017;6(4):e1294299.
260. Patel SP, Kurzrock R. PD-L1 Expression as a Predictive Biomarker in Cancer Immunotherapy. *Mol Cancer Ther*. 2015;14(4):847-856.
261. Mahoney KM, Freeman GJ, McDermott DF. The Next Immune-Checkpoint Inhibitors: PD-1/PD-L1 Blockade in Melanoma. *Clin Ther*. 2015;37(4):764-782.
262. Thierauf J, Veit JA, Affolter A, et al. Identification and clinical relevance of PD-L1 expression in primary mucosal malignant melanoma of the head and neck. *Melanoma Res*. 2015;25(6):503-509.
263. Taube JM, Anders RA, Young GD, et al. Colocalization of inflammatory response with B7-h1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci Transl Med*. 2012;4(127):127ra137.
264. McLaughlin J, Han G, Schalper KA, et al. Quantitative Assessment of the Heterogeneity of PD-L1 Expression in Non-Small-Cell Lung Cancer. *JAMA Oncol*. 2016;2(1):46-54.
265. Sato H, Okonogi N, Nakano T. Rationale of combination of anti-PD-1/PD-L1 antibody therapy and radiotherapy for cancer treatment. *Int J Clin Oncol*. 2020.
266. Kleinovink JW, Fransen MF, Löwik CW, Ossendorp F. Photodynamic-Immune Checkpoint Therapy Eradicates Local and Distant Tumors by CD8⁺ T Cells. *Cancer Immunology Research*. 2017;5(10):832-838.
267. Shields JA, Shields CL, De Potter P. Surgical management of conjunctival tumors. The 1994 Lynn B. McMahan Lecture. *Arch Ophthalmol*. 1997;115(6):808-815.
268. Finger PT, Czechowska G, Liarikos S. Topical mitomycin C chemotherapy for conjunctival melanoma and PAM with atypia. *Br J Ophthalmol*. 1998;82(5):476-479.
269. Herold TR, Hintschich C. Interferon alpha for the treatment of melanocytic conjunctival lesions. *Graefes Arch Clin Exp Ophthalmol*. 2010;248(1):111-115.
270. Brouwer NJ, Marinkovic M, Peters FP, et al. Management of conjunctival melanoma with local excision and adjuvant brachytherapy. *Eye (Lond)*. 2021;35(2):490-498.
271. Westekemper H, Anastassiou G, Sauerwein W, et al. [Analysis of ocular surface alterations following proton beam radiation in eyes with conjunctival malignant melanoma]. *Ophthalmologie*. 2006;103(7):588-595.
272. Shields JA, Shields CL, Freire JE, Brady LW, Komarnicky L. Plaque radiotherapy for selected orbital malignancies: preliminary observations: the 2002 Montgomery Lecture, part 2. *Ophthalm Plast Reconstr Surg*. 2003;19(2):91-95.
273. Wuestemeyer H, Sauerwein W, Meller D, et al. Proton radiotherapy as an alternative to exenteration in the management of extended conjunctival melanoma. *Graefes Arch Clin Exp Ophthalmol*. 2006;244(4):438-446.
274. Glossmann JP, Skoetz N, Starbatty B, et al. [Conjunctival melanoma : Standard operating procedures in diagnosis, treatment and follow-up care]. *Ophthalmologie*. 2018;115(6):489-498.
275. Cohen VML, O'Day RF. Management Issues in Conjunctival Tumours: Conjunctival Melanoma and Primary Acquired Melanosis. *Ophthalmol Ther*. 2019;8(4):501-510.
276. Lim LA, Madigan MC, Conway RM. Conjunctival melanoma: a review of conceptual and treatment advances. *Clin Ophthalmol*. 2013;6:521-531.
277. Esmaeli B, Wang X, Youssef A, Gershenwald JE. Patterns of regional and distant metastasis in patients with conjunctival melanoma: experience at a cancer center over four decades. *Ophthalmology*. 2001;108(11):2101-2105.
278. Pfeiffer ML, Ozgur OK, Myers JN, et al. Sentinel lymph node biopsy for ocular adnexal melanoma. *Acta Ophthalmol*. 2017;95(4):e323-e328.
279. Mor JM, Rokohl AC, Koch KR, Heindl LM. Sentinel lymph node biopsy in the management of conjunctival melanoma: current insights. *Clin Ophthalmol*. 2019;13:1297-1302.

280. Grimes JM, Shah NV, Samie FH, Carvajal RD, Marr BP. Conjunctival Melanoma: Current Treatments and Future Options. *Am J Clin Dermatol*. 2020;21(3):371-381.
281. Garbe C, Amaral T, Peris K, et al. European consensus-based interdisciplinary guideline for melanoma. Part 2: Treatment - Update 2019. *Eur J Cancer*. 2020;126:159-177.
282. FDA. TAFINLAR (dabrafenib). 2013. Accessed August 18th, 2020.
283. FDA. ZELBORAF (vemurafenib). 2011. Accessed August 18th, 2020.
284. FDA. BRAFTOVI (encorafenib). 2018. Accessed August 18th, 2020.
285. FDA. MEKINIST (trametinib). 2013. Accessed August 18th, 2020.
286. FDA. COTELLIC (cobimetinib). 2020. Accessed August 18th, 2020.
287. FDA. MEKTOVI (binimetinib). 2018. Accessed August 18th, 2020.
288. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature*. 2010;468(7326):973-977.
289. Whittaker SR, Theurillat J-P, Van Allen E, et al. A Genome-Scale RNA Interference Screen Implicates NF1 Loss in Resistance to RAF Inhibition. *Cancer Discovery*. 2013;3(3):350-362.
290. Wagle N, Van Allen EM, Treacy DJ, et al. MAP kinase pathway alterations in BRAF-mutant melanoma patients with acquired resistance to combined RAF/MEK inhibition. *Cancer Discov*. 2014;4(1):61-68.
291. Catalanotti F, Cheng DT, Shoushtari AN, et al. PTEN Loss-of-Function Alterations Are Associated With Intrinsic Resistance to BRAF Inhibitors in Metastatic Melanoma. *JCO Precis Oncol*. 2017;1.
292. Lin L, Sabnis AJ, Chan E, et al. The Hippo effector YAP promotes resistance to RAF- and MEK-targeted cancer therapies. *Nat Genet*. 2015;47(3):250-256.
293. Kim MH, Kim J, Hong H, et al. Actin remodeling confers BRAF inhibitor resistance to melanoma cells through YAP/TAZ activation. *Embo j*. 2016;35(5):462-478.
294. Kim MH, Kim CG, Kim SK, et al. YAP-Induced PD-L1 Expression Drives Immune Evasion in BRAFi-Resistant Melanoma. *Cancer Immunol Res*. 2018;6(3):255-266.
295. Larkin J, Ascierto PA, Dréno B, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med*. 2014;371(20):1867-1876.
296. Long GV, Stroyakovskiy D, Gogas H, et al. Combined BRAF and MEK inhibition versus BRAF inhibition alone in melanoma. *N Engl J Med*. 2014;371(20):1877-1888.
297. Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med*. 2015;372(1):30-39.
298. Pahlitzsch M, Bertelmann E, Mai C. Conjunctival Melanoma and BRAF Inhibitor Therapy. *J Clin Exp Ophthalmol*. 2014;5(1).
299. Weber JL, Smalley KS, Sondak VK, Gibney GT. Conjunctival melanomas harbor BRAF and NRAS mutations--Letter. *Clin Cancer Res*. 2013;19(22):6329-6330.
300. Griewank KG, Westekemper H, Schilling B, et al. Conjunctival melanomas harbor BRAF and NRAS mutations--response. *Clin Cancer Res*. 2013;19(22):6331-6332.
301. Maleka A, Astrom G, Bystrom P, Ullenhag GJ. A case report of a patient with metastatic ocular melanoma who experienced a response to treatment with the BRAF inhibitor vemurafenib. *BMC Cancer*. 2016;16:634.
302. Pinto Torres S, Andre T, Gouveia E, Costa L, Passos MJ. Systemic Treatment of Metastatic Conjunctival Melanoma. *Case Rep Oncol Med*. 2017;2017:4623964.
303. Rossi E, Maiorano BA, Pagliara MM, et al. Dabrafenib and Trametinib in BRAF Mutant Metastatic Conjunctival Melanoma. *Front Oncol*. 2019;9:232.
304. Dagi Glass LR, Lawrence DP, Jakobiec FA, Freitag SK. Conjunctival Melanoma Responsive to Combined Systemic BRAF/MEK Inhibitors. *Ophthalmic Plast Reconstr Surg*. 2017;33(5):e114-e116.

305. Welsh SJ, Corrie PG. Management of BRAF and MEK inhibitor toxicities in patients with metastatic melanoma. *Ther Adv Med Oncol.* 2015;7(2):122-136.
306. Nareyck G, Wuestemeyer H, von der Haar D, Anastassiou G. Establishment of two cell lines derived from conjunctival melanomas. *Exp Eye Res.* 2005;81(3):361-362.
307. Keijser S, Maat W, Missotten GS, de Keizer RJ. A new cell line from a recurrent conjunctival melanoma. *Br J Ophthalmol.* 2007;91(11):1566-1567.
308. Joensuu H, DeMatteo RP. The management of gastrointestinal stromal tumors: a model for targeted and multidisciplinary therapy of malignancy. *Annu Rev Med.* 2012;63:247-258.
309. Carvajal RD, Antonescu CR, Wolchok JD, et al. KIT as a therapeutic target in metastatic melanoma. *Jama.* 2011;305(22):2327-2334.
310. Guo J, Si L, Kong Y, et al. Phase II, open-label, single-arm trial of imatinib mesylate in patients with metastatic melanoma harboring c-Kit mutation or amplification. *J Clin Oncol.* 2011;29(21):2904-2909.
311. Hodi FS, Corless CL, Giobbie-Hurder A, et al. Imatinib for melanomas harboring mutationally activated or amplified KIT arising on mucosal, acral, and chronically sun-damaged skin. *J Clin Oncol.* 2013;31(26):3182-3190.
312. Germann UA, Furey BF, Markland W, et al. Targeting the MAPK Signaling Pathway in Cancer: Promising Preclinical Activity with the Novel Selective ERK1/2 Inhibitor BVD-523 (Ulixertinib). *Mol Cancer Ther.* 2017;16(11):2351-2363.
313. Sullivan RJ, Infante JR, Janku F, et al. First-in-Class ERK1/2 Inhibitor Ulixertinib (BVD-523) in Patients with MAPK Mutant Advanced Solid Tumors: Results of a Phase I Dose-Escalation and Expansion Study. *Cancer Discov.* 2018;8(2):184-195.
314. Posch C, Moslehi H, Feeney L, et al. Combined targeting of MEK and PI3K/mTOR effector pathways is necessary to effectively inhibit NRAS mutant melanoma in vitro and in vivo. *Proceedings of the National Academy of Sciences.* 2013;201216013.
315. Algazi AP, Esteve-Puig R, Nosrati A, et al. Dual MEK/AKT inhibition with trametinib and GSK2141795 does not yield clinical benefit in metastatic NRAS-mutant and wild-type melanoma. *Pigment Cell & Melanoma Research.* 2018;31(1):110-114.
316. Humer J, Ferko B, Waltenberger A, et al. Azidothymidine inhibits melanoma cell growth in vitro and in vivo. *Melanoma Research.* 2008;18(5):314-321.
317. Bachmann IM, Halvorsen OJ, Collett K, et al. EZH2 expression is associated with high proliferation rate and aggressive tumor subgroups in cutaneous melanoma and cancers of the endometrium, prostate, and breast. *J Clin Oncol.* 2006;24(2):268-273.
318. Zingg D, Debbache J, Schaefer SM, et al. The epigenetic modifier EZH2 controls melanoma growth and metastasis through silencing of distinct tumour suppressors. *Nature Communications.* 2015;6(1):6051.
319. Cao J, Pontes KC, Heijkants RC, et al. Overexpression of EZH2 in conjunctival melanoma offers a new therapeutic target. *J Pathol.* 2018;245(4):433-444.
320. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med.* 2011;364(26):2517-2526.
321. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol.* 2015;16(4):375-384.
322. Finger PT, Pavlick AC. Checkpoint inhibition immunotherapy for advanced local and systemic conjunctival melanoma: a clinical case series. *J Immunother Cancer.* 2019;7(1):83.
323. Kini A, Fu R, Compton C, Miller DM, Ramasubramanian A. Pembrolizumab for Recurrent Conjunctival Melanoma. *JAMA Ophthalmol.* 2017;135(8):891-892.

324. Ford J, Thuro BA, Thakar S, et al. Immune Checkpoint Inhibitors for Treatment of Metastatic Melanoma of the Orbit and Ocular Adnexa. *Ophthalmic Plast Reconstr Surg*. 2017;33(4):e82-e85.
325. FDA. YERVOY (ipilimumab). 2020. Accessed April 8th, 2020.
326. FDA. KEYTRUDA (pembrolizumab). 2020. Accessed April 28th, 2020.
327. FDA. OPDIVO (nivolumab). 2020. Accessed April 28th, 2020.
328. Mocellin S, Lens MB, Pasquali S, Pilati P, Chiarion Sileni V. Interferon alpha for the adjuvant treatment of cutaneous melanoma. *Cochrane Database Syst Rev*. 2013(6):Cd008955.
329. Vann RR, Karp CL. Perilesional and topical interferon alfa-2b for conjunctival and corneal neoplasia. *Ophthalmology*. 1999;106(1):91-97.
330. Karp CL, Galor A, Chhabra S, Barnes SD, Alfonso EC. Subconjunctival/perilesional recombinant interferon alpha2b for ocular surface squamous neoplasia: a 10-year review. *Ophthalmology*. 2010;117(12):2241-2246.
331. Finger PT, Sedek RW, Chin KJ. Topical interferon alfa in the treatment of conjunctival melanoma and primary acquired melanosis complex. *Am J Ophthalmol*. 2008;145(1):124-129.
332. Benage MJ, Morrow NC, Janson BJ, Greiner MA. Evaluation of interferon alpha 2b as adjunctive therapy for conjunctival melanoma. *Am J Ophthalmol Case Rep*. 2019;15:100467.
333. Rafique I, Kirkwood JM, Tarhini AA. Immune checkpoint blockade and interferon-alpha in melanoma. *Semin Oncol*. 2015;42(3):436-447.
334. Baron S, Tyring SK, Fleischmann WR, Jr., et al. The interferons. Mechanisms of action and clinical applications. *Jama*. 1991;266(10):1375-1383.
335. Curtsinger JM, Valenzuela JO, Agarwal P, Lins D, Mescher MF. Type I IFNs provide a third signal to CD8 T cells to stimulate clonal expansion and differentiation. *J Immunol*. 2005;174(8):4465-4469.
336. Davar D, Wang H, Chauvin JM, et al. Phase Ib/II Study of Pembrolizumab and Pegylated-Interferon Alfa-2b in Advanced Melanoma. *J Clin Oncol*. 2018;Jco1800632.
337. Kiyohara T, Tanimura H, Miyamoto M, et al. Two cases of BRAF-mutated, bulbar conjunctival melanoma, and review of the published literature. *Clin Exp Dermatol*. 2020;45(2):207-211.
338. Chaves LJ, Huth B, Augsburger JJ, Correa ZM. Eye-Sparing Treatment for Diffuse Invasive Conjunctival Melanoma. *Ocul Oncol Pathol*. 2018;4(4):261-266.
339. Chang M, Lally SE, Dalvin LA, Orloff MM, Shields CL. Conjunctival melanoma with orbital invasion and liver metastasis managed with systemic immune checkpoint inhibitor therapy. *Indian J Ophthalmol*. 2019;67(12):2071-2073.
340. Weber JS, Hamid O, Chasalow SD, et al. Ipilimumab increases activated T cells and enhances humoral immunity in patients with advanced melanoma. *J Immunother*. 2012;35(1):89-97.
341. Weber JS, Postow M, Lao CD, Schadendorf D. Management of Adverse Events Following Treatment With Anti-Programmed Death-1 Agents. *Oncologist*. 2016;21(10):1230-1240.
342. Byrne EH, Fisher DE. Immune and molecular correlates in melanoma treated with immune checkpoint blockade. *Cancer*. 2017;123(S11):2143-2153.
343. Sophie R, Moses GM, Hwang ES, Kim JE. Fundus Hypopigmentation and Loss of Choroidal Nevi Pigmentation Associated With Nivolumab. *JAMA Ophthalmol*. 2019;137(7):851-853.
344. Tarhini A. Immune-mediated adverse events associated with ipilimumab ctla-4 blockade therapy: the underlying mechanisms and clinical management. *Scientifica (Cairo)*. 2013;2013:857519.
345. Zimmer L, Goldinger SM, Hofmann L, et al. Neurological, respiratory, musculoskeletal, cardiac and ocular side-effects of anti-PD-1 therapy. *Eur J Cancer*. 2016;60:210-225.
346. Papavasileiou E, Prasad S, Freitag SK, Sobrin L, Lobo AM. Ipilimumab-induced Ocular and Orbital Inflammation--A Case Series and Review of the Literature. *Ocul Immunol Inflamm*. 2016;24(2):140-146.

347. Dalvin LA, Shields CL, Orloff M, Sato T, Shields JA. CHECKPOINT INHIBITOR IMMUNE THERAPY: Systemic Indications and Ophthalmic Side Effects. *Retina*. 2018;38(6):1063-1078.
348. Wong RK, Lee JK, Huang JJ. Bilateral drug (ipilimumab)-induced vitritis, choroiditis, and serous retinal detachments suggestive of vogt-koyanagi-harada syndrome. *Retin Cases Brief Rep*. 2012;6(4):423-426.
349. Brouwer NJ, Haanen J, Jager MJ. Development of Ocular Rosacea following Combined Ipilimumab and Nivolumab Treatment for Metastatic Malignant Skin Melanoma. *Ocul Oncol Pathol*. 2017;3(3):188-192.
350. Fierz FC, Meier F, Chaloupka K, Boni C. Intraocular Inflammation Associated with New Therapies for Cutaneous Melanoma - Case Series and Review. *Klin Monbl Augenheilkd*. 2016;233(4):540-544.
351. Kellermann N, Maier M, Feucht N. [Vitreous metastasis of malignant cutaneous melanoma during treatment with ipilimumab]. *Ophthalmologe*. 2017;114(2):163-166.
352. Francis JH, Berry D, Abramson DH, et al. Intravitreal Cutaneous Metastatic Melanoma in the Era of Checkpoint Inhibition: Unmasking and Masquerading. *Ophthalmology*. 2020;127(2):240-248.
353. Brouwer NJ, Konstantinou EK, Gragoudas ES, et al. Targeting the YAP/TAZ Pathway in Uveal and Conjunctival Melanoma With Verteporfin. *Invest Ophthalmol Vis Sci*. 2021;62(4):3.