Major review

Advances in the management of conjunctival melanoma

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ABSTRACT

Malignant melanoma of the conjunctiva is a rare but serious condition. Over the last several years, there have been important advances in the classification, diagnosis, and treatment of this condition. Recent cytogenetic and immunohistochemical studies are increasing understanding of its tumorigenesis. Diagnosis, although still made via histopathology, has been aided with imaging techniques such as ultrasound biomicroscopy and anterior segment optical coherence tomography. Primary treatment consists of surgical excision. But adjuvant treatments with cryotherapy, topical chemotherapy, and radiation therapy have shown increased success. Sentinel lymph node biopsy has shown early promise of detecting micro-metastasis. Long term follow-up of patients with conjunctival melanoma with systemic surveillance is necessary to detect recurrences and metastases.

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1. Case presentation

An 86-year-old man presented with a pigmented conjunctival lesion on the left eye that he reported had grown in size over the last 4 years. On examination, a pigmented, elevated lesion was present at the superior limbus and associated with a feeder vessel (Fig. 1). On gonioscopy, the angle was opened, and there was no intraocular extension. He underwent excisional biopsy with alcohol epithelial keratectomy and double freeze-thaw cryotherapy to the conjunctival margins. Histopathologically, the lesion was consistent with malignant melanoma focally arising from primary acquired melanosis with severe atypia (American Joint Committee on Cancer [AJCC] stage T1b), with tumor-free margins. A systemic survey with liver function tests and computed tomography (CT) body scan was negative for metastatic disease. No adjuvant therapy was administered, and after 16-month follow-up, he has no signs of recurrence or metastasis.

2. Epidemiology

 Conjunctival melanoma comprises 2% of all ocular malignancies. The annual incidence of conjunctival melanoma in...
 Conjunctival melanomas may arise de novo (12%–47%), from preexisting nevi (1%–26%), or from primary acquired melanosis (10%–74%). De novo melanomas carry a higher risk of metastasis and death. When arising from primary acquired melanosis (PAM), the first sign is thickening. Although PAM may be benign in nature, Gloor and Alexandrakis recommended excision of lesions that are widespread, thickened and dark in appearance, palpebral in location, involving the cornea, associated with unusual vascularity, and progressive in nature. Histopathologically, PAM with mild atypia has less likelihood of transformation to melanoma than PAM with severe atypia. Shields and colleagues reported no risk of progression to melanoma for PAM without atypia or with mild atypia, compared to 13% for PAM with severe atypia. Nevi that are present in childhood and increase in size in an adult should raise suspicion for malignant transformation.

5. Staging

In 2009, the AJCC revealed the seventh edition of its universal staging classification aimed to stratify mortality risk from conjunctival melanoma. The AJCC divides melanomas clinically into tumors of the bulbar conjunctiva (T1), the nonbulbar (palpebral, fornical, and caruncular) conjunctiva (T2), or either/or both with local invasion of globe, orbit, eyelid, or sinus (T3) or central nervous system (T4). Each of these stages is subdivided depending on size (see Table 1). The AJCC does use invasion into the substantia propria in its classification scheme. N1 identifies regional lymph node metastasis and M1, distant metastasis. One major change from previous staging was to define melanoma confined to the conjunctival epithelium, formerly known as PAM with severe atypia, as melanoma in situ (Tis).

Pathologic staging is based on vertical thickness and depth of invasion. The thickness is classified as follows: ≤0.5 mm, 0.5 mm–1.5 mm, and >1.5 mm. The Breslow scale of tumor depth, previously used to characterize cutaneous melanomas, is not routinely used to classify conjunctival melanomas. As discussed later, tumor depth does implicate greater risk for metastasis and mortality, but the Breslow stages have not yet been translated to the conjunctival melanomas.

Shields and colleagues used the AJCC seventh edition staging system to review 343 patients diagnosed with conjunctival melanoma. They reported stage T2 and T3 conjunctival melanomas had higher rates of recurrence, lymph node metastasis, distant metastasis, and mortality than stage T1 melanomas. Another smaller retrospective review of 42 patients also concurred with the predictive value of the AJCC seventh edition staging system for local recurrence and lymphatic and distant metastases. They mentioned that this staging system does not predict the higher risk of recurrence in multifocal tumors.

Renaming PAM into different histopathologic categories of "conjunctival melanocytic intraepithelial neoplasia (C-MIN)" has also been proposed as a more objective and reproducible labeling scheme. In addition to a clinical diagnosis of PAM (with or without atypia), the C-MIN histopathologic diagnosis delineates the lesion on the neoplastic spectrum and grades the gravity of the diagnosis. C-MIN 0 corresponds to
hyperpigmentation with melanocytic proliferation, and C-MIN 1–10 requires increasing amounts of horizontal and vertical spread of melanocytic proliferation and increasingly atypical cytological features. C-MIN 6 or greater corresponds to conjunctival melanoma in situ. This new terminology for PAM echoes Jakobiec’s initial concern about describing a lesion casually as a proliferative process, rather than premalignant. Jakobiec proposed new histopathologic terminology for PAM, given that the term “melanosis” is vague and does not distinguish between melanin overproduction and melanocytic proliferation. He recommended subdividing PAM without atypia into “intraepithelial nonproliferative melanocytic pigmentation” and “intraepithelial melanocytic proliferation without atypia.” In addition, he also recommended PAM with atypia to be renamed “intraepithelial melanocytic proliferation with atypia.” Folberg furthers these proposed changes by reiterating that PAM is useful clinically, but not descriptive enough histopathologically.

### 6. Biologic data

Recent studies have shown that conjunctival melanomas share molecular commonalities with cutaneous melanoma and seem to be distinct from uveal melanomas. These biologic data are important not only for understanding the pathophysiology of the disease process, but also have implications for therapy and enrollment of patients in clinical trials of new treatments.

The v-raf murine sarcoma viral oncogene homolog B1 (BRAF) is an oncogenic mutation in one of the RAF genes and a known contributor to the formation of cutaneous melanoma. BRAF has also been detected in conjunctival melanomas, implying a separate oncogenic pathway. Griewank and colleagues conducted a large genetic analysis of 78 conjunctival melanoma samples using oncogene hotspot...
array to screen for known cancer-relevant mutations and found BRAF (of which 91% were V600E) mutations in 29% of the tumors and NRAS in 18% of the tumors, similar to cutaneous melanomas. GNAQ and GNA11 mutations, often found in uveal melanomas, were not detected in the conjunctival melanomas. In addition, no known activating KIT mutations were found. Lake and colleagues also detected the BRAF V600E mutation in 50% of primary tumor samples and 67% in the metastatic samples.

Copy number alterations have also been studied in conjunctival melanomas. Griewank and colleagues analyzed 30 conjunctival melanomas using array-based comparative genomic hybridization and found that the pattern was similar to cutaneous and mucosal melanomas (with gains of 1q, 3p, 7, 17q and losses of 9p, 10, 11, and 12q), but different from uveal melanomas (which often have losses of 1p, 3, and 6q, and gains of 6p and 8q). Vajdic and colleagues used comparative genomic hybridization in 2 conjunctival melanoma samples and found multiple chromosomal changes, most notably 10q and 16q loss that is also found in cutaneous melanomas.

Using multiplex ligation-dependent probe amplification assays, Lake and colleagues reported frequent copy number amplifications of CDKN1A and RUNX2 in primary tumors (6p21.2), both of which have been implicated in cutaneous melanoma tumorigenesis.

Fluorescence in situ hybridization studies have also revealed similarities in chromosomal aberrations between conjunctival and cutaneous melanomas. Busam and colleagues reported gains in RREB1 (6p25) in all 6 tumors studied and cyclin D1 (11q13) in 4 of the 6 tumors, both of which are commonly found in cutaneous melanoma. McNamara and colleagues reported that the aberrations seen with fluorescence in situ hybridization studies are different from those of uveal melanomas (Specifically, monosomy 3 has been found in uveal melanomas, whereas multiplication rather than deletions occur in conjunctival melanoma), indicating different genetic mechanisms for the 2 ocular tumors.

Recent immunohistochemical studies have helped to further characterize conjunctival melanomas. Zoroquiain and colleagues found that p16 expression is similar in conjunctival and cutaneous melanoma and differs from other conjunctival melanocytic lesions. The p16 protein encoded by the CDKN2A gene is involved in cell cycle regulation. Mutation of the CDKN2A gene, resulting in decreased p16 protein production, leads to increased mitotic activity and replication and hence melanoma genesis. In other immunolabeling studies, S100 levels, which are calcium-binding proteins, have been found to differ between conjunctival and uveal melanomas.

Finally, through similar immunohistochemical techniques, Westekemper and colleagues reported that heat shock protein 90, an antiapoptotic antigen and target in new cancer therapies, is overexpressed in conjunctival melanomas when compared to benign nevi, similar to cutaneous melanomas.

Four cyogenetic and immunohistochemical studies not only are important for understanding tumorigenesis, but also can help guide therapy. Treatments such as BRAF inhibitor therapy could be used, especially in metastatic disease. It has been suggested that PLX4023 (vemurafenib), a BRAF inhibitor specific for the V600E mutation, could be used for treatment of metastases. Briceno and colleagues showed that VEGFC, VEGFD, and VEGR3 are diffusely expressed by conjunctival melanoma cells, most intensely at the invasive tumor edge. Similarly, CXCL12, CXC4, CCL21, and CCR7 were also most intensely expressed at the invasive edge, where there is the highest density of lymphatic vessels. These expression patterns suggest that these mediators of tumor-associated lymphangiogenesis warrant further investigation as potential therapeutic targets in conjunctival melanoma.

7. Clinical workup

7.1. Ophthalmic examination

Clinical examination should include a detailed history with risk factors such as history of sun exposure, previous cancers, family history of cancer, systemic medications, and review of old photographs. Examination should not only be limited to the eyes but also include preauricular, submandibular, and cervical lymph node palpation. Evaluation should involve slit-lamp examination of the entire anterior ocular surface, including double eversion of eyelids to visualize the bulbar and tarsal conjunctiva, as well as a dilated fundus examination. Vital dyes such as rose bengal and lissamine green that may be
helpful in recognizing ocular surface squamous neoplasia are not helpful in diagnosing conjunctival melanoma.

7.2. Imaging

Although the standard of care in the diagnosis of conjunctival lesions is clinical examination and excisional biopsy if there is concern for malignancy, noninvasive imaging technology may revolutionize the landscape. Although imaging is unlikely to replace excisional biopsy for definitive diagnosis, it can guide whether to pursue further invasive testing. There is currently no evidence that these imaging modalities improve clinical outcome; however, this is being studied.

7.2.1. Slit-lamp photography and clinical drawing

A detailed clinical drawing or slit-lamp photography is important for documenting the location and size of the lesion. Photography should be performed at every visit. This helps to monitor the progression or regression of the lesion.

Clinical drawings are useful for surgical planning because photographs may not always demonstrate subtle pigmented areas. The coaxial illumination of the surgical microscope may also bleach out pigmentation, and thus a drawing can help guide excision of the entire lesion. Drawings should indicate examination of the tarsal and bulbar conjunctival surfaces, medial canthal regions, and the fornix. Concurrent photographs may not always demonstrate subtle pigmented features such as episclera and sclera.

7.2.2. In vivo confocal microscopy

In vivo confocal microscopy using near infrared laser light is used to image conjunctival melanocytic lesions. Messmer and colleagues demonstrated that in vivo confocal microscopy discerned PAM with or without atypia and melanoma with high sensitivity and specificity when compared with histopathologic specimens of conjunctival melanocytic lesions. In in vivo confocal microscopy, hyperreflective granules were confined to the basal epithelium in most PAM without atypia, whereas PAM lesions with atypia demonstrated these granules and patches throughout the epithelium. PAM with atypia had large networks of hyperreflective dendritic cells. In addition, melanomas exhibited hyperreflective large cells with hyperreflective nuclei with hyporeflective nucleoli.

7.2.3. Anterior segment optical coherence tomography

Anterior segment optical coherence tomography can be useful in certain conjunctival melanomas (Fig. 2). Nanji and colleagues recently described the use of commercially available high resolution, spectral-domain optical coherence tomography (RTVue, Optovue, Fremont, CA, USA). They reported difficulty in differentiating benign pigmented lesions from melanomas. Both nevi and melanomas had hyperreflective basal epithelial layers, whereas nevi had cysts and melanomas had increased shadowing of the sublesional tissue. Similarly, Shousha and colleagues used custom-made ultrahigh resolution anterior segment optical coherence tomography in various ocular surface lesions, including 5 amelanotic melanomas. Although this imaging modality did not distinguish PAM with and without atypia, the images showed hyperreflectivity in the basal epithelium without invasion of subepithelial layers, which corresponded with melanocytes on histopathology. For the melanomas, the optical coherence tomography images revealed hyperreflective subepithelial lesion with significant shadowing. For nevi, the images revealed hyperreflective and well-circumscribed lesions with cysts in the subepithelial layer. Given the lack of epithelial involvement and mostly subepithelial nature of the lesion, they were able to distinguish amelanotic melanoma from ocular surface squamous neoplasia and noted evidence of epithelial cleavage in the amelanotic melanomas. Although useful in some instances, anterior segment optical coherence tomography imaging of pigmented lesions is limited owing to light scatter that prevents visualization of underlying structures such as episclera and sclera.

7.2.4. Ultrasound biomicroscopy

Ultrasound biomicroscopy (UBM) can be used to assess extent of a conjunctival lesion (Fig. 3). In a small case series, Ho and colleagues demonstrated feasibility and relative accuracy (within 0.0 to 0.5 mm of thickness measured histologically) of using UBM to estimate conjunctival melanoma tumor depth. UBM is used to evaluate intraocular extension of ocular surface tumors as well. When compared with anterior segment OCT, UBM provided better resolution and visualization of all tumor margins in pigmented conjunctival lesions with less posterior tumor shadowing. UBM can be especially useful when assessing for intraocular invasion or distinguishing conjunctival melanoma from extraocular extension of an underlying uveal melanoma.

7.2.5. Pump-probe microscopy

A newer noninvasive technology currently being tested on histopathologic specimens of conjunctival melanocytic lesions uses patterns of pigment chemistry. Pump-probe microscopy, used more extensively in cutaneous melanoma research, uses a 2-color pulsed laser source to distinguish different melanin subcomponents based on the differences in

Fig. 2 – Anterior segment OCT image of an amelanotic melanoma. OCT, optical coherence tomography.

Fig. 3 – Ultrasound biomicroscopy image of conjunctival melanoma.
transient excited state photodynamics. A recent report demonstrated differences in pigment chemistry between nevi, PAM, and conjunctival melanoma lesions (Fig. 4). This technology has potential as a diagnostic tool for in vivo analysis of pigmented conjunctival lesions.

7.2.6. Other
Although not routinely used, impression cytology is a diagnostic tool for conjunctival melanomas and other melanocytic lesions. Conjunctival melanoma can be quite friable and cells can be liberated easily with gentle manipulation or swabbing. In addition, tear cytology may be useful in detecting atypical melanocytes in advanced conjunctival melanomas.

7.3. Systemic workup
After a full ophthalmic examination, a complete physical examination, including palpation of the parotid, preauricular, submandibular, and cervical lymph nodes, should be done. Given that the common sites of conjunctival melanoma metastasis are regional lymph nodes, brain, lungs, and liver, baseline abdominal imaging (CT, MRI, or ultrasound) is warranted. Positron emission tomography–CT scanning may be useful in detecting regional lymph nodes and distant metastases, allowing proper staging and treatment. Given the propensity of conjunctival melanoma for brain, liver, and lung metastases, baseline liver imaging (CT, MRI, or ultrasound), brain MRI, and chest CT or positron emission tomography–CT scanning should be considered.

8. Treatment
8.1. Surgical treatment

Although excisional biopsy techniques vary among surgeons and surgical centers, there are few basic principles we recommend following (see Fig. 5). In 1997, Shields and colleagues described a widely-used “no touch” surgical technique and provided guidelines for surgical management to ensure complete tumor removal and minimize the likelihood of recurrence or metastasis. The “no touch” technique without direct manipulation of tumor and wide margin excision with en bloc removal is used to avoid tumor cell seeding. Damato and Coupland hypothesized that inadequate surgical treatments leads to recurrence and metastases, likely from iatrogenic seeding of the tumor. Given this risk, Damato and Coupland discourage incisional biopsy and recommend the “no-touch, en bloc tumor excision” technique and reconstruction with clean instruments to prevent such seeding. The conjunctival part of the tumor is excised with at least 3-mm margins, although some surgeons attempt 5- to 7-mm margins when possible. Furthermore, the underlying Tenon capsule down to the level of bare sclera is included in the excision of superficial lesions. This allows histopathological evaluation of the deep margin to ensure there is no involvement. Care should be taken to mark or orient specimens of the limbal lesions that overhang the cornea because these areas might appear as transected lesions (deep margins positive) if the pathologist does not know the orientation of the tissue. Hemostasis can be achieved with bipolar cautery. If the tumor appears to involve the Tenon capsule, or there is any evidence of scleral adhesion or pigment, then a lamellar dissection of the sclera should also be performed. Some surgeons recommend treating the exposed base with absolute alcohol and then scraping the sclera with a blade or applying mitomycin C. Intraoperative adjuvants for the scleral margin are not always used, but we strongly recommended treating the conjunctival margins of the tumor area with double freeze-thaw cryotherapy to destroy possible remaining tumor cells. If the scleral bed appears clear, conjunctival reconstruction can be performed by undermining the conjunctiva and Tenon capsule bluntly with clean instruments. These conjunctival flaps allow for adequate closure of the conjunctival defect and prevent the restriction of extraocular motility or fornical shortening.

Fig. 4 – Pump-probe imaging of conjunctival melanoma. A: Pump-probe image of conjunctival melanoma demonstrating distribution of melanin components. B: Corresponding H&E and C: unstained section of lesion. (Courtesy of Warren Laboratory, Duke University.)
For any corneal component, a localized superficial corneal epitheliectomy without disruption of the Bowman membrane layer is used. Cryotherapy to the limbal margin can be performed. Lamellar corneal excision is not usually necessary for smaller lesions because Bowman membrane commonly resists deep invasion. In advanced and aggressive lesions, lamellar corneal excision may be required for complete resection. This does increase the risk of postsurgical corneoscleral thinning and intraocular invasion because of disruption of Bowman membrane’s natural barrier. When deep corneal resections are necessary, reconstruction with lamellar keratoplasty can be used to repair the wound.

For fornical and palpebral conjunctival melanomas, it is necessary to ascertain the extent of the lesion. Involvement of the eyelid margin or extension onto the skin may further change the surgical approach. Fornical lesions should be resected with wide margins and cryotherapy applied to the conjunctival borders. Lesions involving the tarsal conjunctiva may require a posterior lamellar eyelid resection with possible replacement with mucosal membrane or amniotic membrane graft. Those lesions extending onto the eyelid margin and skin require more extensive full thickness resections and reconstruction.

The intraoperative use of sodium hypochlorite was originally proposed by Oosterhuis and de Wolff-Rouendaal in 1983. Missotten and colleagues tested sodium hypochlorite, known to have less side effects to the ocular surface than ethanol, on a conjunctival melanoma cell line CM2005.1 and found promising results with complete cell death after 3 minutes of treatment. This chemical is not used as adjuvant chemotherapy, but rather as a cytotoxic agent to avoid intraoperative seeding of tumor cells. After surgical resection, they recommend rinsing the ocular surface with sodium hypochlorite 5% for 2 minutes to kill any remaining melanoma cells.

Often primary closure of the conjunctiva is adequate at the end of the procedure; however, in large resections ocular surface reconstruction via conjunctival, amniotic membrane or oral mucosal grafting reduces the risk of scarring and symblepharon formation. The most commonly used graft is amniotic membrane, available in freeze or air-dried and cryopreserved forms and in various thicknesses.

Appropriate handling of the pathology specimen is crucial for diagnosis. To prevent tangential cutting of the specimen and to allow the examination of the deep margin of the tumor and the evaluation of the tumor depth, the specimen should be flattened out over a piece of filter paper with a drawing of its location on the globe and labeled carefully for orientation. The specimen margins may roll, thus inking the margins before resection can help identify the cut edge. Alternatively, the lesion can be oriented carefully on filter paper, with the ends tagged with suture or the margins marked with ink. Whichever method is used, accurate drawings and history should always be communicated to the pathologist.

8.2. Orbital exenteration/enucleation

Orbital exenteration or modified enucleation with wide conjunctival margins may be necessary in cases of extensive tumors that are difficult to control locally with surgical excision and cryotherapy. Intraocular extension is rare but has been seen in eyes with limbal lesions or those that underwent multiple surgical resections. In these cases, enucleation or exenteration was required. In a review of 151 patients with conjunctival melanoma, Shields and colleagues reported that...
orbital exenteration was necessary in 13% of cases presented to a tertiary eye care center from 1974 to 1997. They recommend exenteration in extensive tumor recurrence, nonresectable tumor without evidence of metastasis, or patients with painful eyes and unacceptable cosmetics. Parsons, however, reported a case of nasal and orbital recurrence 21 years after initial exenteration.

In rare cases, primary acquired melanosis or melanoma may extend although the punctum into the nasal lacrimal mucosa and be overlooked. Before and during exenteration, evaluation of the nasal lacrimal system for signs of disease should be done to guide the resection. The surgical margin of the nasolacrimal sac should be marked to aid the pathologist’s evaluation. Exenteration procedures have significant morbidity and should be used when complete resection is possible and before the evidence of regional or distant metastasis. In cases where the disease is purely conjunctival or orbital, a lid sparing exenteration can offer a good cosmetic appearance and a socket that will easily accept a prosthesis.

8.3. Adjuvant cryotherapy

Cryotherapy works by freezing the cells and then producing ischemia from disruption of the microvasculature. This causes epithelial sloughing, including atypical melanocytes, and simultaneously preserves the substantia propria, which helps to decrease scar formation. Sceral melts are a rare complication of cryotherapy. Thus, it is advisable to freeze only the conjunctiva and not the sclera. As mentioned earlier, the base margins can be treated with alcohol or other techniques.

It is now widely accepted practice to apply cryotherapy to tumor margins after the excision. The “Finger-tip” cryoprobe with has a spatulated tip has been used by Paul Finger to treat large areas with minimal exposure to adjacent structures. Adjuvant cryotherapy has been shown to be superior in preventing the tumor recurrence than surgical excision alone as 52% of patients initially treated with excisional biopsy alone developed a local recurrence, compared to 18% of patient initially treated with excisional biopsy with cryotherapy.

Cryotherapy, previously used as a separate modality to help treat diffuse PAM with atypia as well as multinodular melanoma, is effective in treating PAM with atypia, but has high recurrence rates in multinodular malignant melanoma and thus should be reserved as an adjunctive procedure. Cryotherapy alone can be used in patients with recurrence, positive margins found after surgery, or those with a flat tarsal conjunctival lesion.

8.4. Adjuvant topical chemotherapy

Topical chemotherapy has several advantages in the treatment of conjunctival tumors as it treats the entire ocular surface in eyes with poorly defined tumor margins, which allows for the treatment of diffuse or multifocal lesions, or occult areas. Topical chemotherapy can also be used to treat diffuse corneal involvement in cases where complete resection would compromise limbal stem cell function. In addition, topical chemotherapy is easily repeatable.

Topical chemotherapy, usually considered as an adjuvant therapy for conjunctival melanoma if surgical margins demonstrate PAM with atypia, can be considered as a primary treatment when the patient is resistant to surgery or in cases of diffuse PAM with atypia. If margins are positive for invasive melanoma, repeat surgical treatment rather than adjuvant topical chemotherapy should be performed. Here, we briefly review the latest adjuvant treatments for conjunctival melanoma.

8.4.1. Mitomycin C

Mitomycin C (MMC), a nonspecific antibiotic isolated from Streptomyces caesiporus, acts as an alkylating agent to stop DNA synthesis and promote breakage of single-stranded DNA. Despite the potential topical irritation from MMC, Kim and Abramson recommend patients and family members wear latex gloves when handling this medication. MMC is a potent agent and up to 18% of patients initially treated with excisional biopsy alone developed a local recurrence, compared to 52% of patients initially treated with excisional biopsy with cryotherapy.

A delay of a few weeks after surgical treatment is recommended before initiating MMC treatment to allow for wound healing and prevent scleral melting. A common treatment pattern is a 2- to 4-week cycle of 0.04% MMC 4 times daily, followed by 1 week of rest or topical steroid use. The combination of MMC with interferon-alpha-2B is likely most effective for intraepithelial and superficial melanoma.

The most common complications of topical MMC are keratoconjunctivitis, tearing, and pain, whereas the most significant ones are limbic stem cell deficiency with keratopathy and punctal stenosis. The formation of an intumescent cataract after MMC treatment has also been reported. Given that the ocular surface toxicity, a drug holiday is often given, a few days to a week off treatment to allow for the ocular surface to heal. Mild topical corticosteroid (such a fluorometholone ophthalmic suspension 0.1%) is used 4 times daily during the course of treatment along with artificial tears.

Recurrence rates after treatment with adjuvant MMC range from 33%–50%. In 1 case series, nodular and subepithelial nests of conjunctival melanoma were resistant to treatment. Given that recurrences of tumor were more likely to have originated from deeper layers and pseudoglands of Henle, it has been suggested that MMC is likely most effective for intraepithelial and superficial melanoma.

8.4.2. Interferon-alpha-2B

Although the mechanism of action is not completely known, interferon-alpha-2B is a glycoprotein molecule that acts at the cell surface to produce antitumor activity. Topical interferon-alpha-2B (1 million U/mL) has been used as a less toxic adjuvant treatment of PAM with atypia and conjunctival melanoma after initial surgical excision. The most common

S U R V E Y O F O P H T H A L M O L O G Y  6 2 ( 2 0 1 7 )  2 6 – 4 2
### Table 3 – Comparison of studies using MMC for primary treatment of PAM with atypia and conjunctival melanoma

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>No of eyes</th>
<th>Type of study</th>
<th>History</th>
<th>MMC dose (%)</th>
<th>Treatment protocol</th>
<th>Results</th>
<th>Average follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger et al 1993</td>
<td>37</td>
<td>Case report</td>
<td>Recurrence in 8 PAM with atypia, 1 recurrence of CoM 17 years after excisional biopsy</td>
<td>0.04</td>
<td>q.i.d. × 28 days, 1-month holiday, second cycle</td>
<td>Scant pigment remained, requiring cryotherapy, negative biopsy</td>
<td>39 months</td>
</tr>
<tr>
<td>Frucht-Pery and Pe’er 1996</td>
<td>1</td>
<td>Case report</td>
<td>Difuse PAM with atypia</td>
<td>0.02, 0.04</td>
<td>q.i.d. × 14 days, then 0.04% q.i.d. × 14 days</td>
<td>Decreased pigmentation and thinning of tumor, underwent excision and cryotherapy, 3 weeks after</td>
<td>21.5 months</td>
</tr>
<tr>
<td>Finger et al 1998</td>
<td>26</td>
<td>Case series</td>
<td>1 diffuse PAM with atypia, 1 recurrence of CoM 17 years after excisional biopsy</td>
<td>0.04</td>
<td>q.i.d. × 14 days (2 cycles), with 14-day holiday</td>
<td>Negative biopsy at 8 months</td>
<td>28 months</td>
</tr>
<tr>
<td>Demirci et al 2000</td>
<td>7</td>
<td>Case series</td>
<td>5 PAM with atypia, 1 CoM, 1 CoM with corneal recurrence</td>
<td>0.04</td>
<td>q.i.d. × 14 days (2 cycles), with 14-day holiday</td>
<td>Negative biopsy 1 month after end of treatment</td>
<td>30 months</td>
</tr>
<tr>
<td>Kurli and Finger 2005</td>
<td>10</td>
<td>Case series</td>
<td>Recurrence in 8 PAM with atypia and 2 CoM</td>
<td>0.04</td>
<td>q.i.d. × 14 days (2 cycles), with 14-day holiday</td>
<td>5 with recurrence</td>
<td>80 months</td>
</tr>
<tr>
<td>Pe’er and Frucht-Pery 2005</td>
<td>12</td>
<td>Retrospective</td>
<td>9 PAM with atypia, 3 CoM</td>
<td>0.02, 0.04</td>
<td>q.i.d. × 14 days, 2–5 cycles (with 14-day holiday)</td>
<td>Negative biopsy 1 month after end of treatment</td>
<td>23 months</td>
</tr>
</tbody>
</table>

CoM, conjunctival melanoma; MMC, mitomycin C; PAM, primary acquired melanosis; q.i.d, 4 times daily.

### Table 4 – Comparison of studies using adjunctive MMC for PAM with atypia and conjunctival melanoma

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>No of eyes</th>
<th>Type of study</th>
<th>History</th>
<th>MMC dose (%)</th>
<th>Treatment protocol</th>
<th>Results</th>
<th>Average follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Finger et al 1998</td>
<td>6</td>
<td>Case series</td>
<td>Excision and cryotherapy immediately before, CoM versus PAM not delineated</td>
<td>0.04</td>
<td>1 drop q.i.d. × 7 days</td>
<td>No recurrence</td>
<td>33 months</td>
</tr>
<tr>
<td>Demirci et al 2000</td>
<td>5</td>
<td>Case series</td>
<td>Excision and cryotherapy immediately before, 2 PAM with atypia, 2 PAM with focal invasion, 1</td>
<td>0.04</td>
<td>1 drop q.i.d. × 7 days</td>
<td>2 developed recurrence (both CoM)</td>
<td>52 months</td>
</tr>
<tr>
<td>Ditta et al 2011</td>
<td>15</td>
<td>Retrospective</td>
<td>CoM underwent excisional biopsy and cryotherapy</td>
<td>0.04</td>
<td>1 drop q.i.d. × 3-week cycles separated by a week of steroids</td>
<td>5 with recurrence, 3 with metastases</td>
<td>23.8 months</td>
</tr>
<tr>
<td>Kurli and Finger 2005</td>
<td>6</td>
<td>Case series</td>
<td>CoM underwent excisional biopsy and cryotherapy</td>
<td>0.04</td>
<td>1 drop q.i.d. × 7 days</td>
<td>3 with recurrence</td>
<td>95 months</td>
</tr>
</tbody>
</table>

CoM, conjunctival melanoma; MMC, mitomycin C; PAM, primary acquired melanosis; q.i.d, 4 times daily.
side effect is keratoconjunctivitis, which resolves after stopping therapy. There are minimal data supporting the efficacy and long-term results of treatment with interferon-alpha-2b, but it may have some use as an adjunct agent in certain cases.

8.4.3. Future directions in topical chemotherapy agents

5-Fluorouracil, an antimetabolite that prevents DNA and RNA synthesis in the S phase, has been used to treat ocular surface squamous neoplasia, but has not been reported in the use of ocular surface melanocytic lesions. Given the good results seen in ocular surface squamous neoplasia; however, this drug may prove to be useful as adjunctive treatment in conjunctival melanoma.

Westekemper and colleagues exposed 2 established conjunctival cell lines (CRMM-1 and CRMM-2) from recurrent conjunctival melanoma to various established chemotherapies. Cisplatin, an organometal and classic alkylating agent used in the treatment of a variety of malignancies, and MMC effectively inhibited growth of the cell lines as single agents. In addition, they reported the combination of imatinib—a selective tyrosine kinase inhibitor most commonly used in treatment of chronic myelogenous leukemia with promising results in malignant melanoma, and MMC had synergistic effects in cell growth inhibition.

A similar study was done by the same group with newer chemotherapeutic agents. They reported 4 agents that inhibited growth of CRMM-1 and CRMM-2 in vitro. Bortezomib, the agent with the lowest ICSO, is a proteasome inhibitor that blocks nuclear factor κB and has been used in cutaneous malignant melanoma. The second agent, clusianone 502, also known as nemorosone, is an antitumor agent from the plant Clusia rosea studied in vitro on pancreatic carcinoma. Ranpirnase, a ribonuclease extracted from the frog Rana pipiens studied on malignant mesothelioma, also effectively inhibited growth of the conjunctival melanoma cells. Finally, sorafenib, a multikinase inhibitor used in renal cell and hepatocellular carcinoma and being tested in malignant melanoma, also produced promising results. These 4 chemotherapeutic agents may serve as potential adjuvant treatment in conjunctival melanoma.

Cunneen and colleagues tested sodium butyrate and trichostatin A, both histone deacetylase inhibitors that induce G1 cell cycle block, on CRMM-1 and CRMM-2 as well as on Tenon capsule fibroblasts, and compared results to MMC. They determined that these 2 agents effectively induced cell death in a dose-dependent fashion, with minimal damage to the fibroblasts.

Although these studies provide hope for future treatments, these cell lines were exposed to the chemotherapeutic agents for multiple hours at a time. To achieve similar results on the ocular surface, a depot would be needed, as topical drops would not achieve similar concentrations. In addition, these studies showed cell growth inhibition but are limited in the ability to demonstrate side effects in vivo.

8.5. Adjuvant radiotherapy

8.5.1. Brachytherapy

Brachytherapy involves putting radioactive elements close to a tumor to allow energy from β or γ ray emitting isotopes to target malignant cells. Plaque brachytherapy, where a device filled with radioactive isotope is sutured to the ocular surface, has been used as adjuvant therapy after surgical excision with cryotherapy to the margins. Radiation therapy is usually delayed until the conjunctiva has healed after excision. Unlike topical chemotherapy, plaque brachytherapy has the ability to treat deep in the sclera. In contrast, the more superficial structures absorb more radiation than deeper structures, thereby minimizing damage to the lens and other structures. For limbal lesions, Wals-Conway and Conway recommend positioning the plaque directly on the cornea, which may predispose patients to transient epithelial defects. As is common with radiotherapy, treatments may be fractionated to decrease side effects. Karim and Conway suggest that using brachytherapy postexcision to treat remaining tumor cells allows for more conservative surgical margins, thereby avoiding many complications of extensive surgery.

Another positive result from radiotherapy is the antivascular effects that reduce vascularization and conjunctival injection, leading to improvement in ocular surface irregularity.

Brachytherapy with iodine-125 has been used widely. Adjuvant brachytherapy with iodine-125 was reported in 19 patients with melanoma in situ or early-invasive conjunctival melanoma (defined as invading substantia propria less than 1.5 mm) 1 month after surgical excision and cryotherapy to the margins. An apex dose of 100 Gy to a depth of 1.5–3.0 mm over the area of highest disease involvement was applied. There were no recurrences in the 2- to 4.5-year follow-up period, but 3 patients developed new PAM with atypia lesions distant from treatment site. Six patients developed transient corneal epithelial defects postoperatively from physical trauma from the plaque. The same group found favorable medium-term results in patients with more advanced involvement (n = 5).

Plaque brachytherapy with ruthenium-106 has also been used at many centers. In 1993, Zehetmayer and colleagues described the successful treatment of an epibulbar conjunctival melanoma with surgical excision followed by a total dose of 290 Gy delivered to the tumor bed. With no recurrence at 50-month follow-up, but scleral rarefaction and cataract formation developed. The current treatment dose, as described by Damato and Coupland, is 100 Gy at 1–2 mm after the conjunctiva has healed from excisional biopsy. They favor adjunctive ruthenium brachytherapy rather than cryotherapy or the excision of the scleral bed for invasive malignancy. They have found that adjunctive radiotherapy significantly lowers the risk of recurrence at site of primary tumor when compared to patients treated without radiotherapy.

Stannard notes (personal communication with Sauerwein) that the ruthenium-106 plaque can also be sutured to the sclera immediately after tumor removal. The dose delivered is 130 Gy at 2 mm.

An alternative to brachytherapy with an attached plaque, which involves an additional surgical procedure, is using strontium-90 beta radiotherapy via applicator. The process is noninvasive, and treatment is administered using a handheld applicator after topical anesthesia. The treatment protocol involves the delivery of 60 Gy to surface in 4 weekly fraction, or 50 Gy in 5 daily fractions. Cohen and colleagues reported favorable results with a success rate of 90% using strontium-90 beta radiotherapy as adjuvant treatment in 20 patients with
incompletely excised conjunctival melanoma. These patients remained tumor free in the treatment area for the follow-up period, whereas 2 patients (10%) developed recurrences at 15 and 37 months. They treated all patients with 5 fractions of 10 Gy each at the scleral surface. Complications included dry eyes, episcleritis, and descemetocele. Another team previously reported treating 15 patients with 9 fractionated sessions of 6 Gy each, with 46% success rate. With relatively low complication rate and potentially higher success rate, Strontium-90 brachytherapy is a possible forerunner in adjuvant therapy, however, access to this treatment is limited as the applicator and devices are available at only a few oncology centers.

Brachytherapy has also been delivered with an I-125 interstitial implant made of radioactive seeds in a plastic tube implanted in the eyelid to treat palpebral conjunctiva and eyelid melanoma involvement. A stainless steel shield was attached to the extraocular muscles to protect the ocular surface and deeper structures.

Finally, phosphorus-32 has recently been reported to treat diffuse, recalcitrant conjunctival tumors such as ocular surface squamous neoplasia, sebaceous carcinoma, and lymphoma. A phosphorus-32 impregnated film was applied to patients with recurrent or residual tumors, delivering a median dose of 15 Gy in 1 fraction at 1 mm. Marr and colleagues reported 75% recurrence-free survival. This therapy may be considered for recalcitrant conjunctival melanomas as well.

8.5.2. Proton radiotherapy
Treatment of diffuse growth pattern and involvement of non-bulbar conjunctiva can be challenging. In cases with extensive palpebral, fornical, conjunctival, or caruncular involvement, an alternative to extensive surgical treatment or exenteration is proton beam irradiation. Proton beam irradiation allows for more precise treatment than other irradiation modalities owing to the “ballistic properties” of protons, which allows for accurately treated volume to a specific depth; however, the patient must be immobilized for accurate planning and delivery of treatment.

Wuestemeyer and colleagues treated 20 patients with extensive conjunctival melanoma (mostly T3) not amenable to plaque brachytherapy with proton radiotherapy with 31 Gy in 6 fractions and an additional 2 fractions up to 45 Gy in highest tumor volume area after excisional biopsy and conjunctival mapping. They noted 30% recurrence rate and 30% metastasis rate. Complications included dry eye (95%), focal cataract (35%), limbal stem cell deficiency (20%), and eyelash loss in all cases where the eyelids were irradiated.

9. Sentinel lymph node biopsy
Metastases can be detected with full body [18f]-fluorodeoxyglucose positron emission tomography-CT imaging. Moreover, careful palpation of the regional lymph nodes, high-resolution ultrasound imaging, and MRI of the head and neck can identify obvious lymphatic metastases. Micrometastasis through the lymphatic system, however, can only be detected with sentinel lymph node biopsy (SLNB). In a population-based, retrospective study of Finnish conjunctival melanoma patients from 1967 to 2000, Tuomaala and Kivela

Fig. 6 – Sentinel lymph node biopsy. A: Identifying the lymph node with γ-probe localization. B and C: Excision of the identified sentinel lymph node. D: The excised lymph node.
reported that median time from primary tumor diagnosis to diagnosis of regional metastases and systemic metastases was 2.3 years and 3.4 years, respectively. In addition, they reported an estimated maximum 10-year cumulative incidence of lymphatic spread to be 29%.144

Successful SLNB requires a multidisciplinary team with involvement of an ophthalmic surgeon, head and neck surgeon, and nuclear medicine specialist.31 A thorough understanding of the anatomy is crucial to perform SLNB. Superficial conjunctival and deeper lymph channels drain toward the lateral and medial canthi. Generally, pericocular lymphatics drain from the medial canthus to the facial and submandibular nodes, and lateral canthus to the preauricular superficial parotid nodes; however, there is significant anatomical variation.139

Sentinel biopsies of the cervical lymph node chain and parotid gland are recommended for staging of conjunctival melanomas and should be especially considered in patients with tumors with Breslow thickness of greater than 1 or 2 mm (depending on center), a nonlimbal location, and ulceration.15,28,55,99,115,144,145 After preoperative lymphoscintigraphy, the SLNB procedure involves identification of sentinel nodes by imaging and γ-probe localization after injection of technetium Tc-99m nanocolloid into the tumor location (see Fig. 6).15,31,55,99,115 Sentinel lymph nodes are identified as being at least twice as radioactive as the background.29,115 Injection of a methylene blue or isosulfan blue dye has been previously to further help identify sentinel lymph nodes, this technique by itself has largely been abandoned due to low yield, and if used is in conjunction with Tc-99m.15,31,55,99 The main risk to the SLNB procedure is facial nerve injury.15,55,99

Reported positive SLNB rates range from 11%–33%, although this varies by selection criteria.15,55,115 If any biopsies are positive for malignancy, patients may undergo parotidectomy and radical neck dissection to treat the cervical lymph node changes and adjuvant radiation therapy to prevent distant metastases.15,29,115 Elective parotidectomy and neck dissection need not be performed unless there is obvious clinical or imaging evidence of disease.151 There is no evidence that radical surgery improves mortality, and in fact, conservative parotid or submandibular lympectomy followed by external beam radiotherapy may be as effective.139 All patients with negative SLNB should be monitored closely and undergo long-term follow-up given the possibility of later lymphatic metastasis.115 Distant metastasis without evidence of lymph node involvement may occur.73 The metastasis in these cases likely spreads hematogenously.

### Table 5 – Risk factors for recurrence, metastasis, and mortality

<table>
<thead>
<tr>
<th>Risk factors for recurrence</th>
<th>Risk factors for metastasis</th>
<th>Risk factors for mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irregular pigmentation</td>
<td>Tumor thickness &gt; 2.0 mm</td>
<td>Location in palpebral conjunctiva, caruncle, plica, or fornices</td>
</tr>
<tr>
<td>Incomplete surgical excision</td>
<td>Ulceration</td>
<td>Tumor thickness</td>
</tr>
<tr>
<td>Tumor invasion deeper than</td>
<td>Mitotic figure &gt; 1/mm²</td>
<td>Tumor invasion deeper than</td>
</tr>
<tr>
<td>substantia propria</td>
<td></td>
<td>substantia propria</td>
</tr>
<tr>
<td>Nonepibulbar location</td>
<td>Local recurrence</td>
<td>Epithelioid cell type</td>
</tr>
<tr>
<td>Epithelioid cell type</td>
<td>Pagetoid invasion of atypical melanocytes in associated PAM</td>
<td>Nodular growth pattern</td>
</tr>
<tr>
<td>Positive tumor margins</td>
<td>Arising de novo</td>
<td>Lymphovascular invasion</td>
</tr>
<tr>
<td>Extralimbal location</td>
<td>Tumor-associated lymphangiogenesis</td>
<td>Microsatellitosis</td>
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<tr>
<td>Tumor-associated</td>
<td></td>
<td>Tumor-associated</td>
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<tr>
<td>lymphangiogenesis</td>
<td></td>
<td>lymphangiogenesis</td>
</tr>
<tr>
<td>Excision alone as initial therapy</td>
<td></td>
<td>Arising de novo</td>
</tr>
</tbody>
</table>

PAM, primary acquired melanosis.

Sources: Anastassiou et al,4 Esmaeli,33 Fernandes,34 Frucht-Pery and Pe’er,15 Ho et al,55 Norregaard et al,100 Shields et al,127 Shousha et al,131 Van Raamsdonk et al,150 Weiss et al,133 and Yousef and Finger.162

### Table 6 – Differential diagnosis of conjunctival melanocytic Lesions

<table>
<thead>
<tr>
<th>Benign melanosis</th>
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<tbody>
<tr>
<td>Benign nevus</td>
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<tr>
<td>Primary acquired melanosis</td>
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<tr>
<td>Conjunctival blue nevus</td>
</tr>
<tr>
<td>Pigmented conjunctival squamous cell carcinoma</td>
</tr>
<tr>
<td>Conjunctival seborrheic keratosis</td>
</tr>
<tr>
<td>Extraocular extension of uveal melanoma</td>
</tr>
<tr>
<td>Postsurgical hemat cyst</td>
</tr>
<tr>
<td>Uveal prolapse through surgical wound</td>
</tr>
<tr>
<td>Conjunctival inclusion cyst</td>
</tr>
<tr>
<td>Necrotic pyogenic granuloma</td>
</tr>
<tr>
<td>Conjunctival mycosis</td>
</tr>
<tr>
<td>Graphite foreign body granuloma from pencil injury</td>
</tr>
<tr>
<td>Exogenous argyrosis</td>
</tr>
<tr>
<td>Conjunctival mastocarcinoma</td>
</tr>
<tr>
<td>Conjunctival foreign bodies (mullite and illite)</td>
</tr>
<tr>
<td>Retained foreign body from air gun pellet injury</td>
</tr>
</tbody>
</table>

### 11. Prognosis

Although a rare diagnosis, conjunctival melanoma has serious implications with respect to recurrence, metastasis, and mortality. Risk factors for these end points are listed in Table 5.4,32,33,40,52,95,122,124,143,146,155

19. Follow-up

Long-term follow-up is necessary to monitor for recurrence or metastasis. We recommend complete ophthalmic examination and annual imaging of regional lymph nodes. Additional systemic imaging for metastases should be considered based on clinical findings.114 Annual to biannual chest radiograph, brain MRI, complete physical examination, bloodwork including liver enzymes, and possible abdominal or chest CT imaging are also recommended.33,123
Conjunctival melanoma usually metastasizes to the local preauricular, submandibular or cervical lymph nodes, lungs, brain, liver, skin, bones, and gastrointestinal tract.\textsuperscript{13,23,95,123} The bladder and upper urinary tract are a rare site of metastasis.\textsuperscript{97} Distinct from primary nodular conjunctival melanomas, local conjunctival metastases have also been described, developing 3–102 months after appearance of the first invasive melanoma lesion.\textsuperscript{96} Likely spread via lymphatics, local metastatic lesions appear as discrete unencapsulated nodules under the conjunctival epithelium. Local invasion into the cornea has also been reported, usually superficial to Bowman membrane, but occasionally deeper in the stroma.\textsuperscript{103,117,142,147} Intraocular extension is rare but may occur after incomplete surgical excision.\textsuperscript{154}

Based on reported case series, overall risk of tumor recurrence is 36%–56%.\textsuperscript{23,33,79} Risk of metastasis has been reported as 21%–26%.\textsuperscript{124} The reported 10-year cumulative incidence of tumor-related mortality from conjunctival melanoma is between 23%–39%, with survival after systemic metastasis is a median of 8 months.\textsuperscript{33,79,118,143,155} Based on retrospective case series, the 5-year survival probability is 74%–86.3%, 10 year is 41%–77.7%, and 15 year is 67%–68%.\textsuperscript{33,96,107,155}

12. Differential diagnosis

The differential diagnosis for a pigmented ocular surface lesion is limited. Table 6 lists the diagnoses reported in the literature.\textsuperscript{6,12,27,43,49,50,68,83,85,87,88,125,128,140,165}

13. Summary

 Conjunctival melanoma is a rare, but vision and life-threatening condition. Prompt diagnosis with a thorough clinical examination and adjunctive imaging is crucial. Treatment is mainly surgical with options of adjuvant chemotherapy and radiotherapy. Long-term follow-up with ophthalmic and systemic workup is important to detect recurrences and metastases.

14. Method of literature search

The literature review was conducted on a comprehensive Pubmed search of references related to the following key words: conjunctival melanoma, ocular melanoma, conjunctival melanocytic lesion, pigmented lesion of conjunctiva, malignant melanoma of the conjunctiva. The abstracts of these articles were reviewed, and ones relevant to the article, the full-length articles were reviewed in detail. Manual search through references from these articles was also done. All articles published from to 1966 to end of 2015 were reviewed.

15. Disclosures

The authors have no conflicts of interest and funding sources to report.

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