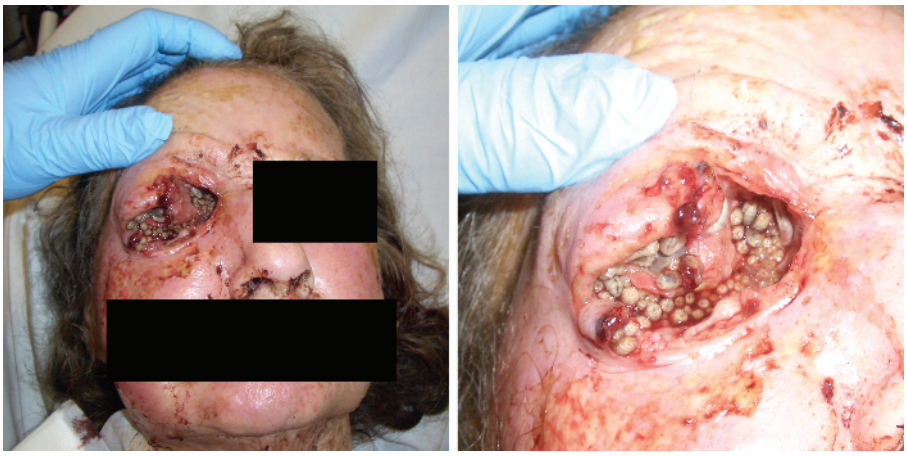

Figure 2 Clinical photograph of patient upon presentation to ER. A higher magnification photograph of the orbit of the right eye (OD) is seen on the right. Informed consent was obtained for publication of this figure.

the globe. The patient again refused exenteration. The patient's vision continued to decline progressively to counting fingers and the mass continued to grow over the next 4 months when the patient again presented to the ER for irritation OD. Repeat CT scan demonstrated significant soft tissue oedema (fig 1). Examination revealed a massive infestation with maggots in the orbit OD (fig 2). Vision was no light perception OD, with a normal exam for the left eye (OS). The patient was debrided at the bedside and several maggots were removed, including over three full-sized maggots from what remained of the globe. The patient was further debrided in the operating room. The patient refused further surgical intervention and was placed in palliative care due to systemic metastases.

Discussion

Although the species of these maggots was unknown, most cases of ophthalmomyiasis are caused by *Dermatobia hominis* (human botfly), *Cochliomyia hominivorax* (screw worm), *Hypoderma bovis* (ox warble fly) and *Oestrus ovis* (sheep botfly).[6] Cuterebra (rodent botfly) larvae are native to North America.[7] In this case treatment with debridement was sufficient, although in cases where this procedure is unsatisfactory, other authors have used petroleum jelly (to suffocate the maggots so they emerge) and paralytic agents such as ivermectin to facilitate extraction.[8] Patients with chronic ulcerative lesions of the eye who refuse treatment, as in our case, should be warned of the rare but blinding consequence of orbital myiasis, and should be taught proper wound dressing and hygiene.

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A new cell line from a recurrent conjunctival melanoma

The incidence of conjunctival melanoma is about 0.2–0.8/1 000 000 cases each year.[1 2] As a consequence our knowledge about this tumour is limited, but cell lines derived from these tumours will give more information about them. So far only three conjunctival melanoma cell lines have been described.[3 4] The first cell line, IPC 292, was described in 1993 by Aubert *et al*.[4] Recently, Nareyeck *et al* developed two additional conjunctival melanoma cell lines (CRMM1 and CRMM2).[3] We wish to report a further, fourth, conjunctival melanoma cell line, CM2005.1.

The conjunctival melanoma cell line CM2005.1 was established from tumour material derived from a 84-year-old male. The primary tumour was located at the medial side of the inner upper eyelid adjacent to the cornea. The tumour was treated initially by local excision together with adjuvant iridium 192 brachytherapy. After 3 years the tumour reappeared on the inner side of the lower eyelid. The tumour extended into the nasal cavity and disseminated further. The patient died 1 year later from the melanoma which had originated in the conjunctiva. Both the primary tumour and the local recurrence were histologically proven conjunctival melanomas.

From the local recurrence two small tumour specimens were available for cell culture. The tumour material was cut into small pieces with scalpels and transferred to several culture plates. Culture plates contained 10 ml/dish RPMI 1640 (Invitrogen-Gibco, Groningen, the Netherlands) supplemented with 10% FCS (Hyclone, Logan, UT), 100 IU/ml penicillin (Invitrogen-Gibco) and 100 µg/ml streptomycin (Invitrogen-Gibco). Cultures were incubated at 37℃ in a humidified atmosphere and a CO_2 content of 5% in air and the culture medium was refreshed every 72–96 h. After 4 months a stable cell line had been developed, which was grown for over 22 passages. Cell doubling time was measured three times by culturing 100 000 cells/plate. The cell count was measured again at days 1, 2, 3, 4 and 5, which showed a cell doubling time of around 35 h (fig 1).

To establish the melanocytic origin of the cultured cells, cytospins were undertaken and immunohistochemically stained for S100, MelanA, NKI-C3 and HMB 45.

Immunohistochemical reactions were performed using the streptavidin-biotin method. The melanocytic origin of the cultured cells was proved by the fact that 95–100% were positive for all four primary antibodies. MelanA, HMB 45 and NKI-C3 strongly stained the cultured cells, while the S100 labelling was slightly less intense.

Cytogenetic analysis was performed to assess the karyotype of cell line CM2005.1. Chromosome preparations were obtained according to standard procedures and stained to produce R or Q banding. Cytogenetic abnormalities were described in accordance with the ISCN.[5]

The following karyogram was found in most cells: 83~96, XX, +X, −Y, −Y, −1, −1, −3, −3, −4, −5, −5, −6, +7, +7, +del (7) (q22), −8, −9, −9, −10, −10, del (11) (q23)x2, −12, +13, +13, +14, −16, −17, −17, −18, −19, +20, −21, −21, −21, −22, +12~16 mar (fig 2).

Cell line CM2005.1 was derived from a recurrent conjunctival melanoma that reoccurred after excision and brachytherapy, which could explain the distorted karyogram and the relatively high cell doubling time of this cell line. However, the cytogenetic analysis was performed 4 months after start of the culture, which could have influenced the karyogram and may therefore not represent the original tumour. Nareyeck *et al* found cell doubling

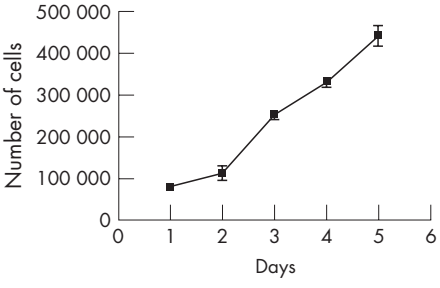

Figure 1 Growth curve of conjunctival melanoma cell line CM2005.1 showing the number of cells at various time points during culture. Cell doubling time was around 35 h.

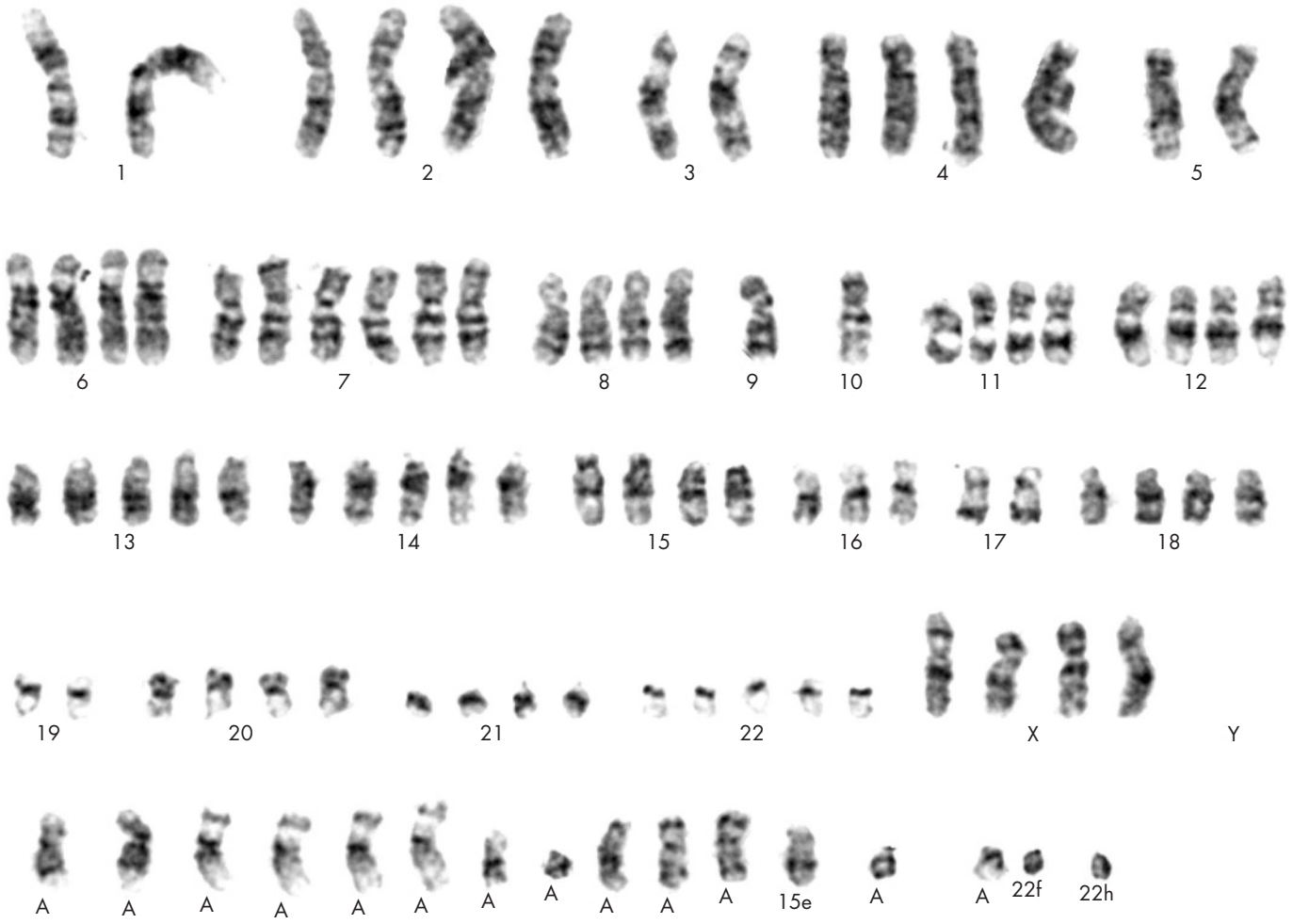

Figure 2 Karyogram of cell line CM2005.1. A very complex karyogram with gains, deletions and rearrangements of almost all chromosomes was observed in the majority of cultured cells.

times of around 60 h for their conjunctival melanoma cell lines CRMM-1 and CRMM-2.[3]

This fourth conjunctival melanoma cell line is now available for research, and can hopefully contribute to expansion of our knowledge of conjunctival melanomas.

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Complications of management in primary acquired melanosis with atypia

The management of conjunctival primary acquired melanosis (PAM) with atypia is in flux. Lesions are treated with biopsy, excision, adjunctive cryotherapy, or topical mitomycin.[1] Acceptable, usually minimal, complications have been reported with these adjunctive therapies.[2] Adverse events associated with topical mitomycin are uncommon and punctual stenosis is observed, especially in cold windy climates in approximately 20% of cases. Limbal stem cell deficiency has been described after the use of this agent on a dose-related basis, but is uncommon.[2 3]

We report a case of recurrent PAM with atypia treated with three courses of mitomycin and two courses of cryotherapy, with subsequent stem cell deficiency and vision loss that was reversed with an autologous stem cell transplant. We received human experimentation committee (CCPMC Human Experimentation Committee) approval for this study.

Case report

A 56-year-old woman presented with an enlarging area of right conjunctival and corneal pigmentation. It started to expand five years ago with marked growth over the past six months. She had 20/30 visual acuity in the involved right eye; the left eye was unremarkable. There was circumferential superficial right limbal pigmentation with a thickened area inferiorly to the inferior fornix, as well as pigment dusting the caruncle. We resected the inferior corneal-conjunctival area of thickened pigmentation, which showed PAM with atypia. The surgical margins were clear. Adjunctive double freeze–thaw cryotherapy was used. She received two one-week courses of postoperative mitomycin C, 0.04% three times a day. There was some regression of the remaining pigmentation over the next few months. Approximately six months later, there was an area of increased limbal pigmentation, which we treated with an additional one-week course of mitomycin. This produced marked diminution of pigmentation.

Five months later she developed increased pigmentation, which was treated with a one-week course of 0.4% mitomycin with a marked decrease of the pigmentation.

Pigmentation remained superficial and stabilized for approximately a year, and then she developed an additional spread of flat pigmentation without new vessels or increased