Int Ophthalmol (2019) 39:295–301
https://doi.org/10.1007/s10792-017-0811-0

ORIGINAL PAPER

Role of topical interferon alpha-2b in 'mitomycin-C-resistant' ocular surface squamous neoplasia: our preliminary findings

Manpreet Singh · Natasha Gautam · Manpreet Kaur

Received: 17 August 2017 / Accepted: 11 December 2017 / Published online: 23 January 2018
© Springer Science+Business Media B.V., part of Springer Nature 2018

Abstract

Purpose To report the clinical presentation of mitomycin-C (MMC)-resistant ocular surface squamous neoplasia (OSSN) and its treatment outcome with topical interferon alpha-2b (IFNα-2b).

Methods A prospective, non-randomised, pilot study enrolling clinically diagnosed OSSN patients. The inclusion criterion was resistance of OSSN to standard topical MMC (0.02%) chemotherapy. The resistance was defined as 'no clinical response' in the terms of reduction in tumour size, extension and vascularity after minimum 6 weeks 'on-cycles' of MMC. Any previous surgical intervention or recurrent OSSN lesions were excluded. Topical MMC was stopped in all, and topical IFNα-2b (1million IU/ml) eyedrops were prescribed to each patient. At first presentation, the clinical features and side-effect profile of MMC was noted and therapeutic effect of IFNα-2b was clinically monitored at each follow-up. Topical immunotherapy was continued for 24 weeks and a minimum follow-up of 12 weeks was observed after stopping IFNα-2b.

Results Six patients with a mean age of 62 years met the inclusion criteria. At presentation, all had unilateral, circumscribed, sessile and unifocal lesions with mean dimensions of 7.67 × 5.17 mm. Four patients had temporal lesions while surface keratin, pigmentation and corneal involvement were noted in three lesions, separately. All lesions had dilated and tortuous feeder vessels. All six tumours resolved completely over a mean tumour resolution time of 16 weeks while the total duration of IFNα-2b treatment was 24 weeks. After stopping immunotherapy, a mean of 14.5 weeks follow-up was observed. None showed any recurrence. The approximate cost of total therapy session was 8400 Indian rupees.

Conclusion The OSSN lesions showing 'less or no response' to topical MMC may be shifted to topical recombinant IFNα-2b before proceeding for a surgical excision.

Keywords Ocular surface squamous neoplasia · Interferon alpha-2b · Topical mitomycin-C · Chemotherapy resistance

Introduction

In the management of ocular surface squamous neoplasia (OSSN), the traditional surgical excision with 'no-touch technique' has limited indications in the current era. The recent literature advocates medical management for the majority of OSSN lesions, since both medical and surgical treatment have shown

M. Singh (✉) · N. Gautam · M. Kaur
Department of Ophthalmology, Post Graduate Institute of Medical Education and Research, Chandigarh 160012, India
e-mail: drmanu83@gmail.com

comparable success as well as recurrence rates [1, 2]. The medical management includes topical chemotherapeutic agents like mitomycin-C (MMC) and 5-fluorouracil (5-FU) and local immunotherapy with interferon alpha-2b (IFNα-2b) [3]. All of these drugs need reconstitution to be used as topical eyedrops [4–6]. Of these, a recent inclination has been noticed towards the local use of recombinant IFNα-2b amongst the ocular oncologists [6]. So, with the upsurge of medical treatment for OSSN, the effectiveness of available topical drugs and their side-effect profile becomes the point of concern.

Not all tumours have similar response to specific anti-tumour drugs. The resistance of tumour cells to the chemotherapeutic agents is a known phenomenon. This resistance can either be an inherent feature of tumour cells itself (cancer cell heterogeneity) or an acquired phenomenon shown after drug exposure [7]. The mechanism of resistance to the conventional antimetabolites is via over-expression of DNA-synthesis gene (DHFR and TYMS) leading to the less-effective action of mitomycin-C (MMC) and 5-fluorouracil (5-FU) [8]. Moreover, the chemotherapeutic agents have been classified according to the cell cycle phase on which they act upon. Hence, the resistance of overall tumour depends upon the stage of cell cycle in which majority of the tumour cells are present [7–9].

MMC is an anti-tumour antibiotic which works best against the cells present in 'G1-S phase' of cell cycle [4]. 5-FU is a structural analogue of thymine and replaces thymine during the 'S-phase' of DNA and RNA synthesis [5]. Hence, these drugs act best on the tumour cells present in their specific cell cycle phases. Probably, the rest of tumour cell mass appears to be relatively safe from these drugs. Moreover, these drugs do not affect the virus laden cells. Additionally, an intrinsic and acquired resistance has been described to methotrexate in diseases like acute leukaemia highlighting the possibility of resistance to chemotherapy [8, 9]. The prominent use of topical MMC for OSSN in the past appears to be one of the reasons to identify more number of patients who showed reduced effectiveness or partial resistance to MMC. This non-response possibly gets masked by the prominent local side-effect profile of MMC. This is in contrary to the local side effects of topical IFNα-2b on the ocular surface.

To the best of our knowledge, no study has described resistance of OSSN to MMC although the mechanism of resistance of OSSN to IFN has been hypothesised by Ashkenazy et al. in immune-suppressed individuals. They concluded that an intact immune system may be an important link between IFNa2b therapy and tumour resolution [10]. In this article, we want to describe the clinical profile and treatment outcomes of diagnosed OSSN patients who showed resistance to topical MMC but clinically responded to topical recombinant IFNα-2b immunotherapy.

Materials and methods

This was a prospective, non-randomised, observational study conducted on the diagnosed patients of OSSN presenting to our ophthalmic plastic surgery clinic from July 2013 to December 2016. Our pilot project adhered to the tenets of Declaration of Helsinki and all the documentation was performed by a single observer (MS). A detailed history (personal, previous treatment, surgery, etc.), tumour characteristics (laterality, location, morphological type, tumour dimensions, presence of keratin/pigment, corneal involvement, etc.) and clinical outcomes were studied.

All the OSSN patients who were resistant to topical MMC 0.02% eyedrops were included in our study. In our routine practice, topical MMC was used as per recommended frequency of QID for 1 week followed by a week 'off drug'. The OSSN was defined 'resistant' to topical MMC eyedrops if there was 'no clinical response' in the terms of reduction in tumour size, extension and vascularity after a minimum of 6 weeks 'on-cycle' (total 12, 6 weeks on and off each) of topical MMC chemotherapy. Patients with recurrent OSSN and any history of surgical intervention for diagnosis or treatment of OSSN were excluded from our study. All the patients were diagnosed clinically (no biopsy performed) and treated by the same ophthalmic oncologist (MS). Topical MMC was stopped in all, and the patients were shifted to reconstituted recombinant interferon alfa-2b injection, 3 million international units/ml, i.e. 3 MIU/ml (Injection Zavinex, Zydus Cadila, Gujarat, India). This prefilled injection was diluted with 2 ml of preservative-free lubricant eyedrops forming a 3 ml solution containing 1 MIU/ml of IFNα-2b. This freshly prepared vial was advised to be stored in a refrigerator at 4–8 °C temperature. Adequate instructions were

given to the patients and relatives about the frequency, cold-chain maintenance, preserving sterility of vial, noting the change in symptoms and regular follow-up visits.

Topical IFNα-2b was reconstituted by the ophthalmic oncologist or the experienced staff under aseptic conditions on the demand of patient or relatives. All patients used a single drop of IFNα-2b 4 times/day and were followed up at 1, 2, 4, 8 weeks and then monthly. All patients were instructed to use one bottle (3 ml) of reconstituted IFNα-2b for 2 weeks after which a fresh vial was prepared. Slit-lamp examination was done at each follow-up visit for tumour dimensions, tumour extension, surface pigment, overlying keratin and tumour vascularity along with any side effects of topical IFNα-2b. Clinical pictures were obtained at each visit for documentation.

Topical immunotherapy was continued for a minimum of 24 weeks or till complete clinical remission was noted and documented. Patients were followed up for a minimum of 12 weeks after cessation of topical IFNα-2b therapy. The success of treatment was defined as the complete clinical resolution of OSSN lesion. Any progression of the above-mentioned clinical features while on treatment was considered as therapy failure. After stopping topical IFNα-2b, any clinically residual or recurrence of the tumour was termed as partial success or recurrence, respectively. A complete tumour resolution with the absence of all characteristic features was considered as complete remission at the final follow-up.

Results

Twenty-five patients of OSSN were examined at our ophthalmic plastic surgery clinic from May 2014 to December 2015. Of them, only 6 (24%) met the inclusion criteria of our study, rest 19 (76%) responded to the primary treatment (either IFN or MMC). The mean age of patients was 62 years, and 5 (83.33%) were males. History of excessive sun exposure (> 50% of waking h) was present in 4 (66.67%), chronic smoking in 3 (50%), skin cancer (squamous cell carcinoma) of the neck in 1 (16.67%) and none of them had any history of cancer in the family. None of them were positive for HIV or other hepatitis virus profiles.

All patients had unilateral lesions (100%). On slit-lamp magnified examination, the tumour location was temporal in 4 (66.67%) and nasal in 2 (33.33%) (Fig. 1). The mean tumour dimensions were 7.67 × 5.17 mm (range 4–11 mm) and all the OSSN lesions were circumscribed, sessile and unifocal (Figs. 2a, 3a, 4a). Half of the lesions had the presence of pigment and surface keratin. Morphologically, the lesions were elevated in 4 (66.67%) (Fig. 2a), had a gelatinous appearance in 3 (50%) and 2 (33.33%) were flat lesions (Fig. 3a). All lesions showed the involvement of conjunctiva and limbus while cornea was involved only in 50%. All OSSN had ≥ 1 feeder vessels supplying and disappearing into the mass. Dilatation and tortuosity were two common components of all the vessels supplying the tumour.

A mean of 7.33 weeks (range 6–8 weeks) of 'on-cycle' of topical 0.02% MMC topical chemotherapy was noted, without any clinical signs of improvement, and was labelled as resistant OSSN. The side-effect profile of topical MMC was the foreign-body sensation in 6 (100%), punctum stenosis causing symptomatic epiphora in 5 (83.33%), superficial punctate keratitis (SPK) in 4 (66.67%) and diffuse conjunctival injection in 3 (50%) patients.

All patients were shifted to reconstituted topical recombinant IFNα-2b eyedrops, i.e. topical immunotherapy. None of the patients underwent an incisional biopsy for histological confirmation of OSSN before starting the immunotherapy. No therapy modification or therapy switch was required in any of the patients. None received any subconjunctival or local injections of IFNα-2b around the tumour.

The clinical response to treatment was noted and documented on each follow-up visit (Figs. 2, 3, 4). The side effects of MMC like foreign-body sensation, diffuse conjunctival injection and SPKs disappeared in every patient at the first week follow-up visit after starting immunotherapy. Epiphora secondary to punctum stenosis resolved at the second week visit in 4 while in one it persisted till 4 weeks. All the patients responded favourably to topical IFNα-2b immunotherapy but the nasal tumours (3–4 weeks) showed a delayed response compared to the temporal (2 weeks) ones. The reduction of tumour mass size, extent, intrinsic vascularity and the reduction in the calibre and tortuosity of the feeder vessel were noted as the signs of tumour regression. Typically, the feeder vessels became straight and of near-normal calibre in

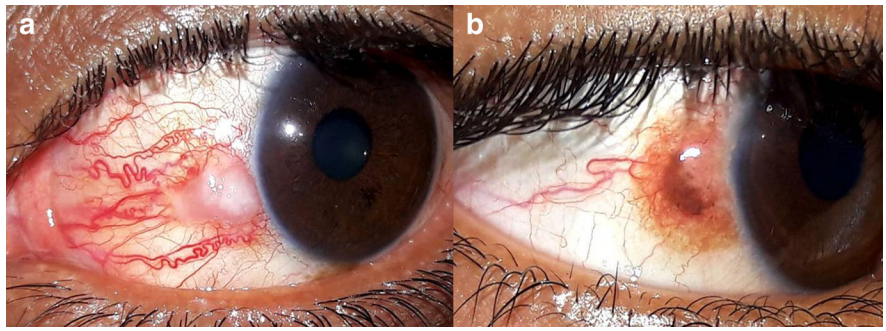

Fig. 1 **a** Left eye showing raised nodular nasal OSSN, extending from 8 to 9 o'clock position. The cornea and limbus appear spared. Tortuous and dilated feeder vessels appear dipping into the tumour mass. Surface keratinisation is prominent, and localised congestion is present. **b** A pigmented temporal lesion of right eye from 8 to 10 o'clock position with a single feeder vessel. Marked pigmentation and gelatinous surface are well appreciated. Limbus and cornea appear clear

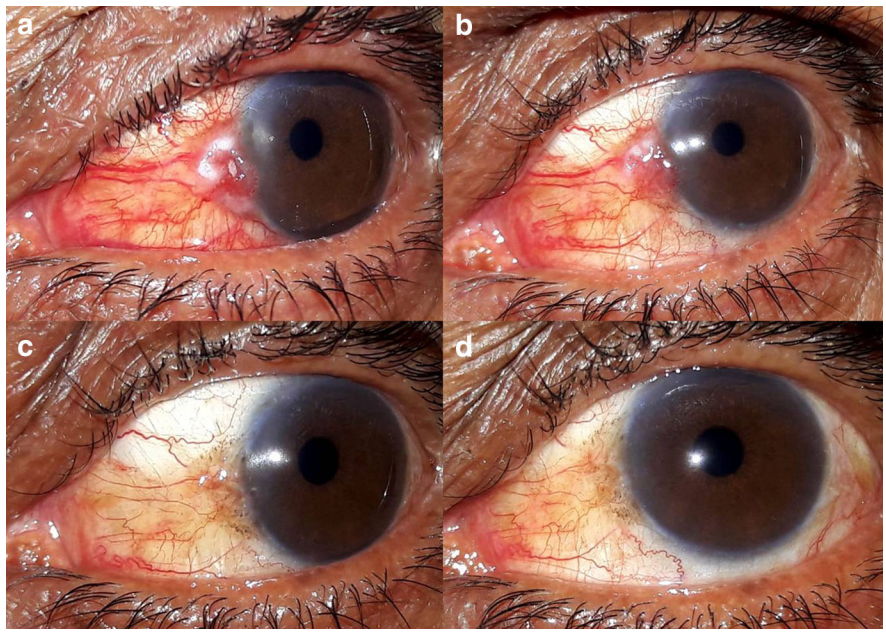

Fig. 2 **a** Left eye shows a nasal lesion (8–10 o'clock) with presence of surface keratin. Dilated and tortuous feeder vessels are seen supplying and disappearing into the tumour mass. Marked conjunctival congestion secondary to topical MMC is evident. Conspicuous limbus and corneal involvement is noticeable. **b** At 4 weeks follow-up, patient showed marked reduction of conjunctival congestion and tumour vascularity. Tumour size and surface keratinisation have also reduced. **c** At 8 weeks, conjunctival congestion, tumour size, calibre of feeder vessels and surface keratinisation has shown near-total reduction. **d** At 12 weeks, the tumour mass, conjunctival congestion, surface keratin has disappeared. The blood vessels have become normal in calibre. Mild pigmentation has appeared at the lesion site

all patients. The keratin present over the surface of OSSN disappeared while minimal residual pigmentation at the end of treatment (Fig. 2d).

All patients used preservative-free carboxymethylcellulose 1% eyedrops QID 15 min before putting reconstituted IFNα-2b eyedrops. Topical immunotherapy had mild and transient adverse effects like foreign-body sensation in 2 and follicular conjunctivitis in 1 patient. Follicular conjunctivitis was treated with a weekly course of mild potency topical steroids. None experienced any systemic flu-like illness or allergic reaction.

The total treatment duration was 24 weeks. The mean time for tumour resolution was 16 weeks (range

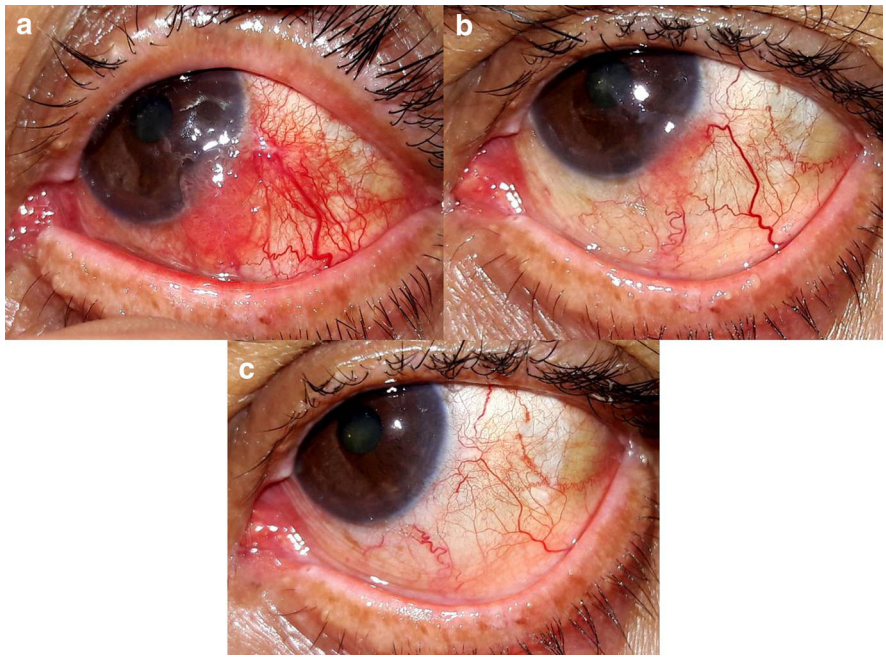

Fig. 3 **a** Left eye of a 62-year-old male showing diffuse conjunctival congestion, an OSSN raised lesion measuring 8.5 × 6 mm with conjunctival (major), limbal and corneal component is evident. Dilated and tortuous feeder vessels are seen along with intrinsic vascularity of tumour. **b** At 4 weeks, marked reduction of conjunctival congestion, tumour size, intrinsic vascularity and feeder vessel calibre and tortuosity. **c** At 12 weeks, the tumour has disappeared with normal conjunctival blood vessel calibre and straightening

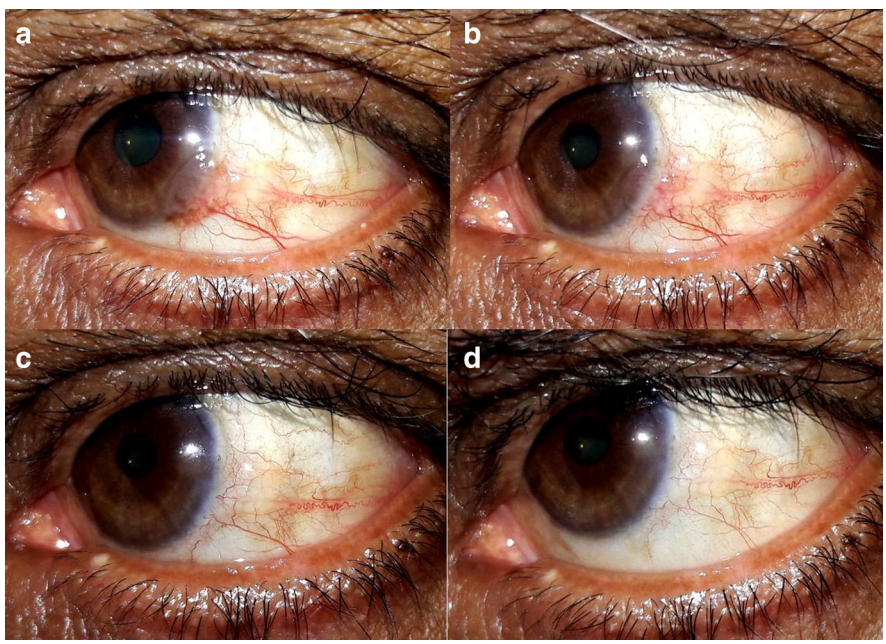

Fig. 4 **a** Left eye shows an infero-temporal, flat, oval-shaped OSSN lesion (3–5 o'clock) with pigmentation at 5 o'clock end. One dilated prominent feeder vessel seen. The conjunctiva is locally congested. **b** At 2 weeks, the lesion size, tumor vascularity, pigmentation and conjunctival congestion has reduced. **c** At 8 weeks, the tumour and pigmentation has nearly disappeared clinically. Minimal feeder vessel dilatation is seen. **d** At 16 weeks, the eye looks normal with no tumour mass and normal conjunctival vasculature

12–20 weeks). At a mean follow-up of 14.5 weeks after stopping topical immunotherapy, no clinical recurrence was observed in any patient (Figs. 2d, 3c, 4d). At the end of treatment, all the patients were asymptomatic and without any ocular congestion. On an average, one reconstituted vial (3 ml) lasted 2 weeks which costs approximately 700 Indian rupees (INR) to the patient. The total therapy session, i.e. 24 weeks (6 months), approximately costs 8400 INR.

Discussion

Over the years, the treatment paradigms for many diseases have changed, i.e. shift from surgery to 'conservative' medical management or from 'routine' surgery to minimally invasive surgery. A similar global trend has been witnessed in recent years for the management of OSSN. Traditional surgical treatment for OSSN included a 'no-touch technique' excisional biopsy ± cryotherapy ± alcohol corneal epitheliectomy. Despite the histopathological clear margins, the surgery has a potential risk of OSSN recurrence up to 33% [11]. If surgical excision is performed for extensive OSSN, it may lead to symblepharon formation and limbal stem cell deficiency [1, 2, 12]. These potential complications have led to the rise of medical treatment which may completely treat the OSSN or adequately reduce the tumour bulk and make it amenable for surgery.

The clinical evaluation of OSSN should include location (clockwise or quadrantic) of the tumour epicentre, basal dimensions (in mm), morphological appearance, surface pigmentation or keratinisation and tumour vascularity (intrinsic and feeder vessels) [13, 14]. All these features help in the tumour assessment and monitoring of the treatment. The OSSN lesions have a 'surface-growing' nature which usually does not correlate with its deeper corneal or scleral penetration [14, 15]. The typical clinical features of OSSN can provide sufficient diagnostic clues for establishing a working diagnosis and guiding the course of management. Though the imaging modalities like anterior segment-optical coherence tomography, ultrasound biomicroscopy and specular microscopy may show an in vivo depth analysis of OSSN, the clinical evaluation remains the basis of diagnosis [16, 17]. In past, an incisional biopsy was routinely performed for the histopathological diagnosis of OSSN but the amount of harvested tissue was always a crucial factor for small/flat OSSN lesions. In recent times, the majority of the OSSN lesions are diagnosed clinically and managed with medical treatment. All of our patients were clinically diagnosed and assessed before, during and after stopping the topical immunotherapy.

In 1994, Maskin first described the use of topical interferon in the regression of limbal epithelial dysplasia [18]. Since then, the topical interferon has been used in the management of OSSN but the higher cost of this drug has always remained an issue. With time, the availability and affordability of IFN has improved. The interferons are endogenously produced glycoprotein molecules secondary to the tumours and viral infections. Hence, IFN has inherited antiviral and anti-neoplastic properties [3, 19]. A recent trend has also been observed for its use in primary or recurrent cases of OSSN and conjunctival papillomas, the latter having human papilloma virus as the aetiology [19, 20]. Being an endogenous glycoprotein, the molecular structure of IFN incites minimal local side effects and is best tolerated by the eyes as compared to the chemotherapeutic agents.

In a case–control study, Nanji et al. [1] observed no statistical difference in the recurrence rate of OSSN in surgical (5%) versus medical (3%) treatment groups using Kaplan–Meier survival analysis for 1-year recurrence. They used topical IFN and found that the initial no-response of immunotherapy might reverse with the longer treatment. The conjunctival involvement of OSSN and higher pathological grade of the tumour were the risk factors for its recurrence. Overall, the recurrence rates of OSSN with topical IFN treatment ranges from 0 to 17% over a follow-up of 7–42 months [2, 6, 13, 19].

The initial flat lesions of OSSN should be clinically diagnosed and managed appropriately. Widespread OSSN renders surgical excision an extremely challenging and damaging task with a risk of limbal stem cell deficiency, symblepharon formation and diffuse conjunctival scarring [21, 22]. The topical drops can target approximately every part of the ocular surface and the drug may act on the dislodged tumour cells as well, hence reducing the tumour recurrence rate. Topical immunotherapy should be initiated in patients with flat or raised OSSN lesions for the purpose of tumour resolution or tumour mass reduction (to make it amenable for surgical resection), respectively.

Ours is the first-ever report mentioning about the possible resistance of topical MMC chemotherapy in the OSSN patients. Though we had a small sample size and limited follow-up period, in our experience, the topical immunotherapy has shown better and promising results for OSSN treatment than the chemotherapeutic agents. Our article also provokes the thoughts and discussion about the need for molecular research on the resistance of topical chemotherapeutic agents used in OSSN treatment. The immunohistochemistry may provide better insights about the resistance of

tumours and may lead to the foundation of future studies. In conclusion, the increasing trend of medical management as compared to surgical excision of OSSN may reveal more cases of non-responding or resistant tumours. We believe that due to prominent local side effects of topical MMC, the ineffective action or possible resistance of MMC might have been underreported. Similar reports and long-term follow-up studies are required to validate our results.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent The written informed consent was obtained from all the participants before starting topical interferon therapy.

References

1. Nanji AA, Moon CS, Galor A, Sein J, Oellers P, Karp CL (2014) Surgical versus medical treatment of ocular surface squamous neoplasia: a comparison of recurrences and complications. Ophthalmology 121:994–1000
2. Sturges A, Butt AL, Lai JE, Chodosh J (2008) Topical interferon or surgical excision for the management of primary ocular surface squamous neoplasia. Ophthalmology 115:1297–1302
3. Poothullil AM, Colby KA (2006) Topical medical therapies for ocular surface tumors. Semin Ophthalmol 21:161–169
4. Wilson MW, Hungerford JL, George SM, Madreperla SA (1997) Topical mitomycin C for the treatment of conjunctival and corneal epithelial dysplasia and neoplasia. Am J Ophthalmol 124:303–311
5. Yeatts RP, Ford JG, Stanton CA, Reed JW (1995) Topical 5-fluorouracil in treating epithelial neoplasia of the conjunctiva and cornea. Ophthalmology 102:1338–1344
6. Shah SU, Kaliki S, Kim HJ, Lally SE, Shields JA, Shields CL (2012) Topical interferon alfa-2b for management of ocular surface squamous neoplasia in 23 cases: outcomes based on American Joint Committee on Cancer classification. Arch Ophthalmol 130:159–164
7. Luqmani YA (2005) Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract 14:35–48
8. Housman G, Byler S, Heerboth S, Lapinska K, Longacre M, Snyder N, Sarkar S (2014) Drug resistance in cancer: an overview. Cancers 6:1769–1792
9. Gorlick R, Goker E, Trippett T, Waltham M, Banerjee D, Bertino JR (1996) Intrinsic and acquired resistance to methotrexate in acute leukemia. N Engl J Med 335:1041–1048
10. Ashkenazy N, Karp CL, Wang G, Acosta CM, Galor A (2017) Immunosuppression as a possible risk factor for interferon nonresponse in ocular surface squamous neoplasia. Cornea 36:506–510
11. Tabin G, Levin S, Snibson G, Loughnan M, Taylor H (1997) Late recurrences and the necessity for long-term follow-up in corneal and conjunctival intraepithelial neoplasia. Ophthalmology 104:485–492
12. Stone DU, Butt AL, Chodosh J (2005) Ocular surface squamous neoplasia: a standard of care survey. Cornea 24:297–300
13. Shields CL, Kaliki S, Kim HJ, Al-Dahmash S, Shah SU, Lally SE, Shields JA (2013) Interferon for ocular surface squamous neoplasia in 81 cases: outcomes based on the American Joint Committee on Cancer classification. Cornea 32:248–256
14. Shields CL, Demirci H, Karatza E, Shields JA (2004) Clinical survey of 1643 melanocytic and nonmelanocytic conjunctival tumors. Ophthalmology 111:1747–1754
15. Kao AA, Galor A, Karp CL, Abdelaziz A, Feuer WJ, Dubovy SR (2012) Clinicopathologic correlation of ocular surface squamous neoplasms at Bascom Palmer Eye Institute: 2001–2010. Ophthalmology 119:1773–1776
16. Kieval JZ, Karp CL, AbouShousha M, Galor A, Hoffman RA, Dubovy SR, Wang J (2012) Ultra-high resolution optical coherence tomography for differentiation of ocular surface squamous neoplasia and pterygia. Ophthalmology 119:481–486
17. Xu Y, Zhou Z, Xu Y, Wang M, Liu F, Qu H, Hong J (2012) The clinical value of in vivo confocal microscopy for diagnosis of ocular surface squamous neoplasia. Eye 26:781–787
18. Maskin SL (1994) Regression of limbal epithelial dysplasia with topical interferon (letter). Arch Ophthalmol 112:1145–1146
19. Boehm MD, Huang AJ (2004) Treatment of recurrent corneal and conjunctival intraepithelial neoplasia with topical interferon alfa 2b. Ophthalmology 111:1755–1761
20. Singh M, Gautam N, Gupta A, Kaur M (2016) Interferon alfa-2b in the management of recurrent conjunctival papillomatosis. Indian J Ophthalmol 64:778–780
21. Peksayar G, Altan-Yaycioglu R, Onal S (2003) Excision and cryosurgery in the treatment of conjunctival malignant epithelial tumours. Eye (Lond) 17:228–232
22. Siganos CS, Kozobolis VP, Christodoulakis EV (2002) The intraoperative use of mitomycin-C in excision of ocular surface neoplasia with or without limbal autograft transplantation. Cornea 21:12–16