Vaccination with human tyrosinase DNA as a therapy for equine intra-ocular melanoma - 4 cases: 2016-2021

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Summary

This report describes four cases of intraocular melanoma which were treated with a course of human tyrosine DNA canine melanoma vaccine1. Two cases continued to show progression of the tumour. One case presented with bilateral intra-ocular melanomas and demonstrated clinical progression in one eye within five months, and apparent quiescence of disease in the other, though subtle deterioration was noted at two year follow-up. The final case presented with recurrent ulceration due to an intraocular melanoma and demonstrated a good clinical response with a reduction in the frequency and severity of the episodes of ulceration. The small number of cases in this report precludes firm conclusions, but these reported outcomes suggest limited justification for the routine recommendation of melanoma vaccines to horses with intra-ocular melanomas.

Keywords: horse, melanoma, Oncept, vaccine, ocular Introduction

Melanomas are common tumours in grey horses and the proportion of horses affected has been reported to increase linearly with increasing age (MacKay, 2019). Most equine melanomas exhibit benign behaviour, displaying rare mitotic figures, marked pigmentation and ambiguous cellular atypia (Fleury *et al.*, 2000). Frequently reported predilection sites

include the perianal area , prepuce and penis, the lips, the eyelids and invasion of the parotid salivary glands (Fleury *et al.*, 2000; MacKay, 2019)

Melanocytic tumours involving the eye, adnexa or orbital tissues are uncommon and most frequently involve the periocular skin (Myrna and Sheridan, 2019). Intraocular masses are rare, and generally occur concurrently with cutaneous melanomas (Myrna and Sheridan, 2019). Whilst some can be static or slow growing, masses which contact the cornea have the potential to cause corneal oedema, with secondary keratitis, uveitis and recurrent ulceration. Glaucoma may also develop if the irideocorneal angle becomes obstructed (Myrna and Sheridan, 2019). Enucleation is curative but invasive, and not preferred in cases of bilateral ocular pathology. Sector iridectomy has also been successfully reported, but carries a high risk of intra-operative complications such as haemorrhage or incomplete excision, which leaves a high potential for regrowth (Scotty *et al.*, 2008).

There are no medical treatment options with proven efficacy for intraocular melanomas. A xenogenic DNA vaccine encoding human tyrosinase1 is licensed for treatment canine oral melanoma. Equine tyrosinase shares 90% homology to human, and is over-expressed in melanomas (Phillips *et al.*, 2012). The treatment concept relies on the vaccine’s ability to induce tyrosinase-specific immunoreactivity against the melanoma tumour cells (Lembcke *et al.*, 2012). This has received attention as a non-invasive adjunct or alternative to surgical excision of cutaneous melanoma. One recent study compared melanoma growth between a group of vaccinated and unvaccinated horses using a linear mixed model, and found no significant increase in tumour volumes in the vaccinated group over a median period of 231 days, compared to the unvaccinated group where significant

 expansion of tumour volume was seen (Echelmeyer, J., 2019). The recommended course consists of four injections at two-week intervals, followed by six monthly booster injections, delivered via a needle-free injection device (Phillips *et al.*, 2011). This case series is the first to report the use of the vaccine in horses with intra-ocular melanoma and provides preliminary evidence regarding its utility as an alternative to surgical excision.

Case 1

A 15-year-old Connemara grey gelding presented in March 2016 for management of multiple melanomas. Ophthalmic examination revealed bilateral intra-ocular masses. Menace response was present in the right eye but absent in the left eye. Dazzle reflexes were present bilaterally.

Examination of the left eye demonstrated a large, pigmented mass occupying the entire temporal region of the pupil, with only the nasal aspect visible (Figure 1). Ultrasound examination of the left eye demonstrated a dense, echogenic mass in contact with the posterior corneal surface. Marked opacity of the lens was noted, consistent with the presence of a mature cataract and the lens appeared displaced posteriorly. Intraocular pressure was measured using a hand-held tonometer2 and was increased in the left eye (27.6mmHg) compared to the right (11mmHg). Examination of the right eye demonstrated a smaller pigmented mass in the inferio-temporal region of the iris (Figure 2a), which had a homogenous echogenic appearance on ultrasound (Figure 2b).

An initial course of the melanoma vaccine was administered, consisting of four vaccines fourteen days apart. At the fourth vaccine appointment (6 weeks after initial presentation); the intra-ocular melanomas were visually and ultrasonographically reassessed and had not changed in size or appearance. The gelding re-presented in June 2016 for surgical removal of melanomas from other locations. Repeat ultrasonographic assessment of the masses was performed at this time, and no change had occurred.

The gelding re-presented in August 2016 following the development of corneal oedema, blepharospasm and excessive lacrimation in the left eye. Based on the development of ocular pain and lack of response to treatment; enucleation of the left eye was performed. Repeat visual and ultrasonographic assessment of the right eye confirmed no change in mass dimensions, but it appeared more heterogenous in structure, and a small protuberance on its nasal aspect was noted.

The horse returned in February 2017 for repeat vaccination. and ultrasound confirmed no change in dimensions of the mass, but several hyperechoic regions were noted in the centre (Figure 3).

A repeat booster vaccination was administered in August 2017, and at last follow-up in March 2018, two years after initial presentation, the gelding presented for a further booster. At this time the mass had changed in dimensions and developed multiple lobes, with the anterior section discoloured white. In addition, a focal area of corneal thickening adjacent to the mass was observed, where previously there had been no corneal pathology, suggesting subtle deterioration (Figure 4).

Case 2

A 9-year-old Irish Sports Horse grey gelding presented in May 2017 for assessment of an intra-ocular mass within the left eye. Ocular examination revealed a moderate area of corneal oedema in the inferior and nasal aspects of the cornea, occupying approximately 20% of the corneal surface. Within this portion of the anterior segment, brown discolouration was evident. In addition to this, adherent to the iris in the superior and slightly nasal aspect of the eye, a second pigmented mass was also evident (Figure 5). Ultrasonography was performed which confirmed masses of homogenous echogenicity, with the ventral mass in contact with the surface of the cornea (Figure 6).

The gelding returned for the initial course of four melanoma vaccinations in July 2017. No significant progression or worsening of the corneal oedema had occurred at this time. The gelding received the first three vaccines in the primary course without incident, but at the fourth vaccine appointment (six weeks after the first) diffusely increased corneal oedema was present. Ultrasonographic assessment was performed which revealed no change in dimensions of the masses, but the cornea had a curved appearance, and the left eye measured 2mm larger in the anteroposterior axis than the right (Figure 7), likely consistent with the development of glaucoma, although intra-ocular pressure measurements were not available. The horse was discharged on a combination of dorzolamide and timolol. Follow-up obtained from the referring vet in September 2017 indicated that the appearance of the corneal oedema had improved but no change in the intra-ocular mass was observed.

Case 3

A 6-year-old Connemara -cross grey mare presented to the hospital in July 2018 for investigation of recurrent ulceration and uveitis in the left eye. The owner reported five episodes of recurrent uveitis and ulceration of the left eye since purchase one year previously. A blemish in the left eye had been noted on the pre-purchase exam.

On presentation, the mare displayed moderate blepharospasm and ocular discharge from the left eye. A large opacity was present in the inferio-nasal cornea, extending to the limbus, with some perilimbal pigment migration. In addition, there were multiple linear endothelial opacities across the cornea (Figure 8). Fluorescein uptake was evident only along the dorsal margin of the opacity and the pupil was partially miotic. Ultrasonography of the left eye demonstrated an echogenic mass in the iridocorneal angle, extending along the posterior surface of the cornea (Figure 9). Intraocular pressure in the left eye was 13mmHg. The miosis and ulceration were treated at that time with topical atropine, chloramphenicol and oral phenylbutazone and resolved within three days.

The mare presented for reassessment five days later. The focal area of corneal opacity was reduced in size and intensity and was now fluorescein negative. The mass could now be visualized and it was noted to be pigmented and consistent in appearance with a melanoma.

An initial course of four vaccinations was administered. During this, the mare had one episode of mild corneal ulceration which was managed medically as before. Ultrasonography at the time of the fourth injection (six weeks after presentation) was very similar.

The horse presented for a booster vaccination in February 2019. The owner reported three episodes of corneal ulceration in the previous 6 months, which responded quickly to treatment. Visually the mass appeared similarly to previous examinations, and on ultrasonography no change in dimensions were noted, but the centre of the mass appeared less echoic than previously.

The mare re-presented in August 2019 for reassessment. Ultrasonographically the mass had reduced in size and altered in shape. The owner reported an increasing interval between episodes of corneal ulceration, with the last episode occurring 17 weeks prior. A further vaccine was given at this time.

The mare returned in February 2020 for repeat booster vaccination. In the six months prior, the owner reported no evidence of corneal ulceration. The corneal oedema on the ventral aspect of the cornea was reduced in size, and ultrasound assessment of the eye confirmed a reduction in the size of the melanoma. In light of the good response, the owner opted to return for booster vaccinations every six months. The most recent visit was in February 2022, at which time the eye was largely unchanged from previous assessments, and the owner reported that there had been two episodes of corneal ulceration in the last 6 months.

Case 4

A 7-year-old Irish Draught grey mare presented in September 2020 for assessment of an intraocular mass in the right eye and associated corneal oedema.

Examination of the right eye revealed a large pigmented mass occupying the temporal and inferior aspects of the anterior segment, and partially obstructing the pupil. Over the central area of the mass there was some diffuse corneal oedema (Figure 10). Intraocular pressure in the right eye was 26mmHg and 16mmHg in the left eye. Ultrasound examination confirmed the mass had an echogenicity consistent with a suspected melanoma (Figure 11). The mare received the initial course of four vaccinations. Initially, the mass was unchanged, but at the fourth vaccine appointment (six weeks after presentation) the corneal oedema was noted to have worsened, though the eye remained comfortable.

The mare presented for reassessment in April 2021. Ocular examination revealed buphthalmos and increased intraocular pressure in the right eye (50mmHg), compared to the left eye (20mmHg), though both eyes retained a menace response and dazzle reflex. Marked corneal oedema extended across most of the surface of the right eye (Figure 12).

Ultrasound examination confirmed enlargement of the melanoma from the previous assessment, and distortion of the globe as well as thickening of the cornea. In light of the deterioration, the owner elected to proceed with enucleation of the right eye.

Discussion

Three out of the four cases in this series demonstrated progression of intraocular melanoma or worsening secondary effects after commencement of the vaccine course. In two cases, deterioration occurred within six weeks of starting the course, and in the other, within 12 weeks of finishing the four primary injections. One case demonstrated some response to the vaccine in the form of marked reduction in frequency of episodes of corneal ulceration, and alteration in size and shape of the melanoma. In three cases where progression was noted, it is impossible to say whether the disease would have progressed faster if the horses had remained unvaccinated – the lack of a placebo control group is a limitation of the study in this respect.

The poor treatment response in this report is likely due in part to the unique immunology of the equine eye. Clinical trials performed in dogs with advanced melanoma have correlated the induction of a peripheral tyrosinase-specific antibody response with observed clinical responses (Liao *et al.*, 2006). The cornea has low antigenicity and possesses multiple immunomodulatory and immunosuppressive factors which modify the behaviour of immune cells which enter the eye. These mechanisms maintain the transparency of the cornea and the reduce the likelihood of inflammatory reactions (Hori et al., 2010). Anterior chamber associated immune deviation (ACAID) is one immunomodulatory pathway which maintains ocular immune privilege, and it has been hypothesized that vaccination could activate this mechanism and therefore dampen the immune response; in this case against the local tumour antigens and prevent tumour regression (Rohrbach *et al.*, 2005). No previous work has reported on the presence or absence of anti-tyrosinase antibody within the eye following canine melanoma vaccination, but studies measuring *Leptospira* antibodies in the aqueous following systemic vaccination, provide evidence that antibodies can cross an intact blood-ocular barrier (Rohrbach *et al.*, 2005).

The apparent response of Case 3 is an interesting finding. This horse initially presented with multiple episodes of recurrent ulceration and uveitis and with the eye in a pro-inflammatory state. It is possible that the mechanisms which usually act to maintain immune privilege in the eye had become dysregulated in the presence of active inflammation; allowed the anti-tumour immune response to act within the cornea without suppression. This phenomenon has been reported in humans undergoing corneal transplants; where the presence of prior inflammation and therefore compromise of immune privilege causes allograft rejection, as at any non-privileged site (Hori, Vega and Masli, 2010).

The canine melanoma vaccine is not licensed in horses and can only be obtained by certified specialists, which limits its utility to general practitioners. One clinical trial has been conducted which reports suppression of tumour growth in vaccinated horses compared to unvaccinated; but this is limited to a single population of Lipizzaner horses with cutaneous melanoma (Echelmeyer, J., 2019). In the UK, the initial four vaccine course is costly, and to then continue with booster vaccinations every six months is a significant long-term financial commitment. Other non-surgical treatment modalities which have received attention include oral cimetidine, a histamine H2 receptor antagonist, administration of which in an early case series resulted in partial to complete remission in 3 horses (Goetz *et al.*, 1990), however a more recent controlled study documented no clinical effect (Laus *et al.,* 2010).

This case series has several limitations. Firstly, the outcomes of the small number of cases reported is not sufficient to provide high quality evidence either supporting or contravening vaccine use. Case follow-up is 18 months to two years in three out of four cases, but Case 2 developed secondary changes suspicious of glaucoma within the eye and was lost to follow-up within a few months of presentation. Secondary ocular signs which developed despite three vaccine doses suggests lack of efficacy, though the corneal oedema did improve in response to dorzolamide and timolol. The lack of longer-term follow-up precludes firm conclusions in this case, since it was not established if any change to the mass had been observed following completion of the course.

Furthermore, in the cases where clinical progression was noted either during or after the vaccine course, it is impossible to say whether the speed of deterioration was affected by its administration – a placebo control group would have been required to evidence this. Histological confirmation of tumour type was also not performed in any case. Differential diagnoses of pigmented masses within the anterior chamber can include uveal and corpora nigra cysts and iris hypoplasia (Barnett and Platt, 1990). The characteristic appearance, secondary ocular signs (e.g. corneal oedema) and presence of concurrent cutaneous melanomas observed in each of these cases was deemed sufficient to reach a diagnosis of melanoma. Biopsy of intraocular tissues would have provided a definitive diagnosis. This is extremely invasive however, and the histopathological appearance of equine melanomas is highly variable, and provides little information on clinical behaviour (Myrna and Sheridan, 2019).

In conclusion, the outcomes in this case series are unconvincing to justify routine recommendation of the canine melanoma vaccine for intra-ocular melanoma. It remains the only non-invasive treatment alternative for melanomas in this location, and is associated with minimal reported adverse effects (Lembcke *et al.*, 2012). Careful case selection is warranted prior to recommendation.

Manufacturer’s addresses

1. Oncept; Merial, Merial Limited, Duluth, GA
2. Tonovet, iCARE, Icare Finland Oy, Aryitie 22/Tuike, FI-01510 Vantaa, Finland

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Figure Legends

Figure 1: A heavily pigmented mass occupying the entire temporal region of the left eye of Case 1, obstructing most of the pupil.

Figure 2a: A small, pigmented mass is present in the ventro-temporal region of the right eye of Case 1

Figure 2b: Ultrasound confirms the mass in the right eye of Case 1 to be homogenous and echogenic. Dorsal is to the left of the image.

Figure 3: Ultrasonographic image of the right eye of Case 1 in February 2017. The centre of the mass has several hyperechoic regions, giving a more heterogenous appearance. Nasal is to the left of the image.

Figure 4: The mass in the right eye of Case 1, 2 years after initial presentation. The anterior section is discoloured, and the mass has changed in dimensions and shape. A focal area of corneal thickening was observed, indicating subtle deterioration.

Figure 5: An elliptical area of dense corneal oedema, occupying the ventral and nasal aspect of the left eye of Case 2

Figure 6: Ultrasonographic image of the left eye of Case 2, demonstrating a dorsal echogenic mass on the iris and a ventral mass in contact with the cornea. Dorsal is to the left of the image.

Figure 7: The left eye of Case 2, 3 months after presentation. Diffuse corneal oedema is evident obscuring visualisation of the anterior chamber of the left eye. This eye measured 2mm greater in anteroposterior axis than the right eye, consistent with the development of glaucoma.

Figure 8: A large opacity is present in the ventronasal cornea of the left eye of Case 3, extending to the limbus. Perilimbal pigment migration is evident, along with multiple linear endothelial opacities across the surface of the cornea.

Figure 9: Ultrasonographic image of the left eye of Case 3. A homogenous echogenic mass is present at the ventral irideocorneal angle, extending along the posterior surface of the cornea. Dorsal is to the left of the image.

Figure 10: A large pigmented mass occupies the temporal and ventral portions of the anterior segment of the right eye of Case 4, partially obstructing the pupil. Diffuse corneal oedema is evident over the central portion of the mass.

Figure 11: Ultrasonographic image of the right eye of Case 4, demonstrating a mass of soft tissue echogenicity occupying the width of the anterior chamber and contacting the corneal surface. Dorsal is to the left of the image.

Figure 12: The right eye of Case 4 approximately six months after initial presentation. Marked corneal oedema extends over most of the corneal surface with straie present, suggesting secondary glaucoma due to obstruction of the drainage angle.