Cytokine-enhanced vaccine and suicide gene therapy as adjuvant treatments of metastatic melanoma in a horse

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EQUINE melanomas occur most commonly in grey horses five years of age or older (Smith and others 2002). Affected horses often have encapsulated nodules, where malignant and benign melanomas can be distinguished by microscopy. The features of the disease in grey horses include spontaneous progression, with development of metastases in other organ systems (Baker and Leyland 1975), and the occurrence of disease in genetically predisposed individuals (Rieder and others 2000). Surgical excision can stimulate rapid regrowth of tumours, and is therefore not performed, and cryosurgery has displayed only limited success (MacGillivray and others 2002). In addition to the use of intralesional cisplatin reported by Théon and others (2007), there are no other standard treatments for equine melanoma.

The efficacy of DNA vaccines against a viral disease (rabies) in horses has been demonstrated (Fischer and others 2003), but only one attempt at gene therapy in equine cancer has been reported (Heinzerling and others 2001). In that study, established melanoma metastases in seven grey horses were injected with a plasmid encoding the immunostimulatory human interleukin-12. This treatment produced a significant regression of the treated lesions (approximately 60 per cent) and appeared to be safe.

In dogs with melanomas, intratumour injections of lipid-complexed plasmid DNA encoding the herpes simplex virus thymidine kinase (HSV*tk*) suicide gene sensitised transfected cells to ganciclovir (Finocchiaro and others 2008). Even when only a minority of tumour cells were transfected, the treatment induced substantial regression of the tumours due to the bystander effect (Mesnil and Yamasaki 2000). Furthermore, the release of previously unrecognised tumour antigens by lysis of tumour cells enabled recognition, processing and presentation of these antigens by antigen-presenting cells. The administration of irradiated xenogeneic cells genetically modified to secrete human interleukin-2 (hIL-2) and human granulocyte-macrophage colony-stimulating factor (hGM-CSF), together with formolised tumour extracts to non-immunosuppressed dogs with melanoma was proposed by Finocchiaro and Glikin (2008a) as a coadjuvant of suicide gene therapy and a booster of the immune response against melanoma. This strategy halted the state of relative immune tolerance and promoted a strong immune response against a broader array of tumour antigens, leading to an inhibition of tumour growth, or tumour rejection. The repeated induction of a potent localised immune stimulus by xenoantigens, together with the secretion of cytokines by γ-irradiated engineered xenogenic cells, was able to elicit a powerful antitumour response that delayed or prevented recurrence and metastasis of the tumours (Finocchiaro and Glikin 2008a).

Based on the experiences with spontaneous melanoma in dogs described above, the combination of local suicide gene therapy with a systemic anticancer vaccine was used in a grey stallion with metastatic melanoma, following surgical removal of superficial tumours.

The horse was chosen for the study after the diagnosis of melanoma was confirmed histopathologically. During the study, the horse did not receive chemotherapy or any other potentially antitumour or immunosuppressive medication. The horse's owner was notified about the experimental nature of the treatment, and gave written informed consent.

Superficial tumours were removed surgically while the horse was under general anaesthesia. Multiple grouped superficial melanoma lesions on the foreskin and, 90 days later, in the perianal region were removed. The excised tumours were used to prepare a vaccine that was subcutaneously injected into the flanks as described below. Immediately after surgery, the surgical margins of the cavity left after removal of the tumours were infiltrated with lipoplexes (plasmid DNA:DMRIE/DOPE) carrying the HSV*tk* gene (10 mg DNA) codelivered with 100 mg ganciclovir, evenly distributed at multiple sites in the surrounding areas and/or in the residual tumour masses. From one week after the first surgery, the horse was treated once a week for five weeks with subcutaneous injections of a vaccine composed of autologous formolised tumour cells (0·5 ml pellet) and live irradiated xenogenic Chinese hamster ovary (CHO) cells producing 20 to 30 µg of hIL-2 and hGM-CSF. Both hIL-2 and hGM-CSF have been reported to have effects on cells of the equine immune system (Stott and Osburn 1988, Hammond and others 1999). At the same time as these injections, the subcutaneous tumours (but not the surgical margins) that were not removed surgically (described below) received multiple injections of lipoplexes carrying the HSV*tk* gene (1 to 4 mg DNA) codelivered with 10 to 40 mg ganciclovir, depending on the size of the tumour. These treatments continued until signs of local disease had disappeared. After these injections, the horse was treated with the subcutaneous vaccine every 14 days up to day 105, and then at intervals of 28 days, up to day 245. Follow-up continued for 33 months. A clinical evaluation was performed on every treatment day. The culture of cytokine-producing CHO cells, plasmids, liposome preparation, in vivo lipofection and tumour vaccine preparation was carried out as described by Finocchiaro and Glikin (2008a).

Isolated subcutaneous melanoma masses in the horse's neck, back and shoulders were not removed surgically but injected with the HSV*tk* gene-carrying plasmid plus ganciclovir. After five weekly applications of the treatment to six tumour masses (average diameters ranging 3 to 6 cm), four of the tumours developed draining fistulae that healed and dissolved completely; the other two decreased in size by approximately 50 per cent and subsequently stabilised. Three additional lesions, which were untreated, decreased in size until they disappeared completely.

Multiple injections into the tumour beds of the lipoplexes carrying the suicide gene, together with the subcutaneous administration of cytokine-secreting xenogenic cells, was apparently safe and non-allergenic, could be applied repeatedly, and completely prevented a local postsurgical relapse. No undesirable side effects were observed. The staff caring for the horse reported that the combined treatment restored the horse's quality of life towards what it was before the disease developed, with improvements in its vigour, activity, mood, appetite, state of alertness and general welfare. Thirty-three months after starting the treatment, the horse was reported to have maintained its improved quality of life, had had full reproductive

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function during three seasons, and showed no local relapses of melanoma in the areas that had been treated surgically.

The success of the experimental approach used in this horse was probably due to four factors. First, the surgical margin of the cavity left after removing each tumour was infiltrated with a lipid-complexed plasmid bearing a suicide gene, co-administered with ganciclovir, evenly distributed at multiple sites in the margin. The new margin generated by the suicide gene significantly delayed or prevented post-surgical recurrence of the tumours (Finocchiaro and Glikin 2008a). Secondly, injections of lipid-complexed suicide gene into the remaining tumours circumvented tolerance and immunosuppression mediated by the tumour, providing an immunostimulatory microenvironment necessary for immune cells activated by the vaccine to more efficiently destroy the residual tumour (Ramesh and others 1996). Immunostimulatory sequences in the plasmid DNA (Roman and others 1997) and the cationic lipids (Li and others 1998) would have increased the immunogenicity of the tumour. The postsurgical suicide gene therapy, together with the subcutaneous vaccine, had a powerful and synergistic antitumour effect, shown by a significant reduction (ranging from approximately 50 per cent to 100 per cent) in the size of subcutaneous tumours and the lack of observed local postsurgical recurrence. Thirdly, as a co-adjuvant to the suicide gene therapy and to boost the immune response against the melanoma, a subcutaneous autologous vaccine enhanced with engineered hGM-CSF and hIL-2 secreting xenogenic cells was administered (Soiffer and others 1998, Rochlitz and others 2002). This whole-tumour vaccine immunised the horse against a broad array of tumour surface antigens, increasing the likelihood of effective immunostimulation. Finally, the combined treatment did not produce significant adverse side effects, such as acute xenograft rejection or delayed-type hypersensitivity.

Due to its experimental nature, this particular treatment requires specially trained veterinary professionals, working in accordance with the laws and regulations of the country where it is performed.

To be accepted for clinical practice, any new anticancer therapy should not only increase the survival time but also improve the quality of life; this experimental treatment achieved at least the second of these. In addition, the findings support the usefulness of studies on spontaneously occurring tumours in companion animals as a valid translational model for the evaluation of novel therapies for use in larger domestic animals and, possibly, human patients (Finocchiaro and Glikin 2008b). The encouraging results described here suggest the need for a clinical trial of the treatment in a larger number of horses, to assess its suitability to prevent and/or treat advanced metastatic equine melanoma.

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