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Case report

ECHO-7 oncolytic virus Rigvir® in an adjuvant setting for stage I uveal melanoma; A retrospective case report

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ABSTRACT

Purpose: To describe a case of choroidal melanoma treated with Rigvir® virotherapy in an adjuvant setting.
Observations: A female patient born in 1956 presented with a small choroidal melanoma in October 2007. 34 months after transpupillary thermotherapy the state of her eye worsened until tumor growth was visualized. Despite photodynamic therapy and transpupillary thermotherapy the tumor continued to grow locally. In October 2016 enucleation was performed. Since gene expression profile testing disclosed a tumor (class 2) with a high risk of metastasis formation in 5 years, the patient sought options to prevent progression of the disease. In December 2016 virotherapy with Rigvir® was started with 3 administrations for 3 consecutive days. Therapy was continued once per week until March 2017, when the administrations were changed to once per month. The patient is being monitored by an ophthalmologist. She is stable with the virotherapy ongoing and magnetic resonance cholangiopancreatography (7 May 2018) and abdominal ultrasound (23 March 2019) imaging excludes metastasis formation. The quality of life is high.
Conclusions: To the best of our knowledge, this is the first documented case of uveal melanoma treatment with virotherapy as an adjuvant therapy. Considering the few if any available treatments and the encouraging results of the present treatment, virotherapy should be evaluated more extensively as a potential treatment of uveal melanoma.

1. Introduction

Uveal melanoma is considered a rare disease, arising from choroidal melanocytes, iris or ciliary body. The disease ultimately leads to development of metastases in almost 50% of patients. Even with intensive management, circulating uveal melanoma cells may still be found in the patients, even if there are no clinically detectable lesions present.[1] Since uveal melanoma spreads hematogenously, metastases form mostly in the liver, less frequently in lungs, skin/soft tissue, and bones, thus significantly worsening the prognosis.[1,2] Although blurred vision is a common symptom for primary uveal melanoma, as many as 30% of patients do not present any symptoms, leading to late diagnosis of the disease.[1]

The choroid is the most common to develop cancer in the eye. According to the AJCC studies of staging for nonmetastatic primary choroidal and ciliary body melanomas, the 5-year survival rate decreases drastically with every stage. The 5-year survival rate for stage I is 96–97%, for stage IIIC it is only a mere 25–26%, with the 50% overall survival being slightly above 2 years. The expected 10-year survival for stage I patients is 88–94% and for stage IIIB patients 27–50%. In case of metastatic disease, the 50% overall survival is about 5–18 months, depending on the substage.[3]

Enucleation is frequently used to treat large tumors. In most cases, if possible, conservative methods such as radiotherapy are preferred.[3] Treatments used presently are known to have fairly good results in local control, but not without quite frequent complications.[1] New treatment options are actively sought.

Oncolytic viruses are presently being investigated as a treatment for cancer. Oncolytic virotherapy has shown good tolerability without severe toxicity.[4]

Rigvir® is an oncolytic, nonpathogenic, not genetically modified ECHO-7 virus selected and adapted for melanoma.[5] In a retrospective study Rigvir® showed a substantial improvement in overall survival with a 4.39- to 6.57-fold lower mortality in melanoma stage IB-IIC patients.[6]

The aim of this report is to describe a case of a choroidal melanoma

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patient that has been treated with Rigvir® after radical surgery in an adjuvant setting.

2. Case report

In October 2007 a mildly hyperpigmented lesion in the left eye was spotted in a fundus photo of a female patient born in 1956. The lesion was located just outside the inferior arcade. An ultrasound (US) was performed, showing a lesion with an elevation of close to 2 mm and high internal reflectivity. The patient was asymptomatic – no floaters, flashes or pain had been observed. Taking into consideration the strong family history of malignant melanoma (mother and aunt), the patient was submitted to a doctor who specializes in ocular oncology for further examination. A repeated dilated fundus examination (11 October 2007) disclosed a small choroidal melanoma in the posterior pole inferior to the inferior arcades. There was some evidence of exudation in the macula, as well as orange pigment overlying the lesion. A B-scan US showed the lesion to be approximately 1.3 mm in elevation and about 4.5 mm in diameter.

On 15 October 2007 the patient underwent transpupillary thermotherapy (TTT). A positron emission tomography/computed tomography (PET/CT) scan on 19 October 2007 excluded metastatic disease. At the next follow-up examination on 27 November 2007, a B-scan US showed that the tumor appeared to be sclerotic and flattened (approximately 1.0 mm in thickness). Subsequently, the patient had follow-up visits to the doctor every 4 months; the tumor was stable, without any new symptoms or complaints from the patient (Fig. 1).

In August 2010 the patient experienced conjunctivitis-like symptoms. A primary care physician prescribed eye drops. However, the eyes got worse with blurry vision (without flashes or floaters), followed by strong redness and itchy burning. The use of the drops was stopped but the vision did not improve. After 3 months the patient visited an ophthalmologist. The symptoms, however, remained until December 2013, then the vision got worse, when floaters appeared in the left eye in February 2014. The visual acuity continued to worsen until February 2016, when a floater in the direct line of vision became stable. Fundus photography and enhanced depth imaging (EDI) on 16 February 2016 showed tumor growth with fluid leakage (0.285 mm of growth since the last checkup). The patient underwent photodynamic therapy (PDT) (2 March 2016). One month after PDT the vision of the left eye slightly improved but the floater was still present (EDI showed less fluid in the left eye). Six weeks later visual acuity once again became worse. Fundus photography and optical coherence tomography (OCT) visualized orange pigment and mild subretinal fluid centrally in the left eye. During the next 5 months the vision continued to deteriorate, while the tumor slowly continued to grow, and reached 1.3 mm in thickness on 13 September 2016.

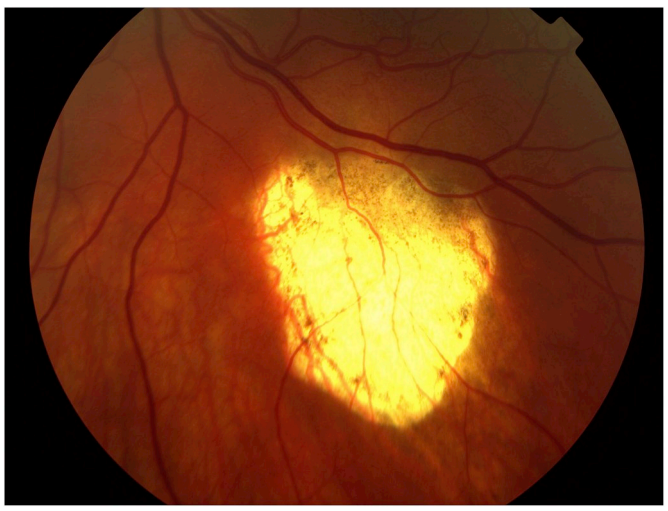

Fig. 1. Fundus photo of the lesion on 3 September 2009.

It was decided to take a fine needle aspirate biopsy (21 September 2016) and send the sample to gene expression testing (23 September 2016). The patient was examined by DecisionDx-UM primary tumor gene expression profile (GEP) testing. This test is used by over 90% of US ocular oncology institutions to individualize the patients' care plans after eye surgery.[7] In this assay RT-PCR is used to detect the expression of 12 marker genes (CDH1, ECM1, EIF1B, FXR1, HTR2B, ID2, LMCD1, LTA4H, MTUS1, RAB31, ROBO1, SATB1) and 3 control genes (MRPS21, RBM23, SAP130) in tumor tissue.[8–10] The test provides classification into class 1A (very low risk, with a 2% chance of the eye cancer spreading over the next 5 years), class 1B (low risk, with a 21% chance of metastasis over 5 years), and class 2 (high risk, with 72% odds of metastasis within 5 years). The DecisionDx-UM test is performed in a College of American Pathologists (CAP)-accredited, Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory[11] in accordance with published guidelines.[12,13] The test is approved by the New York State Department of Health.[14] Very small (0.1–1.5 μg total RNA) amounts of tissue have been shown to be sufficient for accurate molecular profiling.[15] Only samples containing at least 80% tumor nuclei density are used for the test.[16] Technical success for the clinical test measured by the number of samples with a reportable Class 1 or Class 2, has been reported to be over 97%.[9] The GEP test has been clinically validated in a prospective study,[8] and is mentioned in the AJCC[3,17] and included as a prognostic test in the NCCN uveal melanoma guidelines.[18,19] The present GEP test yielded a molecular signature class 2 tumor with high risk of formation of metastases.

Several treatment options were offered to the patient: radiation plaque therapy, PDT, TTT, enrollment in clinical trial and enucleation. As per recommendation, on 27 September 2016, TTT was performed. However, during the following month the patient had intermittent pain in the left eye and her central vision was affected. Then, taking into account the class 2 GEP test result, family history of melanoma, and concomitant heart disease, the patient decided in favor of enucleation. Enucleation of the left eye with removal of the optic nerve was performed on 20 October 2016. The primary malignant melanoma tumor was diagnosed as pT1a; no regional lymph nodes (pNX) and no distant metastases (pMX) were found. Histological examination showed ocular choroidal tumor cells composed of spindle and epithelioid cells with pigmented cytoplasm and large polymorphic nuclei with prominent nucleoli. The tumor was attached to the inner surface of the posterior wall of the globe with the largest basal and vertical diameters 5.4 mm and 1.3 mm, respectively. There was visible growth of tumor into the sclera (< 0.1 mm) but no growth into the ciliary bodies. Mitotic activity was 3 mitoses per 10 high power field (HPF). Extracellular periodic acid Schiff (PAS+) staining loops were detected from at least 3 neighboring micronodular structures. Immunohistochemistry demonstrated weak, moderate or strong cytoplasmic HMB45 staining in 90% of tumor cells, and strong nuclear Ki67 staining in 3% of tumor cells (Fig. 2).

Taking in consideration the results of the gene expression profile (GEP) assay and the family history, the patient had fears about cancer recurrence. The patient was against undergoing radio- and chemotherapy, so other options were sought. Virotherapy appeared appropriate and on 28 December 2016 virotherapy with Rigvir® was started with 3 administrations for 3 consecutive days. Subsequently, administrations were once per week until 7 March 2017, when the administrations were reduced to once per month. The virotherapy is still ongoing and is well tolerated. Serum clinical chemistry parameters were graded according to NCI CTCAE. Values above grade 2 were not observed during Rigvir® therapy. LDH is within reference range. The patient had phosphohexose isomerase tested before virotherapy; on 6 December 2016 it was 61.8 U/L. After 3 months of virotherapy, on 17 March 2017, it was 24.3 U/L, which is within the reference range. The patient regularly visits the ophthalmologist and pays attention to her health; no negative changes have been observed post-surgery. A

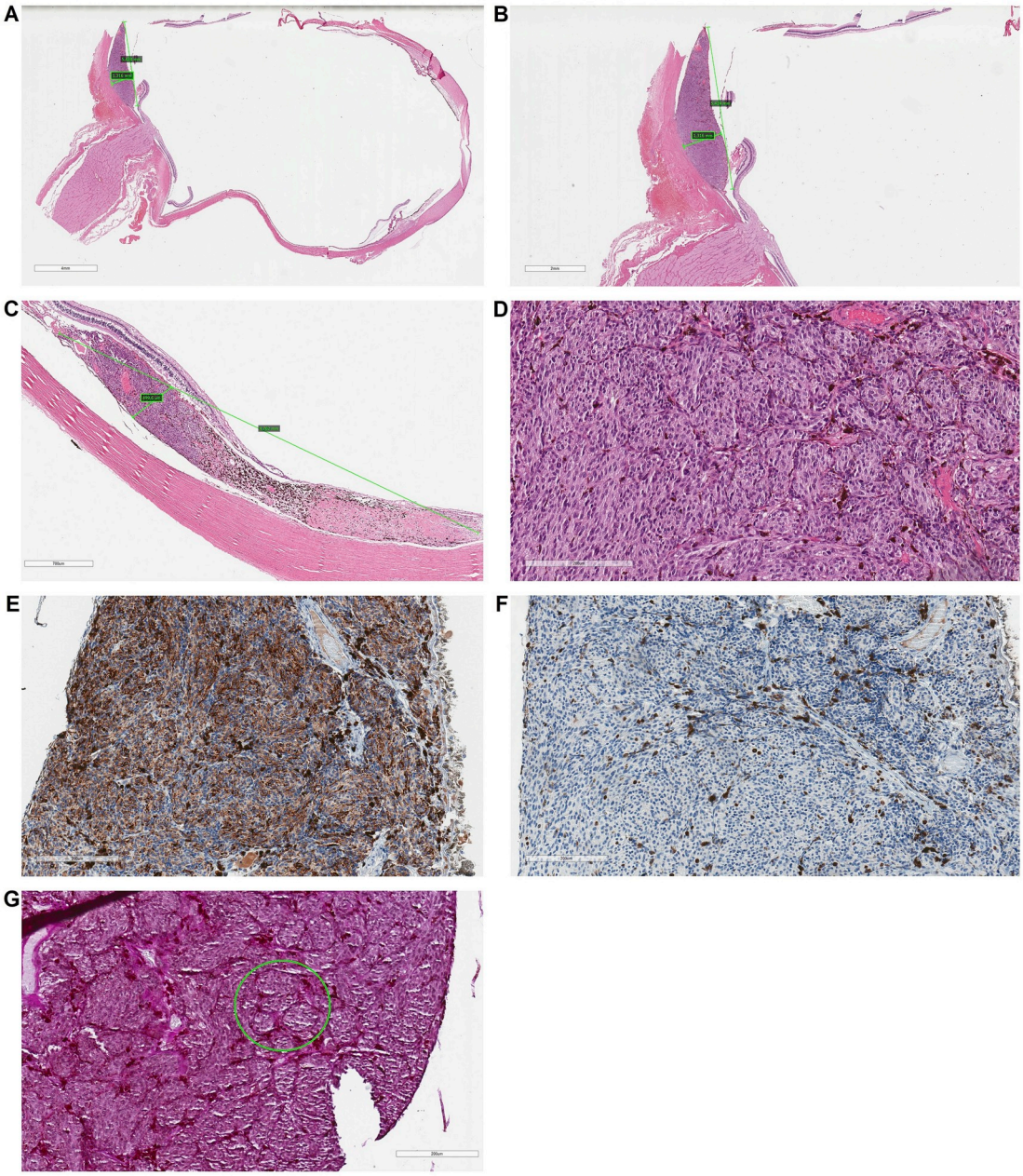

Fig. 2. Representative photomicrographs of choroidal melanoma, hematoxylin-eosin staining.
A, B, C. Tumor attached to the inner surface of the posterior wall of the globe with the largest basal and vertical diameter is 5.4 mm and 1.3 mm. Scale bars are 4 mm, 2 mm and 700 μm, respectively.
D. The tumor is composed of spindle and epithelioid cells. Scale bar is 200 μm.
E. Weak, moderate and strong cytoplasmic HMB45 staining. Scale bar is 200 μm.
F. Strong nuclear Ki67 staining. Scale bar is 200 μm.
G. Extracellular PAS-positive loops. Scale bar is 200 μm.

magnetic resonance cholangiopancreatography (MRI MRCP) scan (7 May 2018) and abdominal US (23 March 2019) also do not show any evidence of disease. The quality of life was assessed in June 2019 by the Functional Assessment of Cancer Therapy – General (FACT-G) Version 4 questionnaire, achieving a FACT-G Total score of 102 of maximal 108.

The patient described in this report used to work as a teacher. She has an active and healthy lifestyle. She has had tonsillectomy, open heart surgery to manage an atrial septal defect (in 2012). Due to heart problems she has hypoxemia in the blood. She eats mostly organic non-processed foods and avoids taking sugar or other sweeteners, does not consume alcohol or smoke, avoids direct sunlight and tanning beds. Although there is not any eye disease in the family history, there is a history of malignant melanoma; her mother had melanoma on the calf by the ankle at the age of 85 and her aunt had it in the hip area; the aunt was diagnosed in late stage with distant metastases and died at the age of 78.

3. Discussion

Prognosis has a very important role in the management of uveal melanoma. There are several aspects involved in metastatic risk prediction such as cytogenetic, transcriptomic, clinical and histopathological factors. It has been observed that 8q and 6p rearrangements and chromosome 3 loss, which are found in most uveal melanoma patients,

worsens the prognosis and increases the metastatic risk. Monosomy 3 is most often used as an indicator for uveal melanoma metastases since it is rarely found in other cancers.[1,2] Monosomy 3 is said to occur quite early in the course of the disease and may initiate isochromosome 8q and 6p formation.[20]

Comparing the gene expression profile (GEP) to standard prediction methods, there are some differences that makes GEP more accurate. Firstly, methods involving chromosomal testing require a generous quantity of tumor tissue, which can only be achieved after enucleation. Secondly, there are sampling error risks due to intratumoral heterogeneity. DecisionDx-UM demonstrates lower intratumoral heterogeneity than the monosomy 3 test, which is considered as one of the most important metastatic risk indicators.[21,22]

While local disease management is usually quite successful, prevention and treatment of metastatic disease is very problematic. Presence of dormant uveal melanoma cells is not uncommon. It is said that immunosurveillance may have a role in preserving the dormant state of the micrometastatic lesions.[23]

Malignant uveal melanoma contains tumor infiltrating lymphocytes, the most dominant being $CD3^+/CD8^+$ or $CD4^+$ and $CD8^+$ cells. It has been suggested that $CD8^+$ cell activation might help to destroy uveal melanoma cells.[24] Cytotoxic T cell accumulation at tumor sites have been observed in cases of oncolytic adenovirus treatment, as oncolytic viruses are known to activate antitumoral immune response.[25]

The lack of efficacious therapies is reflected in the large number of clinical trials. Many treatment options are being investigated, for example, ipilimumab, interferon and vaccine therapy for adjuvant setting and anti-CTLA-4 antibodies, anti-PD-1/PD-L1 antibodies, dendritic cell vaccination and liver directed therapies for metastatic disease. In general, the response of uveal melanoma to treatment is very limited with rare or no response observed. Although immune checkpoint inhibitors have shown good results in treating cutaneous melanoma, still, due to differences in tumor biology, the impact on uveal melanoma is not comparable, most probably because of the low mutational burden.[26]

The oncolytic adenovirus oncorine in combination with dacarbazine had a synergistic effect in uveal melanoma cell lines *in vitro*.[27] There have also been studies of combining oncorine therapy with Bcl2 pathway downregulation[28] and GNAQ expression downregulation,[29] using small interfering RNA.

The cytolytic effect of Rigvir® has been tested on uveal melanoma cell lines 92-1, MP41 and MEL202 *in vitro*. The maximal inhibition of cell growth by 1% and 10% Rigvir® compared to control (PBS) was 92.37% and 95.54% for 92-1, 86.38% and 85.88% for MP41, and 99.34% and 99.40% for MEL202 at 96 h. The results suggest that Rigvir® *in vitro* reduces the viability of uveal melanoma cells (Tilgase et al., submitted; 2020).

The present case report shows good results in a choroidal melanoma patient, primarily treated with TTT, PDT, enucleation and with Rigvir® virotherapy as an adjuvant therapy. The patient was diagnosed more than 12.3 years ago with a relapse 3.9 years ago. The patient started Rigvir® therapy 2 months after enucleation, considering the high-metastasis-risk result obtained in the GEP test and family history. She is stable and feeling well according to the recent FACT-G (version 4) questionnaire.

To our knowledge, this is the first documented case of oncolytic virotherapy as an adjuvant therapy for uveal melanoma. Considering the lack of treatments in the adjuvant setting for uveal cancer, this case report could be considered as noteworthy. Future research in the field is required, since there are not many studies focused on oncolytic viruses as a treatment for uveal melanoma in any type of setting – as monotherapy, in combination with other drugs, as an adjuvant, or as metastatic disease treatment, respectively. Emphasis should be put on the interplay between the tumor microenvironment and the immune system since in uveal melanoma cases a distinct interaction between the cancer cells and immune cells has been observed and it plays a part in the development and spread of the disease, as well as its susceptibility to certain treatments.[30]

4. Conclusions

Since the management of uveal melanoma is very challenging and the overall survival ratings are poor, it is very important that new treatment options are being investigated. This case report describes a patient that was diagnosed in 2007, had a relapse in 2016, when the tumor was classified high metastatic risk class 2 in a GEP test, and has after enucleation been treated with Rigvir® oncolytic virotherapy with good tolerability. At the time of writing, in January 2020, the patient is stable, feeling well, with the virotherapy still ongoing. Considering the encouraging results, studies involving oncolytic viruses as a treatment for uveal melanoma should be considered.

Patient consent

A written consent to publication has been obtained from the patient.

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Authorship

All authors attest that they meet the current ICMJE criteria for Authorship.

Declaration of competing interest

IJ and LB are employees of AmberLife Cancer Clinic. AR and PA are employees of the Rigvir group. DP has no financial disclosures.

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