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Case Report

Long-term treatment with the oncolytic ECHO-7 virus Rigvir of a melanoma stage IV M1c patient, a small cell lung cancer stage IIIA patient, and a histiocytic sarcoma stage IV patient-three case reports

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Oncolytic virotherapy is a recent addition to cancer treatment. Here, we describe positive treatment outcomes in three patients using Rigvir virotherapy. One of the patients is diagnosed with melanoma stage IV M1c, one with small cell lung cancer stage IIIA, and one with histiocytic sarcoma stage IV. The diagnoses of all patients are verified by histology or cytology. All patients started Rigvir treatment within a few months after being diagnosed and are currently continuing Rigvir treatment. The degree of regression of the disease has been determined by computed tomography. Safety assessment of adverse events graded according to NCI CTCAE did not show any value above grade 1 during Rigvir® treatment. Using current standard treatments, the survival of patients with the present diagnoses is low. In contrast, the patients described here were diagnosed 3.5, 7.0, and 6.6 years ago, and their condition has improved and been stabile for over 1.5, 6.5, and 4 years, respectively. These observations suggest that virotherapy using Rigvir can successfully be used in long-term treatment of patients with melanoma stage IV M1c, small cell lung cancer stage IIIA, and histiocytic sarcoma stage IV and therefore could be included in prospective clinical studies.

Key words: Histiocytic sarcoma; immunotherapy; melanoma; oncolytic virotherapy; Rigvir; small cell lung cancer; virotherapy.

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Oncolytic virotherapy is an active immunotherapy in cancer treatment (1, 2). Rigvir is the first virus that has been approved as an oncolytic cancer treatment. It has been registered for melanoma treatment in Latvia since 2004 and is included in the national guidelines for melanoma treatment in Latvia where approximately 75% of melanoma patients are treated with Rigvir (3–5). Oncolytic virotherapy has recently been added as a cancer treatment tool in the USA (6, 7). Rigvir has been shown to significantly reduce the mortality 4.39- to 6.57-fold in melanoma stage IB-IIC patients in a retrospective study (3, 4). Untoward side effects and discontinuation of treatment are rare. Rigvir has also been used in other cancers, for example, in gastric and rectal cancer patients, where 5-year survival was improved (3, 8–10).

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Malignant neoplasms such as melanoma, small cell lung cancer, and histiocytic sarcoma are all characterized by aggressive progression (11–15). The incidence of melanoma has been increasing in the last decades, and it is a major health concern. Despite research and progress in melanoma therapy, mortality rates still remain high and melanoma is one of the most common cancers in the Western world. Small cell lung cancer is considered to be the most aggressive lung cancer subtype of neuroendocrine origin. Small cell lung cancer response to chemotherapy and radiation therapy is high; however, recurrence is common and long-term survival does not exceed 5% (16–18). Histiocytic sarcoma is a rare hematopoietic disease characterized by malignant proliferation of cells that resemble mature hepatocytes according to their phenotypic, morphological, and immunological properties (19, 20). Since only a

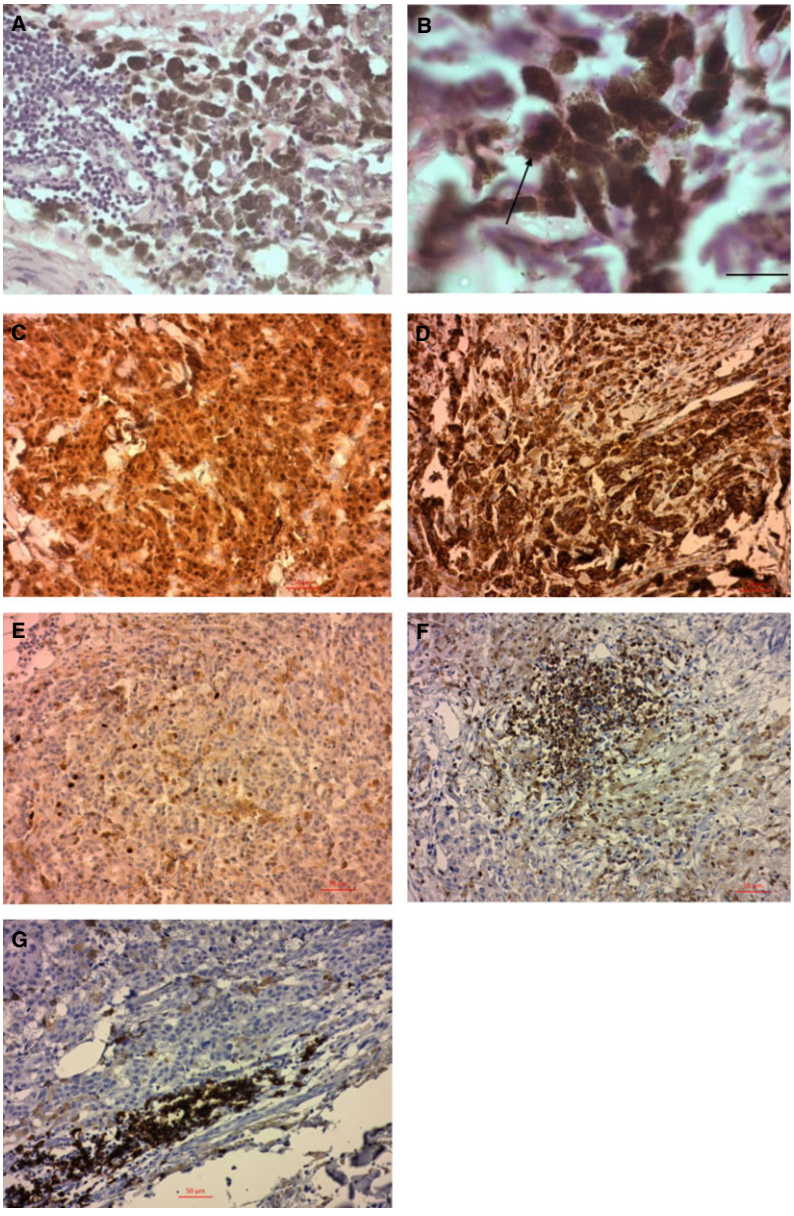

Fig. 1. Malignant epithelioid and nevoid melanoma cells. Hematoxylin and eosin stain, magnification (A) ×200, and (B) ×400. Arrow indicates melanoma cells. Scale bar is 100 μm (A and B). (C) S-100 antigen-positive cells. (D) Human melanoma black 45 (HMB45) antigen-positive cells. (E) Ki-67 antigen-positive cells. (F) Intratumoral lymphoid infiltrate with strong CD3 membrane staining. (G) Intratumoral lymphoid infiltrate with strong CD8 membrane staining. Scale bar is 50 μm (C–G).

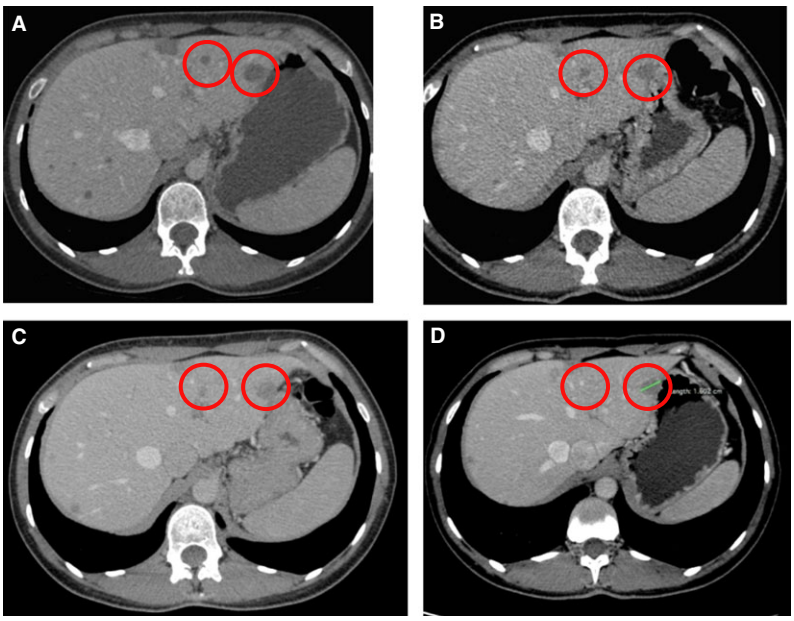

Fig. 2. Liver CT of the melanoma patient. Contrast-enhanced CT late phase (contrast) shows no visible change in multiple metastases in liver parenchyma (encircled). (A) August 22, 2013, (B) December 1, 2014, (C) May 25, 2015, (D) April 14, 2016.

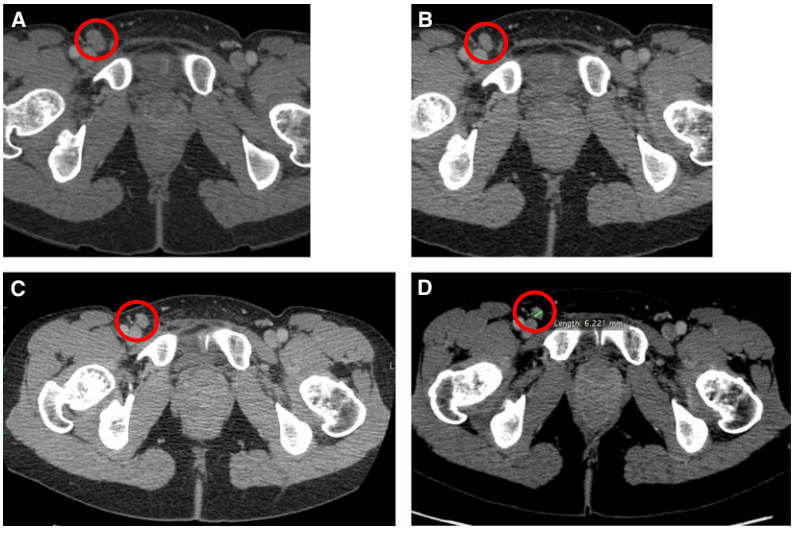

Fig. 3. Inguinal lymph node CT of the melanoma patient. Abdominal contrast-enhanced CT late phase (contrast) shows an enlarged inguinal lymph node (encircled) on the right side that is reduced in size by half between August 22, 2013, and December 1, 2014, and that subsequently has stabilized in size. (A) August 22, 2013, (B) December 1, 2014, (C) May 25, 2015, (D) April 14, 2016.

limited number of histiocytic sarcoma cases have been described (less than 100) and often has poor response to therapy, the disease lacks an accepted standard treatment (11, 14, 21). The aim of this study was to describe long-term virotherapy with Rigvir of a melanoma stage IV M1c patient, a small cell lung stage IIIA cancer patient, and a patient with histiocytic sarcoma stage IV.

MATERIALS AND METHODS

The patients were diagnosed at their respective hospital and subsequently approached the International Virotherapy Center. The study has been approved by the local ethics committee. Written consent has been obtained from the patients for anonymous publication of material relating to them.

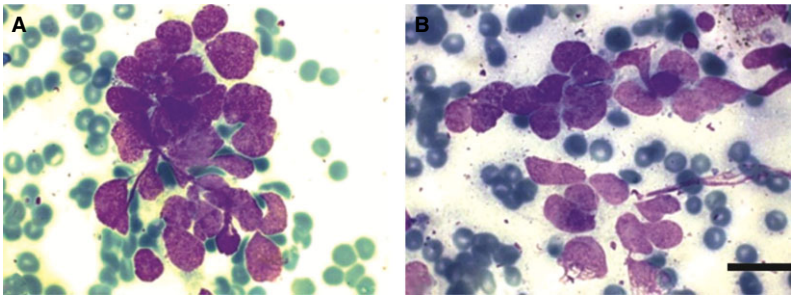

Fig. 4. Cytology of biopsy samples of the small cell lung cancer patient. Lymph node small cell cancer cells (purple) and erythrocytes (blue-green). Light microscopy. Giemsa stain. Scale bar is 100 μm.

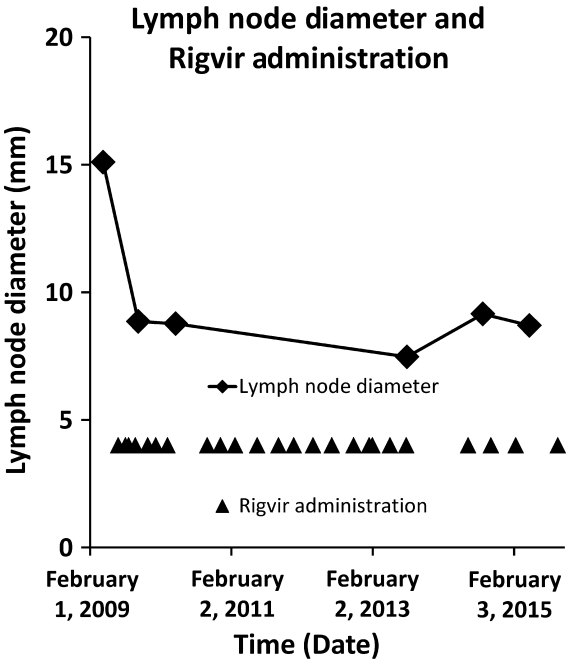

Fig. 5. Thoracic lymph node diameter change and therapy dates of the small cell lung cancer patient. The diameter of an enlarged lymph node that decreased in size with time and stabilized during Rigvir treatment (▲) is shown.

Rigvir was obtained from the marketing authorization holder SIA Latima, Kūdras iela 7-8, LV-2114 Olaine, Latvia.

Rigvir characteristics

Rigvir is a 2 mL frozen solution of an adapted and selected ECHO-7 virus strain; *Picornaviridae* family, *Enterovirus* genus, Enteric Cytopathic Human Orphan (ECHO) type 7, group IV, positive-sense single-stranded RNA virus produced under GMP. The titer is $\geq 10^6$ $TCID_{50}$/mL in sodium chloride for injection and is administered intramuscularly regionally. While Rigvir therapy can be individualized, physicians that have been certified to use Rigvir by the International Virotherapy Center and the Latvian Virotherapy Association are provided with guidelines on the use of Rigvir that they may consult to make practical and informed decisions about specific details of treatment (22).

Safety

In the previous clinical studies, a few side effects were reported, for example, subfebrile temperature (37.5 °C for a couple of days), pain in the tumor area, sleepiness, and diarrhea. In this study, serum clinical chemistry parameters were recorded and graded according to NCI CTCAE (23).

RESULTS

Case 1: Melanoma IV M1c

A woman born in 1972 diagnosed with malignant melanoma in the lumbar paraspinal region had the tumor surgically removed in December 2012; lymph nodes were left untouched. Histological examination of the surgical material confirmed the diagnosis of stage IV M1c melanoma cutis dorsi, Clark V, Breslow 8–9 mm, pT4bNxM1c, S-100 antigen positive, human melanoma black 45 (HMB45) antigen positive, Ki-67 index 10–15%, intratumoral lymphoid infiltrate with strong CD3 and CD8 membrane staining, with liver and inguinal lymph node metastasis (Fig. 1).

Postsurgery in January 2013, the patient received one palliative chemotherapy course of Lomustine (200 mg per os) and ondansetron. From week 5, the patient had fever for 2 weeks where antipyretic therapy was ineffective, accompanied by weakness, nausea, vertigo, lack of appetite, impairment of coordination, hallucinations, and inguinal lymph node swelling. The chemotherapy was discontinued.

Rigvir therapy was started in February 2013 with daily administrations for 3 days. After 4 weeks, another three daily administrations were made. Subsequently, administration was regular

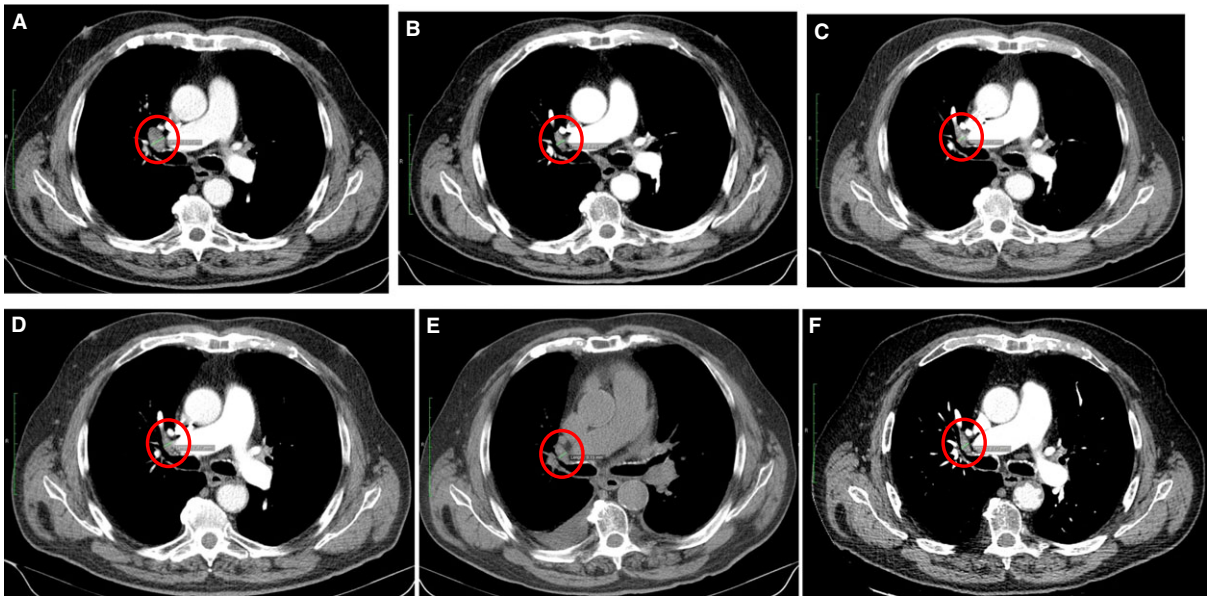

Fig. 6. CT of the small cell lung cancer patient. Contrast-enhanced CT late phase (contrast) shows an enlarged lymph node (encircled) on the right side that decreased in size with time and stabilized. (A) April 9, 2009, (B) October 8, 2009, (C) April 20, 2010, (D) July 29, 2013, (E) August 25, 2014, (F) April 23, 2015.

every week. After 24 months, the administration interval was reduced to once every 2 weeks. Rigvir treatment is being continued. The patient has received no other concomitant treatment. The number and size of liver lesions show no visible change (Fig. 2). Moreover, comparison of the size of an inguinal lymph node shows a reduction in size ca. twofold, followed by stabilization (Fig. 3).

When tested starting about 6 months after surgery, circulating lactate dehydrogenase (LDH) levels are within the normal reference range of the laboratory and the S-100 antigen levels are below the reference threshold.

Lymphocyte subpopulations were measured in blood samples taken in April 2016. The CD3+ (absolute count), activated T-lymphocytes CD3+HLA-DR+ (absolute and relative count), CD8+ (absolute count), and HLA-DR+ (absolute count) were below the reference range. CD38+ (relative levels) and the ratio of T helpers CD4+/T suppressors CD8+ were above the reference range. Serum clinical chemistry parameter values above grade 1 according to NCI CTCAE (23) were not observed.

Thus, the patient′s condition has improved and has been stable since December 2014.

Case 2: Small cell lung cancer stage IIIA

A man born in 1934, long-term and still a smoker, with no comorbidity, had shortness of breath, dyspnea, and cough and was diagnosed with right upper lobe small cell lung cancer (pT2N2M0) in May 2009. Metastases were found in the thoracic mediastinal lymph nodes. Fiber bronchoscopy showed enlarged lymph nodes in the right lung. Cytological examination of biopsy samples of lymph nodes showed poorly differentiated small cell lung cancer (Fig. 4); the patient was diagnosed with small cell lung cancer by two cytologists.

The patient has been treated with Rigvir since June 2009, and the treatment is continuing (Fig. 5). During the first month, Larifan was also prescribed weekly; the patient has not received any other concomitant treatment.

Lymphocyte subpopulations were measured in blood samples taken in June 2009 and June 2016. In comparison, the levels of natural killer cells (CD16+ and CD56+), CD4+, and the ratio of T helpers CD4+/T suppressors CD8+ are normalized. The levels of CD3+, CD8+, CD19+, and CD45+ were all within reference range.

Comparison of CT scans shows that the diameter of enlarged lung lymph nodes decreased in size and subsequently normalized (Fig. 6).

Serum clinical chemistry parameter values above grade 1 according to NCI CTCAE (23) were not observed.

Thus, the patient′s condition has improved and has been stable since October 2009.

Case 3: Histiocytic sarcoma IV

A man born in 1970 had pain in the right side of the abdomen, fever, and lost about 10 kg of body weight in a short period of time. Histology of biopsy samples from lymph nodes of the left side of the neck in October 2009 shows characteristic histiocytic sarcoma (Fig. 7). The biopsy samples were positive for the specific markers CD68, CD163, and lysozyme (14, 24) as well as for CD8, CD43, S-100, and leukocyte common antigen (LCA). Ki-67 index was 80%; the cells were negative for cytokeratin AE1/AE3 (Ck AE1/AE3), epithelial membrane antigen (EMA), CD4, CD56, and HMB45, as determined by immunohistochemistry.

The patient has not had surgery. In October 2009, he was prescribed symptomatic treatment. Rigvir has been administered on average every 3 weeks for 6 years, except for two intermissions, and the treatment is continuing.

In the autumn of 2011, the patient was referred by the Virotherapy Center to the Oncological Center for a medical examination. He was then treated with radiotherapy applied to the lymph nodes of the neck, the para-aortic and iliac lymph nodes, and of the mediastinum, with 6 courses of doxorubicin and cyclophosphamide. At the Oncological Center, he has received Helixor P for some time besides Rigvir (cf. (25)).

Lymphocyte subpopulations were measured in blood samples taken in November 2009 and June 2016. In comparison, the levels of CD3+ (absolute count), CD4+ (absolute and relative count), and the ratio of T helpers CD4+/T suppressors CD8+ are decreased. The levels of CD3+ (relative value),

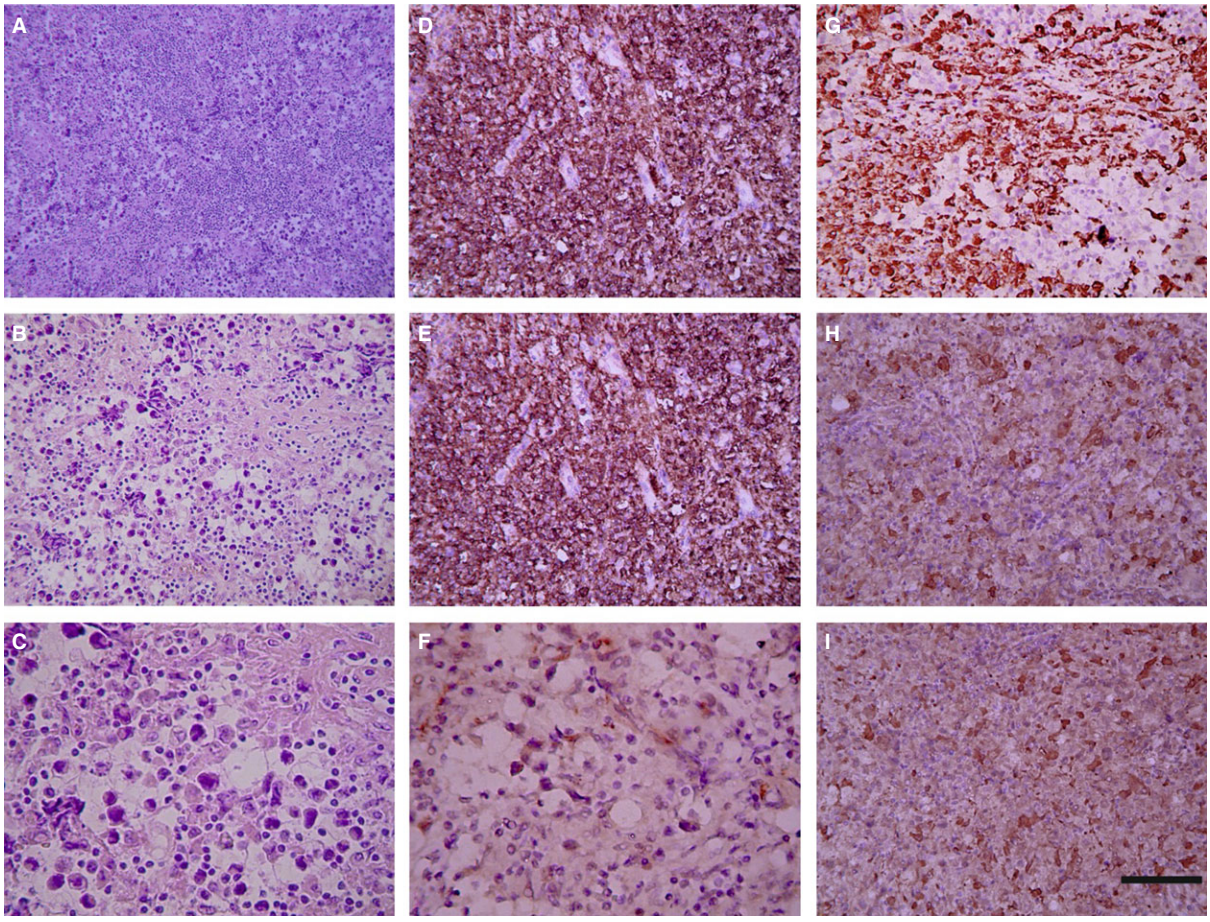

Fig. 7. Lymph node biopsy samples of the histiocytic sarcoma patient. (A) Hematoxylin–eosin stain, magnification ×40; (B) hematoxylin–eosin stain, magnification ×200; (C) hematoxylin–eosin stain, magnification ×400; (D) LCA-positive tumor cells, immunohistochemistry, magnification ×200; (E) CD20-positive tumor cells, immunohistochemistry, magnification ×200; (F) S-100-positive tumor cells, immunohistochemistry, magnification ×200; (G) CD68-positive tumor cells, immunohistochemistry, magnification ×200; (H) CD163-positive tumor cells, immunohistochemistry, magnification ×200; (I) lysozyme-positive tumor cells, immunohistochemistry, magnification ×200. Scale bar is 50 μm.

natural killer cells (CD16+ and CD56+), CD8+, CD19+, and CD45+ were all within reference range.

The size of abdominal, neck, and thorax lymph nodes has been reduced (Figs 8 and 9).

Serum clinical chemistry parameter values above grade 1 according to NCI CTCAE (23) were not observed during Rigvir treatment. However, during chemotherapy and radiotherapy, values of grade 2–3 were observed.

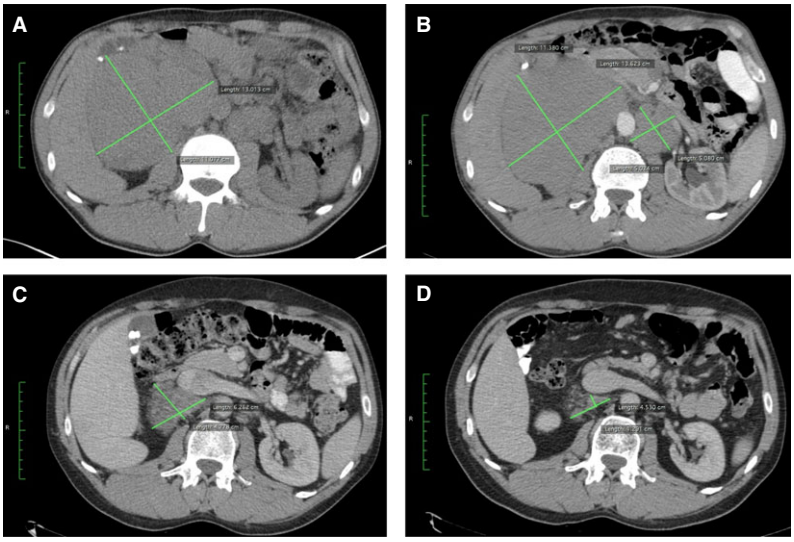

Fig. 8. CT of the histiocytic sarcoma patient. Contrast-enhanced CT late phase (contrast) of abdominal lymph nodes that decreased in size with time and stabilized. (A) July 28, 2011, (B) October 13, 2011, (C) April 2, 2012, (D) October 1, 2015.

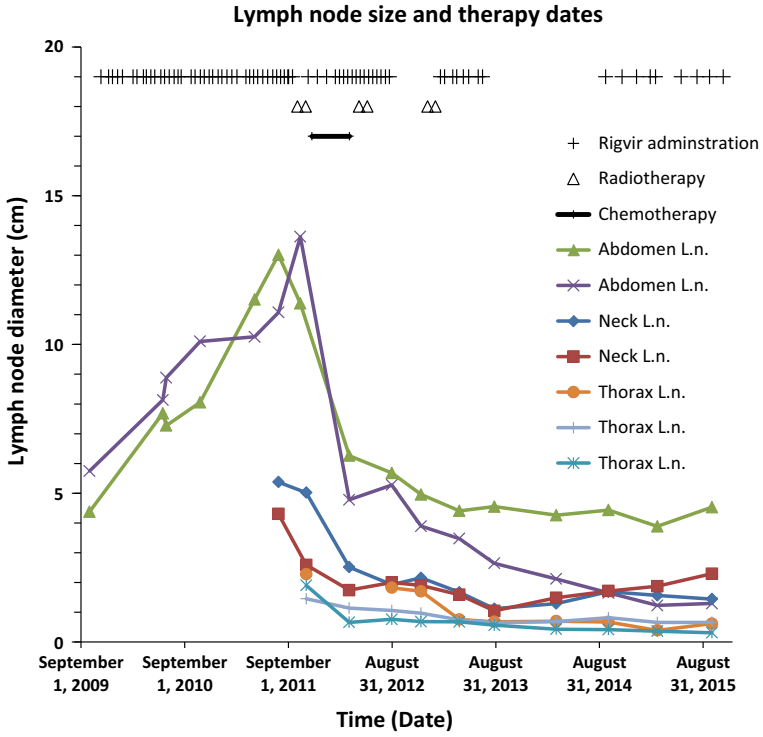

Fig. 9. Lymph node size and therapy dates of the histiocytic sarcoma patient. Abdominal, neck, and thorax lymph node (L.n.) diameters peaked and then decreased in size with time and stabilized. Dates of Rigvir administration (+), chemotherapy (—), and radiotherapy (▲) treatment.

Thus, the patient's condition has improved and has been stable since April 2012.

DISCUSSION

The three patients described here have been diagnosed with melanoma stage IV M1c, small cell lung cancer stage IIIA, and histiocytic sarcoma stage IV. They have been long-term treated with Rigvir virotherapy starting from 1 to 2 months after diagnosis during 3.5, 7.0, and 6.6 years, respectively.

The expected 3-year survival of stage IV melanoma patients treated according to current standard treatments is approximately 15% (26). The predicted 3.5-year survival for melanoma stage IV M1c group patients is approximately 10% (12, 13); however, the latter estimate does not include subgrouping by normal and elevated serum LDH levels at the time of diagnosis. In melanoma stage IV patients with normal LDH levels independent of M1 subgroup, the 3.5-year survival is approximately 30% (12, 13). In a recent study, the 3-year survival in stage IVb and IVc melanoma patients was approximately 19–23% (6). While the expected survival time for half of the stage IV M1c melanoma patients is approximately 6 months (13), the present patient was diagnosed more than 42 months ago.

The 5-year survival for small cell lung cancer stage III with current standard treatment has been calculated to be 8.2% (15, 27). While the expected survival time for half of these patients is approximately 12 months (27), the present patient was diagnosed more than 85 months ago.

Histiocytic sarcoma is a rare disease with only few cases described in the literature, and consequently, there is no accepted standard treatment (14, 21). The relative 5-year survival for the age 25–64 group to 77% (28). A recent retrospective study calculated the overall 5-year survival to 45% (N = 9) (14). Histiocytic sarcoma is often characterized by aggressive progression with little response to therapy (11, 14, 21). The present result might suggest that in this particular patient Rigvir pretreatment slowed down the disease progression and sensitized the tumor tissue to chemo- and radiotherapy.

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