Adapted ECHO-7 virus Rigvir immunotherapy (oncolytic virotherapy) prolongs survival in melanoma patients after surgical excision of the tumour in a retrospective study

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An oncolytic, nonpathogenic ECHO-7 virus adapted for melanoma that has not been genetically modified (Rigvir) is approved and registered for virotherapy, an active and specific immunotherapy, in Latvia since 2004. The present retrospective study was carried out to determine the effectiveness of Rigvir in substage IB, IIA, IIB and IIC melanoma patients on time to progression and overall survival. White patients ($N = 79$) who had undergone surgical excision of the primary melanoma tumour were included in this study. All patients were free from disease after surgery and classified into substages IB, IIA, IIB and IIC. Circulating levels of clinical chemistry parameters were recorded. Survival was analysed by Cox regression. Rigvir significantly ($P < 0.05$) prolonged survival in substage IB–IIC melanoma patients following surgery compared with patients who were under observation (according to current guidelines). The hazard ratio for patients under observation versus treated with Rigvir was statistically significantly different: hazard ratio 6.27 for all, 4.39 for substage IIA–IIB–IIC and 6.57 for substage IIB–IIC patients. The follow-up period was not statistically different between both treatment groups. These results indicate that the patients treated with Rigvir had a 4.39–6.57-fold lower mortality than those under observation. In this study, there was no untoward side effect or discontinuation of Rigvir treatment. Safety assessment of adverse events graded according to NCI CTCAE did not show any value above grade 2 in Rigvir-treated patients. In conclusion, Rigvir significantly prolongs survival in early-stage melanoma patients without any side effect. *Melanoma Res* **25:421–426**

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Introduction

Melanoma is one of the fastest-growing cancers and has the highest mortality rate of skin cancers [1–3]. More than half of melanoma patients experience progression of the disease within 3 years of diagnosis [4,5]. Current clinical practice guidelines for stage I–II melanoma provide few, if any, recommendations for treatment [6–9]. The oncolytic property of viruses has been observed for over a century and is presently being studied intensively [10–16]. An oncolytic, nonpathogenic ECHO-7 virus, adapted and selected for melanoma that has not been genetically modified (Rigvir), was approved and registered in 2004 in Latvia for melanoma therapy [17–27]. The effect of viruses on cancers, including melanoma, has been tested in clinical trials; however, the effectiveness of an approved and marketed virus has not yet been shown in a clinical setting [12,14,16].

In oncolytic virotherapy, Rigvir is a first-in-class. At a later time, a genetically modified adenovirus was approved for head and neck cancer [28,29]. Melanoma is staged with substages from 0 to IV by measuring the thickness of the tumour according to Breslow, by assessment of ulceration, mitotic rate and metastases and by collecting pathologic information on regional lymph nodes [30,31]. On the basis of the stage of the disease, treatment is currently performed according to published guidelines [4,6–9].

The aim of the present study is to test the effectiveness of Rigvir in a retrospective study in substage IB, IIA, IIB and IIC melanoma patients on time to progression and overall survival.

Methods

Retrospective clinical study patients

White patients ($N = 79$) who had undergone surgical excision of melanoma and diagnosis verified histologically during the 4 years between January 2008 and December 2011 were included in this study. All patients were free of disease after surgery and were classified into substages IB,

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Table 1 Study population characteristics ($N = 79$)

	Observation ($N = 27$)			Rigvir ($N = 52$)			
	Mean ± SD	Median	Range	Mean ± SD	Median	Range	*P*
Age at surgery (years)	65.6 ± 13.9	65.0	31–86	56.5 ± 16.6	59.5	19–84	< 0.020
Follow-up (months)	49.5 ± 23.1	50.3	9–76	46.1 ± 12.4	44.9	20–72	NS
Months with no progression	30.0 ± 19.1	27.9	5–70	34.2 ± 15.8	33.8	1–69	NS
Months to progression[a]	23.1 ± 12.8	22.1	5–40	16.9 ± 8.5	16.5	6–31	NS
Female [*n* (%)]	16 (59.3)			35 (67.3)			NS
Progression [*n* (%)]	8 (29.6)			8 (15.4)			NS
Deaths [*n* (%)]	11 (40.7)			4 (7.7)			< 0.001
Substages [*n* (%)]							
IB	5 (18.5)			17 (32.7)			NS
IIA	5 (18.5)			16 (30.8)			
IIB	13 (48.2)			12 (23.1)			
IIC	4 (14.8)			7 (13.5)			

NS, not significant.
[a]Time to progression observed in $N = 8$ in the observation group and $N = 8$ in the Rigvir group.
P-values achieved from Mann–Whitney tests (for continuous variables) and the χ^2-test (for categorical variables).

IIA, IIB and IIC according to the American Joint Committee on Cancer [30,31]. For disease progression, all were followed for a minimum of 3 months until January 2014. The overall survival was checked on 5 June 2014 and considered to reflect the status by 27 May 2014. The detailed study population characteristics of this retrospective study are shown in Table 1.

Current guidelines for melanoma advise no treatment postsurgery for patients who are classified into substages IB and IIA. Patients in substages IIB and IIC are provided three options: participation in a clinical trial, observation and interferon [7,8]. In the absence of strict guidelines, treatment with Rigvir was offered. Thus, 52 study participants received Rigvir and 27 were observed according to the guidelines. The patients who had been treated with interferon were excluded from the present analysis as, in the registry, they were too few to allow for any comparison.

As a part of the safety assessment, serum clinical chemistry parameters were recorded.

The patients in this study were treated in the Latvian Oncology Center of Riga Eastern Clinical University Hospital, the Latvian Virotherapy Center in Riga and the Oncology Clinic of Piejūras Hospital in Liepāja, Latvia.

The study was approved by the respective ethics committee.

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 titre is not less than 10^6 $TCID_{50}$/ml in sodium chloride for injection.

Method of Rigvir administration

Treatment was started after surgical excision of the primary melanoma tumour when the wound had healed. First, Rigvir (2 ml) was administered intramuscularly regionally for 3 consecutive days. After about 4 weeks, administration was repeated for three consecutive days and repeated about 4 weeks later. Subsequently, a single administration of Rigvir (2 ml, intramuscularly) was performed at monthly intervals during the first year, at 6-week intervals during the first half of the second year, at 2-month intervals during the second half of the second year and at 3-month intervals in the third year. Rigvir is not to be used during an acute infection.

Statistical analysis

Statistical analysis of the data was carried out using the SPSS statistical software, V.20 (SPSS Inc., Chicago, Illinois, USA). Mann–Whitney *U*-test and Wilcoxon tests (for continuous variables), Fisher's exact test and the χ^2-test (for categorical variables) were used to test differences between and within groups. Cox proportional hazard survival regression analysis was carried out, which is the most commonly used multivariate model in survival analysis. Thus, any difference between the groups, for example, in age, has been taken into account in the Cox analysis. (This is in contrast to Kaplan–Meier analysis, which is a bivariate analysis that only takes into account one predictor at a time). Hazard ratios (HRs) and 95% confidence intervals were calculated using bivariate and multivariate Cox regression analysis on survival. Endpoints were occurrence of metastases or disease recurrence for time to progression, and death from any cause for analysis of overall survival. Predictors (covariates) used in regression analysis were tumour stages, treatment (Rigvir, observation), sex and age. A *P* value less than 0.05 from a two-sided test was established to indicate statistical significance.

Results

Effectiveness in patients: time to progression

Melanoma patients of substages IB, IIA, IIB and IIC were studied according to the postsurgery management that they had received. One group was treated with Rigvir and the other was managed according to current guidelines by observation (the control group is called

'observation') [6–9]. The follow-up period was not statistically different between both treatment groups (Table 1).

Patients who were free of melanoma after surgical excision and were treated with Rigvir appeared to remain disease free (free of metastases and/or recurrence) for a longer period of time compared with a similar group of patients who did not receive Rigvir. The difference between the treatment groups did not, however, reach statistical significance (Table 2).

Effectiveness in patients: overall survival

The survival of patients who were treated with Rigvir was significantly ($P < 0.05$) longer compared with a similar group of patients who did not receive Rigvir (Fig. 1 and Table 3). The difference between both treatment groups was statistically significant on analysing all four substages together (IB, IIA, IIB, IIC) (Table 3) and on analysing stage II together (substages IIA, IIB, IIC). Adjusting for patient age, sex and substage of disease, the HR was calculated in multivariate Cox regression analysis. The HR for patients treated according to current guidelines by observation versus treated with Rigvir was 6.27 ($P < 0.005$) for all patients, 4.39 ($P < 0.032$) for substage IIA–IIC patients and 6.57 ($P < 0.014$) for substage IIB–IIC patients (Fig. 1). This indicates that the patients who were treated with Rigvir had a 4.39–6.57-fold lower mortality than those treated using current guidelines by observation.

Safety assessment

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 tumour area, sleepiness and diarrhoea. In this retrospective study, however, there was no record of any untoward side effect from Rigvir treatment or its discontinuation.

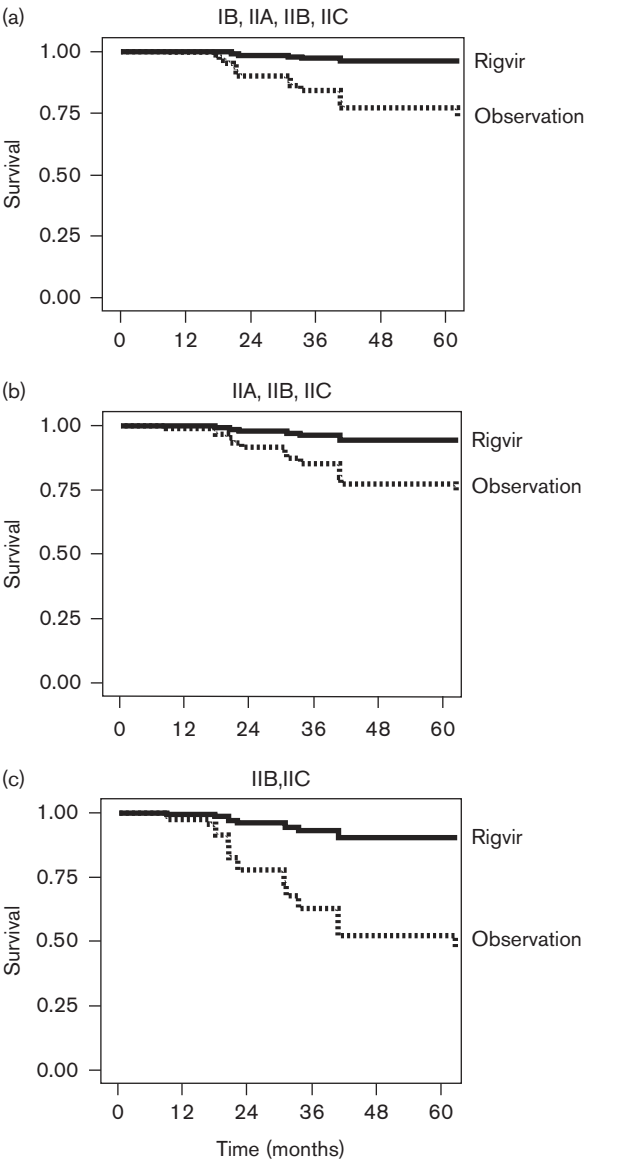
Serum clinical chemistry parameters were recorded and graded according to NCI CTCAE [32] (Table 4). In the observation group, grade 1–3 values were obtained. All grade 3 samples were from two patients obtained within the last few months of life. In one of these patients, progression of the disease was reported simultaneously. In contrast, in the Rigvir-treated patients, values above grade 2 were not observed.

Table 2 Regression estimates from Cox regression analysis of time to progression ($N = 79$)

					95% CI for HR	
	B	SE(*B*)	*P*	e^b (HR)	Lower	Upper
Rigvir[a] 1 = Observation, 0 = Rigvir	0.710	0.578	0.219	2.034	0.655	6.314
Age at surgery	0.039	0.021	0.066	1.040	0.997	1.083
Sex, female = 0, male = 1	0.155	0.581	0.789	1.168	0.374	3.651
Substages[b]			0.012			
IB = 1; (IIA, IIB) = 0	−2.209	0.863	0.010	0.110	0.020	0.596
IIA = 1; (IB, IIB) = 0	−2.410	0.884	0.006	0.090	0.016	0.508
IIB = 1; (IB, IIA) = 0	−1.518	0.695	0.029	0.219	0.056	0.856

B, regression coefficient; CI, confidence interval; HR, hazard ratio.
[a]After adjustment for sex, age and substage.
[b]Compared with substage IIC.

Fig. 1



Cox regression analysis plots of survival of melanoma patients following surgery. *P* is the statistical significance of the difference between the Rigvir (–) group and the observation according to current guidelines (observation) group (---) after adjustment for age, sex and substage; hazard ratio (HR), 95% confidence interval (CI). (a) Substages IB, IIA, IIB, IIC, Rigvir ($N = 52$), observation ($N = 27$), $P < 0.005$, HR = 6.27 (CI: 1.75–22.43). (b) Substages II (A, B, C), Rigvir ($N = 35$), observation ($N = 22$), $P < 0.032$, HR = 4.39 (CI: 1.14–16.98). (c) Substages IIB and IIC, Rigvir ($N = 19$), observation ($N = 17$), $P < 0.014$, HR = 6.57 (CI: 1.47–29.46).

Discussion

Oncolytic virotherapy is one of three forms of virotherapy (the other two being viral vectors for gene therapy and

Table 3 Regression estimates from Cox regression analysis of survival ($N = 79$)

					95% CI for HR	
	B	SE(*B*)	*P*	e^b (HR)	Lower	Upper
Rigvir[a]	1.835	0.651	0.005	6.265	1.750	22.428
1 = Observation, 0 = Rigvir						
Age at surgery	0.061	0.026	0.020	1.063	1.010	1.119
Sex, female = 0, male = 1	0.774	0.561	0.168	2.168	0.722	6.507
Substages[b]			0.026			
IB = 1; (IIA, IIB) = 0	−1.395	0.878	0.112	0.248	0.044	1.387
IIA = 1; (IB, IIB) = 0	−2.791	1.124	0.013	0.061	0.007	0.555
IIB = 1; (IB, IIA) = 0	−1.593	0.661	0.016	0.203	0.056	0.742

B, regression coefficient; CI, confidence interval; HR, hazard ratio.
[a]After adjustment for sex, age and substage.
[b]Compared with substage IIC.

viral immunotherapy, respectively). Early observations of tumour regressions after virus infections have been published starting from the late 19th century (cf. [10–16]). Recently, several oncolytic viruses have been tested clinically [33–35] and *Science* named cancer immunotherapy the breakthrough of the year of 2013 [36]. The melanoma adapted and selected ECHO-7 virus Rigvir is first-in-class in oncolytic virotherapy; it is approved as therapy for melanoma.

The present results show that in substage IB, IIA, IIB and IIC melanoma patients, Rigvir administration after surgery significantly ($P < 0.05$) prolongs survival compared with patients who were managed according to current published guidelines [6–9]. For the Rigvir-treated patients, the HR (risk of death) is 4.39–6.57-fold lower than for the control group treated according to current guidelines by observation. The HR was calculated in multivariate Cox regression analysis adjusting for patient age, sex and substage of disease.

In this study, there was no record of any untoward side effect from Rigvir treatment, which is in agreement with clinical studies using other oncolytic viruses [14,16,33,34, 37]. Moreover, no value higher than grade 2 was recorded in Rigvir-treated patients. This is in contrast to most other cancer therapies, where grades 3 and 4 are frequently observed (cf. [38]).

Administration of virus induces the formation of neutralising antibodies that might potentially influence the efficiency of Rigvir. In previous studies, the titre of neutralising antibodies against ECHO-7 was determined in both healthy individuals and patients before administration of Rigvir. In 94 healthy adult participants tested, the titres were found to be low (1 : 20 to 1 : 62) [39,40]. When tested in 155 adult cancer patients who had not been treated with Rigvir, neutralising antibodies against ECHO-7 were detected in ~50% of the patients [41]. In a local study of 472 individuals, the presence of ECHO-7 antibodies was shown to increase with age in children and

Table 4 Levels of serum clinical chemistry parameters during treatment

	Treatment	Mean	Median	SE	*n*	*N*	Maximum	Minimum	Grade (*N*)
ASAT (IU/l)	Rigvir	20.1	19.6	0.44	101	28	31.0	11.0	–
	Observation	28.4	23.0	3.14	117	23	374.0	13.5	I (9), III (1)
ALAT (IU/l)	Rigvir	18.6	17.1	0.84	110	29	52.0	4.0	I (1)
	Observation	30.9	22.1	3.5	120	23	408.5	6.8	I (10), III (1)
ALP (IU/l)	Rigvir	71.8	69.0	2.1	84	22	142.1	45.0	I (1)
	Observation	93.0	79.0	12.4	80	19	877.0	37.8	I (2), III (2)
Bilirubin (μmol/l)	Rigvir	15.2	12.3	2.9	17	25	45.9	4.9	I (2), II (1)
	Observation	10.5	7.4	2.0	64	23	131.9	2.8	I (2), III (1)
Creatinine (μmol/l)	Rigvir	74.2	74.5	2.0	26	24	92.0	53.0	I (1)
	Observation	79.7	79.0	2.3	53	22	138.0	51.0	I (4), II (1)
Glucose (mmol/l) (fasting)	Rigvir	6.0	5.6	0.4	14	8	9.0	4.7	II (2)
	Observation	5.5	5.5	0.1	57	23	7.4	3.7	I (7)
LDH (IU/l)	Rigvir	182.6	175.0	3.5	171	29	368	29.9	NA
	Observation	192.3	186.0	3.9	83	18	352	143	NA
S-100 (pg/ml)	Rigvir	42.6	37.6	2.5	53	19	121.4	17.9	NA
	Observation	51.6	41.5	5.3	59	12	236.9	11.4	NA
Red blood cells ($\times 10^{12}$/l)	Rigvir	4.5	4.5	0.02	173	29	5.4	3.8	NA
	Observation	4.6	4.5	0.03	138	24	5.4	3.6	NA
Haemoglobin (g/l)	Rigvir	134.5	135.0	1.0	168	29	160	81.0	–
	Observation	137.0	137.0	1.1	139	24	168	104	–
Thrombocytes (platelets) ($\times 10^9$/l)	Rigvir	283.1	270.5	5.8	168	29	620	107	I (1)
	Observation	245.2	253.0	4.8	130	22	525	28	I (1)
White blood cells ($\times 10^9$/l)	Rigvir	7.1	7.0	0.1	169	29	12.7	3.4	I (3)
	Observation	6.6	5.8	0.2	138	24	19.6	3.4	I (3)
Neutrophils ($\times 10^9$/l)	Rigvir	3.8	3.6	0.1	179	29	8.4	1.3	I (8), II (2)
	Observation	3.8	3.3	0.2	134	24	13.0	1.3	I (7), II (1)
Lymphocytes ($\times 10^9$/l)	Rigvir	2.4	2.2	0.06	174	38	5.0	1.0	I (1)
	Observation	2.1	2.0	0.05	135	24	5.8	1.0	–

Number of samples analysed (*n*) from *N* number of patients.
Safety assessment of adverse events graded according to NCI CTCAE (Grade) [32], no record of grade 1 or above (–).
ALAT, alanine aminotransferase; ALP, alkaline phosphatase; ASAT, aspartate aminotransferase; IU/l, international units per litre; LDH, lactate dehydrogenase; NA, not applicable.

level off to a plateau of around 75% in adults [42]. To our knowledge, the prevalence of neutralising antibodies against the ECHO-7 virus in the general adult population has not been reported.

Rigvir is an immunomodulator that affects both the humoral, antibody-mediated, and the cellular immune systems [20–22]. When virus adsorption and penetration to tumour tissue were measured, it was shown that they are not influenced by the presence of neutralising antibodies (titre 1 : 10) [43,44]. Furthermore, in a preliminary study, the levels of neutralising antibodies to Rigvir during the first 18 months of treatment of melanoma patients did not appear to correlate with time to progression after 3 years of follow-up [40]. In that study, the neutralising antibody titre was 1 : 10 before the start of treatment ($N = 34$). After the first dose, the titre was 1 : 25 to 1 : 91 (determined 24–48 h after administration). A month later, before the second dose, the titre was 1 : 250 to 1 : 320 ($N = 30$); after the second dose, it was 1 : 510 to 1 : 850. Two months later, before the third administration, the titre was 1 : 160 to 1 : 895 ($N = 26$) and after the eighth dose, 18 months after the first dose, it was 1 : 280 to 1 : 1350 [40].

Also, after intravenous administration, the correlation between antibody titres varies from one virus to another, and neutralising antibodies do not affect efficacy when local or regional administration is used [14,45,46].

An estimated 14.1 million new cancer cases were diagnosed worldwide in 2012, the latest available. The number is expected to increase to 24 million by 2035. About 232 000 patients are estimated to be diagnosed with melanoma in 2014 [3]. In the 20-year survival data analysis of the American Joint Committee on Cancer (cf. Figure 31.1 of [31]), the majority of all melanoma patients belonged to stage I and stage II, 47 and 24%, respectively [31]. However, at present, clinical practice guidelines suggest postsurgery therapy only for late-stage melanoma (radiation therapy and interferon α) [6–9].

Rigvir has also been used in other types of cancer. *In vitro*, it reduces the viability of melanoma, as well as pulmonary, gastric, pancreatic, bone, and breast cancer cell cultures [47,48]. It is oncolytic in melanoma and rectum cancer patients [49,50] ([26], p. 115) and has been shown to improve the 5-year survival in rectum cancer patients [24].

Taken together, the results suggest that a significant number of melanoma patients would benefit from prolonging the survival with Rigvir treatment. The results also show that this can be achieved without side effects. Results suggest that Rigvir could also be tested in the treatment of other types of cancer.

Conclusion

Rigvir is an oncolytic, nonpathogenic ECHO-7 virus that significantly prolongs survival in early-stage melanoma patients without any side effect.

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Conflicts of interest

Aina Muceniece, Dite Venskus, Jurģis Auziņš and Pēteris Alberts are past and present employees of the Latvian Virotherapy Center. For the remaining authors there are no conflicts of interest.

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