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REVIEW ARTICLE

THERAPEUTIC EXPLOITATION OF ONCOLYTIC VIRUSES IN PEDIATRIC ONCOLOGY

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SUMMARY

Oncolytic viruses are considered as a new class of therapeutic agents inducing preferential killing of malignant cells –both in cell culture and in animal models. In adult patients, clinical trials have mainly demonstrated the clinical safety of oncolytic virotherapy and, in some cases, promising effects against disease progression. The present publication is the first to review the application of any type of oncolytic virus to malignant diseases occurring exclusively or mainly in childhood. The first part gives an overview on the preclinical assessment of oncolytic virotherapy for the 15 most frequent pediatric malignancies. The second part of the article focuses on the specific issues raised by the clinical evaluation of oncolytic virotherapy in children suffering from cancer. In contrast to the extensive preclinical work, only four phase I-II clinical trials have included pediatric or adolescent patients, since clinical research on oncolytic viruses for pediatric patients has just entered the hospitals.

INTRODUCTION

Risk-adapted multimodal therapeutic approaches have improved the outcome of patients in pediatric oncology over the last decades, reaching an overall long-term survival rate of about 80%. However, for certain well-defined subgroups of pediatric patients, such as those with advanced-stage disease or relapsed patients, the prognosis remains poor (1, 2). Since current multimodal treatment protocols basically include surgery, chemotherapy and radiation, novel treatment concepts beyond classical treatment modalities are urgently required to improve the outcome of high-risk disease in pediatric oncology.

Oncolytic viruses are a promising novel class of cancer cell-specific biological agents, infecting and killing transformed cells while sparing normal tissues (3). Moreover, some oncolytic viruses can kill apoptosis-resistant tumor cells and thus do not show crossresistance with cytotoxic drug treatment or radiotherapy (3, 4). In addition to the oncolytic effect observed both in vitro and in vivo, these viruses also provide immunostimulatory signals, inducing the elimination of virus-infected tumor cells. Thus, the innate and adaptive immune systems gain access to tumor antigens, which results in cross-priming and vaccination effects (5, 6).

In adult cancer patients, a number of clinical trials, including several phase III trials, have been performed, mainly showing the clinical safety of oncolytic virus application, and, in some cases, signs of efficacy against disease progression. Five years ago, the worldwide first and only oncolytic virus gained approval for routine clinical application: H-101, a conditionally replicating adenovirus, was admitted by China's State Food and Drug Administration for the treatment of head and neck cancer in combination with 5-fluorouracil (5-FU) and cisplatin therapy. Performing a search for literature reviewing oncolytic virotherapy, solely in PubMed more than 400 publications on adult patients could be retrieved (reference date: November 2010).

In contrast, only two reviews focus on children. One exclusively addresses the application of oncolytic herpesviruses (7). The second is limited to the four oncolytic viruses, HSV-1716, JX-594, NTX-010 and Reolysin®, now entering pediatric phase I clinical trials (8). Here, we summarize the extensive preclinical evaluation of any kind of oncolytic virus for the 15 most frequent pediatric malignancies. Moreover, we describe the conception of clinical trials on oncolytic virotherapy including children or adolescents and the few trials currently being initiated. Among these trials, only one has been completed and a single case of a pediatric patient has been reported (see Table IV) (9). The remaining four trials have just started to recruit

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patients and none has been completed so far. Thus, routine clinical application of oncolytic virotherapy in pediatric patients still remains at the horizon of current research projects.

TUMOR SPECIFICITY OF ONCOLYTIC VIRUSES

Oncolytic viruses exert their therapeutic effects on malignant cells by several mechanisms –frequently combining some of them and thus circumventing the selection of resistant malignant cell clones.

Natural oncospecificity of oncolytic wild-type viruses

Many viruses are known to induce, for their own benefit, crucial changes in cellular proliferation and defense pathways (e.g., inactivation of p53, inhibition of apoptosis, induction of proliferation, suppression of cellular interferon response). These changes are often similar to those acquired by mutation and subsequent clonal selection during the process of malignant transformation. From this convergence, it is not too surprising that some naturally occurring (wild-type) viruses preferentially grow in malignant cells (10). Most of these wild-type oncolytic viruses are animal viruses with selective replication deficiency in nonmalignant human cells, such as Newcastle disease virus, vesicular stomatitis virus, Myxoma virus, Seneca Valley virus, Semliki Forest virus and some rodent parvoviruses, among them parvovirus H-1 (H-1PV). In addition, orthoreovirus, a wild-type virus with low pathogenicity in humans, has also been considered for oncolytic virotherapy (11).

Virus attenuation-dependent oncospecificity

Historically, some attenuated vaccination strains of wild-type viruses were shown to have oncolytic properties. These attenuated strains had been generated by selection processes during serial passages in culture. Despite long-lasting experience with the clinical safety of vaccination strains, e.g., measles virus and vaccinia virus, some concerns about their clinical safety remain, since both have been applied as vectors and therefore had been genetically modified. For vaccination purposes, attenuated strains of measles virus or vaccinia virus had primarily been selected for their ability to induce long-term immunity after a single administration. This feature, however, limits their applicability for repeated or systemic oncolytic therapy. Therefore, combination treatment with immunosuppressive drugs such as cyclophosphamide or rapamycin has been evaluated in order to improve therapeutic efficacy (12-15).

Viruses with genetically engineered oncospecificity

Viruses can also be modified by genetic engineering in order to acquire or amplify their specificity for malignant cells.

Blocking virus replication in normal, nontransformed cells

This can be achieved by impairing the viral ability to counteract antiviral innate immune response mechanisms that are activated in infected normal cells, but are frequently impaired in neoplastic cells (e.g., by inactivating the herpes simplex virus [HSV] gene coding for ICP34.5, a known protein kinase R antagonist). Alternatively, viral genes essential for viral replication in nontransformed cells that are not necessary for lytic replication in malignant cells can be deleted. In adenoviral vectors, e.g., this has been achieved by disrupting the adenoviral E1A. This 24-bp deletion is known to prevent the tumor suppressor protein Rb from binding to E1A, thereby rescuing Rb from binding to E2F and resulting in a repression of the transcription of cellular genes required for cell cycle entry and progression in nonmalignant cells (16). In HSV, the gene for the heavy chain of ribonucleotide reductase, *UL39*, known to supply the nucleotide pool within cells, has been deleted for this purpose (7). These modifications result in the generation of mutant viruses that replicate conditionally in malignant cells.

Targeting oncolytic viruses by enhancing viral entry and replication in malignant cells

The oncotropism of viruses can also be improved by increasing their affinity for surface receptors differentially expressed on malignant cells and/or to intracellular factors driving the viral life cycle (17).

Protease-targeted mutants. In a broad variety of human malignant diseases cancer cells secrete proteases, e.g., matrix metalloproteinases (MMPs) and the serine protease urokinase-type plasminogen activator (uPA), into the extracellular matrix. MMPs and uPA have been shown to increase the ability of cells to migrate and form metastases and therefore represent relevant protease targets for the tumor-selective activation of oncolytic viruses. A measles virus with increased oncoselectivity has been generated by modifying the protease cleavage specificity of the viral envelope fusion protein from being dependent on the ubiquitously expressed protease furin to being dependent on MMPs (18).

Binding site-modified mutants. The specific binding of the wild-type virus to its usual receptor is abolished by genetic modification of the viral capsid, which is further engineered to display additional peptide sequences that enable the virus to bind to surface receptors expressed exclusively or preferentially on malignant cells. Another approach aims at retargeting oncolytic viruses by expression of single-chain antibody motives on their surface, as achieved, e.g., with adeno- or herpesviruses (17, 19).

Promoter-engineered mutants. Viral replication is made to depend on an inserted tissue-specific or tumor-specific promoter. Oncolytic viruses engineered with tissue-specifically activated promoter sequences have been preclinically evaluated for several pediatric malignant diseases. Here, viral gene expression is placed under the *cis*-control of tissue-specifically activated responsive elements derived from heterologous promoters/enhancers known to be activated in specific malignant cells: sarcoma (calponin), hepatoblastoma, hepatocellular carcinoma and some germ cell tumors (α-fetoprotein), nephroblastoma and neuroblastoma (midkine) and non-Hodgkin's lymphoma (NHL) (survivin) (17). Targeting oncolytic HSV to neuroblastoma-initiating cells that express neuronal stem cell markers has been achieved by inserting nestin-responsive elements into the viral promoter (20). Some oncolytic virotherapy approaches use cancer-specific promoter systems such as telomerase (hTERT)-responsive elements to induce selective viral gene expression and subsequent specific viral replication in malignant cells with increased hTERT activity. This approach has recently been evaluated in murine retinoblastoma xenograft models. Moreover, a phase I trial in adult cancer patients has been performed (21).

ONCOSUPPRESSIVE ACTIVITIES OF ONCOLYTIC VIRUSES

Genuine viral oncotoxicity of oncolytic viruses

Oncolytic viruses have an intrinsic cytotoxic activity for which malignant cells are preferential targets due to the above-mentioned proficiency of these cells in virus replication. In addition, malignant transformation may sensitize cells to some viral toxic proteins (22).

Oncolytic virus vectors delivering genes for gene-directed enzyme prodrug therapy

As is the case for viral tropism, the overall viral oncolytic effect can be enhanced by genetic engineering of viruses. One popular strategy consists of using oncolytic viruses as vectors and arming them with suicide genes (23). These genes encode enzymes sensitizing the infected cancer cells to prodrug treatment.

Cytosine deaminase selectively converts the comparatively nontoxic antifungal agent 5-fluorocytosine to the pharmacologically active cytotoxic drug 5′-FU, which is toxic to infected malignant cells. HSV thymidine kinase phosphorylates the virostatic drugs acyclovir and ganciclovir to the highly toxic triphosphates in transduced transformed cells, whereas human thymidine kinase is unable to phosphorylate and activate the prodrug. CYBP21 encodes a cytochrome P450 carboxyl esterase able to convert cyclophosphamide to 4-hydroxycyclophosphamide, which spontaneously converts to the alkylating metabolite phosphoramide mustard. Rabbit carboxyl esterase converts the topoisomerase inhibitor irinotecan to its active metabolite 7-ethyl-10-hydroxycamptothecin.

Oncolytic virus-induced antitumor immune response

The initial cytostatic or cytotoxic effects of oncolytic viruses can initiate but do not necessarily complete the regression of malignant diseases. With their capacity for initiating tumor lysis and thereby promoting the release of not only tumor-associated antigens but also immunostimulatory signals, oncolytic viruses may indeed play the role of adjuvant in anticancer immunity priming. For several oncolytic viruses, such as adenovirus, vaccinia virus, vesicular stomatitis virus, Newcastle disease virus or H-1PV, such immunostimulatory effects have been reported (24). The antitumor immune response can be reinforced by arming oncolytic viruses with transgenes coding for factors such as granulocyte–macrophage colony-stimulating factor (GM-CSF) or interferons known to participate in the recruitment and/or activation of specific components of the immune system. This approach has recently been evaluated applying a conditionally replicating adenoviral vector expressing GM-CSF to adult cancer patients in a phase I clinical trial showing induction of a specific antitumor T-cell response (25). An oncolytic HSV engineered to express GM-CSF (OncoVEX$^{GM\text{-}CSF}$) has also been applied in a phase I clinical trial (26), and a vaccinia virus engineered to express GM-CSF (JX-594) has already completed two phase I trials in melanoma and hepatocellular carcinoma (27). Currently, it is being evaluated in four other clinical trials, and a pediatric phase I trial is starting to recruit patients. Recently, a strategy for enhancing antitumor immunity while reducing antiviral immunity has been described, which is based on the combination of a tumor vaccination with an oncolytic viral therapy approach (28).

Combination treatment comprising oncolytic virotherapy

Oncolytic viruses can be combined with conventional treatment modalities such as cytostatic drugs or irradiation to increase therapeutic efficacy. Additive, or in some cases even synergistic, antitumor effects have been obtained by combining oncolytic viruses with several other classes of therapeutic agents (for review see 29), in particular immunomodulating agents, histone deacetylase inhibitors, antiangiogenic substances, DNA alkylating substances, inhibitors of cellular kinases or radiotherapy (30). The therapeutic potential of some of these combinations is supported by preclinical data (29), justifying the recent start of their evaluation in clinical trials in adult patients (31).

PRECLINICAL EVALUATION OF ONCOLYTIC VIROTHERAPY APPROACHES FOR MALIGNANT DISEASES OF PEDIATRIC RELEVANCE

Clinical aspects in pediatric oncology

In children and adolescents, malignancies of the hematopoietic system constitute about 50% of all cancer cases. Pediatric solid tumors most frequently originate from the nervous system and are mainly located in the brain (astrocytoma, medulloblastoma/primitive neuroectodermal tumors [PNET], germ cell tumors and ependymoma). Among the extracranial solid tumors, the embryonal tumors neuroblastoma and nephroblastoma predominate in infancy. In older children and adolescents, bone tumors such as osteosarcoma and Ewing's sarcoma represent relevant clinical problems. Here, we will review the preclinical evaluation of approaches involving oncolytic viruses with regard to their applicability to the 15 most common childhood cancers (2). An overview of the oncolytic viruses applied is given in Table I. For each potential clinical application, in vitro data clearly indicating oncolytic effects of the viruses are summarized in Table II. The effects of oncolytic virotherapy on the survival of tumor-bearing animals are shown in Table III.

Oncolytic virotherapy for malignant disorders of the hematopoietic system

About 50% of all malignant disorders in children are derived from the hematopoietic system. Among them, about 60% are leukemias and 40% are lymphomas. The survival rates range from 59% in acute myeloid leukemia (AML) to 85% in acute lymphoblastic leukemia (ALL) (2), depending on the immunological phenotype, molecular risk profile, stage and age of the patient.

Leukemia

Childhood acute leukemia, especially ALL, has a relatively good prognosis with current multi-chemotherapy treatment protocols. This is the reason why only a few attempts have been made to extend the spectrum of antileukemic therapeutics to oncolytic viruses. Research on oncolytic virotherapy relevant for pediatric leukemia patients has mainly focused on AML, which is the predominant type of leukemia in adult patients. So far, few oncolytic viruses have been evaluated preclinically for possible therapeutic potential with regard to types of leukemia also occurring in children, i.e., the oncolytic wild-type viruses Seneca Valley virus (32), vesicular stomatitis virus (33) and Myxoma virus (34), and the recombinant conditionally replicating adenovirus SG611-PDCD5 (35).

Table I. *Applications of oncolytic viruses to malignancies of pediatric relevance.*

Virus	Family	Genome	Applicable to	Ref.
DNA viruses				
Herpes simplex virus 1 (HSV-1), recombinant	*Herpesviridae*	dsDNA	Glioblastoma Neuroblastoma Sarcoma Medulloblastoma Hepatoblastoma Rhabdomyosarcoma Osteosarcoma	7, 77
Adenovirus serotype 5, recombinant	*Adenoviridae*	dsDNA	Chronic myelocytic leukemia Medulloblastoma Osteosarcoma Neuroblastoma Glioblastoma	31, 51, 65, 92
Myxoma virus, recombinant	*Poxviridae*	dsDNA	Atypical teratoid rhabdoid tumor Medulloblastoma	13, 93
Adeno-associated virus, recombinant	*Parvoviridae*	ssDNA	Ewing's sarcoma	66
Parvovirus H-1 (H-1PV)	*Parvoviridae*	ssDNA	Burkitt's lymphoma Glioma Neuroblastoma	42, 47, 60
RNA viruses				
Orthoreovirus serotype 3 (strain Dearing), Reolysin®	*Reoviridae*	dsRNA	Lymphoma Glioma Medulloblastoma	41, 44, 45, 54
Seneca Valley virus, NTX-010	*Picornaviridae*	(+)ssRNA	Alveolar rhabdomyosarcoma Ewing's sarcoma Neuroblastoma Retinoblastoma	32, 59, 84
Poliovirus, attenuated and recombinant, JX-594	*Picornaviridae*	(+)ssRNA	Neuroblastoma	62
Semliki Forest virus, recombinant	*Togaviridae*	(+)ssRNA	Glioblastoma Osteosarcoma	46, 71
Vesicular stomatitis virus, attenuated and recombinant	*Rhabdoviridae*	(–)ssRNA	Acute myeloid leukemia Atypical teratoid rhabdoid tumor	33, 93
Measles virus, strain Edmonton, recombinant	*Paramyxoviridae*	(–)ssRNA	Burkitt's lymphoma Glioblastoma Medulloblastoma	19, 55, 94

ALL cell lines showed complete resistance to Seneca Valley virus in vitro (32). In contrast, one pediatric T-cell ALL and four AML cell lines were greater than 1,000-fold more sensitive to vesicular stomatitis virus than peripheral hematopoietic progenitor cells (after in vitro stimulation with GM-CSF). Interestingly, no toxicity for non-neoplastic bone marrow cells was observed in vitro (33).

Fms-like tyrosine kinase 3 (FLT-3) is a receptor tyrosine kinase with important roles in hematopoietic stem and progenitor cell survival and proliferation. In AML, FLT-3 internal tandem duplication mutations confer poor prognosis in both adult and pediatric patients (36). Myxoma virus has been applied to FLT-3 mutant adult AML cells in vitro, where it inhibits colony-forming potential. Ex vivo infection of FLT-3 mutant human AML cells with Myxoma virus 3 h prior to xenotransplantation decreased engraftment rates in immunodeficient mice from 100% in animals with mock-infected xenografts to 10% in animals bearing Myxoma virus-infected xenografts (34). In contrast Myxoma virus infection did not alter the engraftment rates for $CD34^+$ nonmalignant human hematopoietic progenitor cell xenografts (37). However, the implantation of preinfected malignant cells only allows a rough estimation of antineoplastic efficacy, because the experimental setup hardly corresponds to the clinical situation of treating an already established malignant disease.

Chronic myeloid leukemia (CML), a rare malignant disorder in children and adolescents, was preclinically tested for responsiveness to therapy with the adenoviral vector SG611-PDCD5 carrying the proapoptotic transgene *PDCD5*. This conditionally replicating vector harbors a 24-nucleotide deletion within the CR2 region of E1A, and is placed under the triple control of the hTERT promoter for E1A, hypoxia response element for E1B and the cytomegalovirus promoter for *PDCD5*. Tumor-selective replication and cytotoxic efficacy of

Table II. *Cytopathic effects ($TCID_{50}$) of oncolytic viruses in vitro on cell lines of malignant tumors of pediatric relevance.*

Disease	Virus	Minimal $TCID_{50}$ (PFU/cell)	Maximal $TCID_{50}$ (PFU/cell)	Time period (h)	Ref.
Acute lymphocytic leukemia	SVV-001 (NTX-010)	> 10^4	> 10^4	96	32
Acute myeloid leukemia	Vesicular stomatitis virus (attenuated strain)	< 1	< 1	240	33
Chronic myelogenous leukemia	Ad-PDCD5	10	20	168	35
Burkitt's lymphoma	Reovirus serotype 3 (strain Dearing)	20	n.d.	96	41
Burkitt's lymphoma	H-1PV	0.1	10	72	42
High-grade glioma	Reovirus serotype 3 (strain Dearing)	40	< 40	72	44
High-grade glioma	NDV (strain V4UPM)	9	23	72	43
High-grade glioma	H-1PV	n.d.	5	72	95
High-grade glioma	MV-GFP	0.1	0.1	120	94
High-grade glioma	SFV-VA7EGFP	0.001	1	96	46
Medulloblastoma	vMYXGFP	10	>10	48	13
Medulloblastoma	Ad5-Delta 24	1	5	144	57
Medulloblastoma	Reovirus serotype 3 (strain Dearing)	< 1	>100	72	54
Medulloblastoma	MV-GFP	< 0.1	< 0.1	48	55
Atypical teratoid rhabdoid tumor	vMYXGFP	1	40	72	93
Atypical teratoid rhabdoid tumor	VSV(DeltaM51)	< 0.1	0.1	72	93
Neuroblastoma	SVV-001 (NTX-010)	< 0.0001	>10^4	96	32
Neuroblastoma	SVV-001 (NTX-010)	2	40	72	59
Neuroblastoma	H-1PV	0.001	10	144	60
Neuroblastoma	HSVrQT3	n.d.	0.1	96	64
Neuroblastoma	(HSV-1) NV1066	0.001	0.05	144	63
Osteosarcoma	Ad5-Δ24RGD	1	16	168	96
Osteosarcoma	Ad5-Δ24RGD	0.1	1	168-264	75
Osteosarcoma	SFV-VA7EGFP	< 0.2	2	96	71
Osteosarcoma	(HSV-1) G207	< 0.05	0.05	192	77
Osteosarcoma	(HSV-1) NV 1020	< 0.0005	< 0.05	192	77
Ewing's sarcoma	(HSV-1) G207	0.1	5	192	77
Ewing's sarcoma	(HSV-1) NV 1020	0.05	> 5	192	77
Ewing's sarcoma	Ad5 wild type	1	< 10^3	240	78
Ewing's sarcoma	SVV-001 (NTX-010)	< 0.0001	> 10^4	96	32
Rhabdomyosarcoma	(HSV-1) G207	< 0.0005	1	192	77
Rhabdomyosarcoma	(HSV-1) G207	0.01	1	96	80
Rhabdomyosarcoma	(HSV-1) NV 1020	< 0.0005	0.005	192	77
Rhabdomyosarcoma	SVV-001	9.7 x 10-4	> 10^4	96	32
Retinoblastoma	SVV-001	0.1	0.5	48	84

this virus have been shown for several human leukemia cell lines using cell culture models in vitro and a nude mouse xenograft model. In s.c. human CML xenograft models, SG611-PDCD5 alone was able to completely inhibit the development of CML, which correlated with the induction of apoptosis in these s.c.-implanted leukemic cells (35).

Non-Hodgkin's lymphoma

Similar to the incidences in small children, hematopoietic malignancies are also most common in adolescents and young adults, with lymphomas predominating in these older age groups. Survival is high for Hodgkin's lymphoma (93%) and intermediate for NHL (74%) (2). So far, no viruses have been tested for their oncolytic effects on Hodgkin's lymphoma.

Four decades ago, wild-type measles virus had been described to induce remission in Burkitt's lymphoma patients (38), correlating with the natural lymphotropism of this virus. A CD20-targeted and convertase-armed oncolytic measles virus has been developed to target B-cell lymphoma commonly expressing CD20. In a SCID mouse xenograft model of a human pediatric Burkitt's

Table III. *Improvement of survival in animals treated by oncolytic virotherapy.*

Disease	Virus	Animal model	Median survival (mock)	Median survival (viro-therapy)	Ref.
Burkitt's lymphoma	H-1PV	Subcutaneous Namalwa in SCID mice	27 days	> 70 days	42
Burkitt's lymphoma	MV-PNP H^{blind} antiCD20 + F-araAMP	Subcutaneous Raji in SCID mice	10 days	42 days	19
Atypical teratoid rhabdoid tumor	VSV(DeltaM51)	Orthotopic BT-16 in nude mice	21 days	25 days	93
Atypical teratoid rhabdoid tumor	vMYXGFP	Orthotopic BT-16 in nude mice	21 days	> 180 days	93
Glioblastoma	Reovirus serotype 3 (strain Dearing)	Orthotopic U251N in nude mice	42 days	> 90 days	44
Glioblastoma	Reovirus serotype 3 (strain Dearing)	Orthotopic U87LacZ in nude mice	48 days	> 90 days	44
Glioblastoma	H-1PV	Orthotopic RG-2 in Wistar-rats*	18 days	> 90 days	47
Glioblastoma	SVF-VA7EGFP SVF-VA7Rluc	Orthotopic U87Fluc in nude mice	36 days	> 120 days	46
Glioblastoma	vMYXGFP + rapamycin	Orthotopic RG-2 cells in Fischer 344 rats*	18 days	45 days (CED)	14
Medulloblastoma	Reovirus serotype 3 (strain Dearing)	Orthotopic DAOY in nude mice	70 days	160 days	54
Medulloblastoma	vMYXGFP + rapamycin	Orthotopic D341 in nude mice	11 days	25 days	13
Medulloblastoma	MV-GFP	Orthotopic D283med in nude mice	~ 50 days	~ 75 days	55
Neuroblastoma	SVV-001 (NTX-010)	Subcutaneous NB-SD, NB-1771, NB-1643 and NB1691 in SCID mice	7-12 days	> 45days	32
Neuroblastoma	SVV-001 (NTX-010)	Subcutaneous NB-EBc1 in SCID mice	8 days (EFS)	15 days (EFS)	32
Neuroblastoma	Poliovirus A133Gmono-crePV	Subcutaneous Neuro-2aCD155 tg A/J immunized mice	8 days	> 180 days	62
Neuroblastoma	(HSV-1) G47Δ + dendritic cells	Subcutaneous syngeneic N18 in immunocompetent mice	20 days	> 50 days	65
Rhabdomyosarcoma	HSVrRP450 + cyclophosphamide	Subcutaneous Rh30 alveolar RMS in nude mice	~ 8 days	~ 33 days	82
Rhabdomyosarcoma	SVV-001 (NTX-010)	Subcutaneous Rh10, Rh28, Rh30 and Rh30R in SCID mice	11-21 days (EFS)	> 45 days (EFS)	32
Osteosarcoma	SVF-VA7EGFP	Orthotopic K7M3 in nude mice	~ 28 days	~ 37 days	71
Retinoblastoma	SVV-001 (NTX-010)	Orthotopic Y79 in nude mice	28 days	> 84 days	59

*Rat glioblastoma transplant in rats. All other animal models are human xenografts in immunodeficient mice. EFS, event-free survival.

lymphoma cell line, repeated intratumoral injection of this virus (MV-PNP H^{blind}antiCD20) increased the mean survival time of Burkitt's lymphoma-bearing animals from 10 days to more than 30 days (19).

To date, two oncolytic wild-type viruses, reovirus and H-1PV, have been investigated in preclinical models for a possible therapeutic effect in pediatric lymphoma. Activation of the Ras signaling pathway had been reported for NHL, rendering it susceptible to Ras activation-dependent oncolytic viruses, such as HSV-R3616 (39) and reovirus (40). Reoviral infection of a panel of lymphoma cell lines in vitro proved two of four Burkitt's lymphoma cell lines and all diffuse large B-cell lymphoma cell lines to be highly sensitive to the oncolytic effect of the virus. In an s.c. lymphoma xenograft model in SCID mice, tumor growth was significantly impaired by a single intratumoral injection of reovirus (41).

The rodent wild-type parvovirus H-1PV was shown to induce cytopathic effects in NHL, with the highest in vitro toxicity to Burkitt's lymphoma cells, whereas no cytotoxic effect of H-1PV was observed in nontransformed B lymphocytes. Moreover, intratumoral H-1PV treatment of human Burkitt's lymphoma xenografts in SCID mice achieved long-term survival in about 80% of treated animals. In contrast, all nontreated animals died within 30 days after lymphoma cell inoculation (42).

Oncolytic virotherapy approaches for solid tumors occurring in childhood

Brain tumors

Given the poor prognosis, children with disseminated medulloblastomas, high-grade gliomas, intrinsic pontine gliomas, atypical teratoid rhabdoid tumors and ependymomas may benefit from a novel treatment modality such as oncolytic virotherapy.

High-grade glioma. High-grade glioma is one of the main targets in the development of oncolytic virotherapy, since it is the most frequent brain tumor in adults and has an extremely poor prognosis. During the last decade, a number of replicating oncolytic viruses

have been developed for the treatment of adult high-grade glioma patients. Some oncolytic wild-type viruses without relevant pathogenicity in humans have shown promising results in vivo in glioblastoma animal models: Newcastle disease virus (43), reovirus (44, 45), Semliki Forest virus (46), Myxoma virus (14) and rodent parvovirus H-1PV (47). Genetically engineered oncolytic viruses applied to high-grade glioma include HSV (7, 48), measles virus (49), conditionally replicating adenoviral vectors for the delivery of HSV thymidine kinase, human interferon β, or p53, and retroviral vectors delivering HSV thymidine kinase. Clinical trials in adult high-grade glioma patients demonstrating safety have been completed for some of these oncolytic viruses (50). Interestingly, intracranial delivery of HSV thymidine kinase by an adenoviral vector and concomitant ganciclovir treatment was the only approach that has resulted in a significant increase in survival in a phase III clinical trial so far (51).

In childhood, the incidence of high-grade glioma is lower than in adults, and below the age of 3 years it is rare. Like in adult patients, the prognosis of high-grade glioma in children is poor, with 5-year survival of about 20% in grade IV glioma patients, but children below the age of 3 years seem to have a much better prognosis. Survival is mainly determined by the resectability of the tumor and secondarily by response to chemotherapy and radiotherapy. Compared to oncolytic virotherapy in adult high-grade glioma, approaches targeting pediatric high-grade glioma have to face major differences in both the molecular genetic steps inducing carcinogenesis of pediatric glioma (52) and the biology of the surrounding, still-developing normal brain. Especially during the first 2 years of life, the development of the central nervous system is in a particularly vulnerable phase. Oncolytic viruses developed for the treatment of adult glioblastoma patients thus need to be carefully evaluated for toxicity to the still-developing infant brain. To date, the recombinant HSV-1 G207 (a $\gamma_1$34.5-deleted HSV-1 double mutant virus lacking both copies of the neurovirulence gene and the *UL39* gene encoding the large subunit of ribonucleotide reductase) is the only oncolytic virus that has also been tested in newborn laboratory animals for toxicity following intracranial application (7). No alterations in physical development, cognitive performance or exploratory behavior were observed. However, HSV-1 G207 virus infection was associated with an elevated risk of hydrocephalus formation, raising concerns about the safety of oncolytic HSV treatment of children (53). Furthermore, one caveat in the preclinical testing of oncolytic virotherapy concerns the lack of pediatric high-grade glioma cell lines.

Medulloblastoma/PNETs. Medulloblastoma and supratentorial PNETs are the most frequent malignant brain tumors in childhood. Resection of the tumor, chemotherapy and radiotherapy result in long-term survival rates of 50-70%, which is favorable compared to other malignant brain tumors in children. However, the majority of surviving patients suffer from severe treatment-related long-term side effects. At the time of diagnosis, about 30% of the patients have metastatic disease and represent a subgroup with particularly poor prognosis (52). In addition, relapsed patients have a particularly dismal prognosis. For these patients new treatment options are needed.

Three wild-type viruses, reovirus (54), Myxoma virus (13) and Seneca Valley virus (32), have been preclinically evaluated for medulloblastoma treatment. Moreover, a vaccination strain (Edmonton strain) of measles virus engineered to express green fluorescent protein (GFP) (55), and two conditionally replicating genetically modified viruses, HSV-1716 (56) and adenovirus Ad5-Delta24 (57), have been tested. Five viruses showed oncolytic activity in the majority of medulloblastoma cell lines analyzed in vitro, whereas for Seneca Valley virus no in vitro data on medulloblastoma are given. In contrast to the other oncolytic viruses, which showed oncolytic efficacy against medulloblastoma in vivo, for Seneca Valley virus no objective treatment response was observed in three different human medulloblastoma xenograft models in immunodeficient mice (32). HSV-1716 showed significantly improved survival in a murine medulloblastoma xenotransplant model (56). Reovirus-mediated oncolysis showed the expected dependence on Ras activation, in keeping with the involvement of Ras in virus escape from cellular defense mechanisms (58). Reovirus was studied in an orthotopic xenograft model. Here, a single intratumoral injection of reovirus resulted in significantly prolonged survival as compared to treatment with dead virus. Furthermore, repeated intratumoral reovirus administration suppressed the formation of spinal and leptomeningeal metastases, whereas two-thirds of the control group developed CNS metastases (54). Similarly, intratumoral application of Myxoma virus combined with i.p. injections of rapamycin significantly prolonged the animals' survival in murine human orthotopic medulloblastoma xenograft models compared with the control group infected with inactivated virus (13). Recently, the application of the Edmonton strain of measles virus engineered to express GFP (MV-GFP) to medulloblastoma cell lines and xenografts has been described. The applicability of this virus, how-ever, depends on the presence of the CD46 receptor on the respective tumor cells. In an orthotopic medulloblastoma xenograft model established from a CD46-expressing human medulloblastoma cell line, a single intratumoral application of MV-GFP significantly prolonged survival (55).

Extracranial solid tumors

Neuroblastoma. Neuroblastoma is the most frequent extracranial solid tumor in children. Despite the introduction of multimodal treatment concepts, high-risk neuroblastoma patients have an extremely poor prognosis, with long-term survival rates of only about 30%. High-risk neuroblastoma is frequently characterized by amplification of the oncogene *MYCN* and consecutive overexpression of the N-myc protein.

Seneca Valley virus (32, 59) and H-1PV (60) are the only wild-type viruses evaluated so far for their oncolytic efficacy in neuroblastoma. H-1PV has been studied on a panel of 11 neuroblastoma cell lines. We recently demonstrated that under in vitro conditions H-1PV is able to infect, replicate and induce complete lysis in all these neuroblastoma cells, irrespective of their *MYCN* amplification status. In all cell lines analyzed, H-1PV infection induced G_2 arrest and subsequent apoptosis at multiplicities of infection between 0.001 and 1, rendering this virus a good candidate for oncolytic virotherapy in neuroblastoma (60). A systematic in vitro screening for oncolytic effects of Seneca Valley virus in 23 cancer cell lines of the pediatric preclinical testing panel revealed cytopathic effects of Seneca Valley virus against 5 neuroblastoma cell lines analyzed. The in vivo screening showed that a single i.v. injection of Seneca Valley virus induced a complete treatment response in one of five neuroblastoma xenograft models and significantly increased event-free survival (EFS) in four of the five animal models (32).

Virus-mediated delivery of enzyme-directed prodrug therapy has been applied in animal studies for the ex vivo purging of autologous bone marrow transplants and was shown to reduce the tumor cell load with higher efficacy compared to conventional antibody-based purging strategies (61). The therapeutic efficacy of a variety of genetically engineered oncolytic viruses has been evaluated in vivo, namely attenuated poliovirus (62) and HSV (20, 63, 64). Long-term survival could be achieved in about 80% of the poliovirus-treated mice bearing neuroblastoma xenotransplants that expressed the poliovirus receptor (62). The intratumoral administration of an attenuated HSV-1 mutant (63) or a modified oncolytic HSV-1 armed with an MMP-antagonizing transgene led to long-term survival in 20-30% of the xenotransplant tumor-bearing animals (64). Interestingly, the therapeutic efficiency of these oncolytic HSV-1 viruses could be enhanced by sequential intratumoral injections of oncolytic HSV-1 and immature dendritic cells. This combination treatment led to a significant increase in survival of the treated animals via enhancement of antitumor immunity (65). Furthermore, a nestin-targeted oncolytic HSV-1 was constructed. Infection of putative neuroblastoma-initiating cells with this virus before inoculation into the animals prevented these cells from forming tumors in athymic nude mice (20). However, ex vivo infection with a conditionally replicating virus neither reflects the clinical situation of treating an established tumor nor allows to exclude that nontransformed neural stem cells might also be selectively targeted in the case of orthotopic administration of the virus.

Nephroblastoma (Wilms' tumor). Nephroblastoma is the most common renal tumor in children, being most frequently diagnosed in children below the age of 5 years. The current risk-adapted multimodal therapeutic concepts provide a good outcome, with cure rates of about 90%. Therefore, nephroblastoma has not been a focus of research in the field of oncolytic virotherapy.

The only virus-based approach to treat pediatric renal tumors reported so far made use of a nonreplicating recombinant adeno-associated virus vector (rAAV) expressing an antiangiogenic soluble vascular endothelial growth factor (VEGF) ligand (a truncated form of VEGFR-2) for antiangiogenic gene therapy (66). One of the two cell lines used for an orthotopic xenograft model was a rhabdoid tumor of the kidney, whereas the other cell line later turned out to be a Ewing's sarcoma of the kidney (67). To draw any conclusions relevant for the treatment of nephroblastoma, the potential clinical effectiveness of this approach needs to be addressed in a therapeutic model of established nephroblastoma tumors.

Hepatoblastoma. Hepatoblastoma is the most common liver tumor in early childhood (0.7-1 case per million children per year in Western countries). Most patients present before the age of 3 years with a large asymptomatic abdominal mass. In patients with resectable tumors (about three out of four), long-term survival is about 90%. Thus, only a few cases (relapsed or nonresectable tumors) would be eligible for novel therapeutic approaches.

In hepatoblastoma, like in colorectal cancer, malignant cells have been shown to undergo activation of the Wnt signaling pathway, resulting in the activation of TCF-4-dependent promoters. A genetically modified oncolytic HSV (bM24-TE) has been engineered to be driven by the TE promoter, a promoter containing a TCF-responsive element and the human 4F2 gene intronic enhancer. HSVbM24-TE should therefore be conditionally replicating in all malignant diseases with activated Wnt signaling. To date, oncolytic effects of bm24-TE have only been evaluated in vitro and not for hepatoblastoma cells (68).

Osteosarcoma. Osteosarcoma is the most frequent primary bone tumor (incidence of 4-5 per million persons) (69). Osteosarcoma patients have 5-year survival rates of 77% in children and 59% in adolescents and young adults. In contrast, with 5-year survival rates of 67% and 48%, respectively, the prognosis of children and adolescents or young adults suffering from Ewing's sarcoma is comparatively poor (2). Unresectable primary tumor and local or systemic relapse may be considered as indications for the use of oncolytic viruses in patients with bone tumors.

The first application of this strategy in osteosarcoma dates back to 1965, when Helen Toolan performed i.m. injection of wild-type H-1PV in two teenage patients suffering from treatment-resistant osteosarcoma. In both patients, viremia and subsequent seroconversion occurred (70), but the clinical outcome of the patients remained unaffected.

A systematic preclinical evaluation of oncolytic virotherapy approaches for osteosarcoma treatment has been performed using the two wild-type viruses Semliki Forest virus (71) and Seneca Valley virus (NTX-010) (32). In a preclinical pediatric screening approach using six SCID mouse osteosarcoma xenograft models, only a low treatment response was observed for the i.v. treatment with NTX-010. Only one of six xenograft models showed significantly extended event-free survival in the treated mice, whereas data on overall survival were not shown (32). The second wild-type virus, Semliki Forest virus, was shown to significantly increase survival in both a subcutaneous and a second orthotopic osteosarcoma xenograft model in nude mice (71).

Additionally, two recombinant adenoviruses retargeted to osteosarcoma were tested (72, 73). Due the low expression levels of the original coxsackievirus and adenovirus receptor on osteosarcoma cells, the conditionally replicating Ad5-Delta24 derived from adenovirus serotype 5 was retargeted to $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins in order to improve efficiency of viral entry into the host cell (74). The retargeted adenovirus Ad5-Delta24RGD was shown to be highly active in killing human osteosarcoma cell lines (73). Intratumoral injections of Ad5-Delta24RGD into established human osteosarcoma xenografts refractory to chemotherapeutic treatment caused a significant delay of tumor growth (75).

Another conditionally replicating adeno-virus based on Ad5-Delta24, Ad-OC-E1a, expresses the viral E1A gene under the control of the promoter of the bone matrix protein osteocalcin, which targets Ad-OC-E1a replication to cells of the bone matrix and malignant cells derived thereof. Intravenous application of Ad-OC-E1a significantly reduced the size of pulmonary metastatic osteosarcoma nodules in a mouse xenograft model (76). Altogether, these observations give credit to the further clinical evaluation of oncolytic viruses in osteosarcoma patients.

Ewing's sarcoma. Few therapeutic attempts based on oncolytic viruses have been reported so far for Ewing's sarcoma. The genetically engineered oncolytic HSVs NV1020 and G207 had also been tested against a panel of 10 sarcoma cell lines. However, compared to

rhabdomyosarcoma and osteosarcoma cell lines, Ewing's sarcoma cell lines showed the lowest susceptibility to oncolytic HSVs in vitro (77). The susceptibility of Ewing's sarcoma cell lines to adenovirus-mediated transduction could also be demonstrated, since the expression of adenovirus receptor and coxsackievirus/adenovirus receptor was observed in these cell lines. Moreover, a recombinant, conditionally replicating adenovirus induced significant oncolytic effects in Ewing's sarcoma cell lines in vitro (78). In animal models, only two oncolytic viruses were tested: the wild-type virus Seneca Valley virus NTX-010 and a recombinant AAV expressing an antiangiogenic soluble VEGF ligand. In the recently published preclinical evaluation of Seneca Valley virus in pediatric tumors, no significant effects on tumor growth or EFS have been described in four Ewing's sarcoma xenograft models (32). In contrast, the i.v. application of an rAAV expressing an antiangiogenic soluble VEGF ligand into the portal vein of SCID mice induced long-term transduction of hepatocytes. In two-thirds of these animals, the subsequent engraftment of Ewing's sarcoma xenografts could be prevented (66). Since the model used was only preventive, the relevance of this experimental approach to assess its therapeutic efficacy, however, remains limited.

Rhabdomyosarcoma. Rhabdomyosarcoma is the most frequent soft tissue sarcoma observed in children (79). Thirty percent of the patients die within the first 5 years after diagnosis (2). So far, four different genetically engineered oncolytic HSVs, which rely in particular on the deficiency of antiviral defense mechanism, have been preclinically evaluated for their therapeutic efficacy against rhabdomyosarcoma. In a human rhabdomyosarcoma xenotransplant model in nude mice, intratumoral application of HSV-1 G207 induced complete tumor regression in 25% of the animals, and combination treatment with vincristine cured 31% of the treated animals (80). Using the oncolytic HSVs NV1020 and NV1066, the therapeutic efficacy of a single intratumoral oncolytic virus application to rhabdomyosarcoma xenotransplant tumors was further increased, with complete regression in 75% of the treated tumors.

HSV-rRp450 vector-mediated delivery of P450 convertase allowed gene-directed enzyme prodrug therapy with cyclophosphamide. The combination treatment with HSV-rRp450 and cyclophosphamide resulted in a more than doubled survival time for alveolar rhabdomyosarcoma xenotransplant-bearing immunodeficient mice (81, 82). Recently a pediatric preclinical screening revealed complete lysis of two of four rhabdomyosarcoma cell lines at a multiple of infection of 1 PFU/cell. Moreover, a high response to a single i.v. treatment with the Seneca Valley virus NTX-010 and significantly increased EFS were observed in all four alveolar rhabdomyosarcoma xenograft models analyzed (32).

Retinoblastoma. Retinoblastoma is a highly malignant tumor of the eye, which mainly occurs within the first 3 years of life. About 95% of the cases are hereditary, with patients harboring a germline mutation in the *RB1* gene. In European countries, long-term survival is about 95% (2), but extraocular retinoblastoma patients represent a subgroup of patients with an extremely poor prognosis (5-year survival < 10%).

Two oncolytic virotherapy approaches have been subjected to preclinical evaluation for retinoblastoma treatment. The conditionally replicating adenoviral vector was used for gene-directed enzyme suicide therapy (Ad-hTERT-E1A-CMV-HSV*tk*), while the oncolytic wild-type virus Seneca Valley virus was used for oncolytic virotherapy. The Ad-hTERT-E1A-CMV-HSV*tk* is an hTERT promoter-targeted conditionally replicating adenoviral vector armed with the HSV *TK* gene for prodrug therapy with ganciclovir. Combination treatment with ganciclovir and the conditionally replicating adenovirus Ad-hTERT-E1A-CMV-HSV*tk* efficiently suppressed the growth of human retinoblastoma in an orthotopic nude mouse model (83). Similarly, intratumoral application of the wild-type picornavirus Seneca Valley virus resulted in a significant reduction in invasive retinoblastoma growth and intracranial metastasis formation in an immunocompetent orthotopic mouse retinoblastoma model (84).

Tumor-initiating cells as targets of oncolytic viruses

Cancer-initiating cells are considered to be a subpopulation of malignant cells within a tumor responsible for self-renewal and growth in a hierarchical manner. These cells often share the expression of stem cell markers with normal, nontransformed stem/progenitor cells. In several models, cancer-initiating cells have been shown to have the potential for self-renewal and pluripotency and to be resistant to "conventional" cytostatic drugs or irradiation. Thus, local relapse of a tumor or metastasis formation after initial clinical remission is suspected to originate from these cells (85). Therefore, a variety of oncolytic viruses have been engineered to target cancer-initiating cells (for review see 86). Among them, two are already being evaluated preclinically for pediatric malignant diseases. In particular, the nestin promoter-targeted HSV rQNestin34.5 was applied to pediatric tumor-initiating cells of neuroectodermal origin. Under in vitro conditions, this virus was successful in killing human neuroblastoma-initiating cells. Furthermore, ex vivo infection of neuroblastoma neurospheres prior to implantation resulted in a significant reduction in tumor outgrowth in a mouse xenotransplant model (20). However, the significance of animal experiments applying this preventive therapeutic strategy is limited compared to the treatment of already established tumors in the clinical situation.

CLINICAL EVALUATION OF ONCOLYTIC VIROTHERAPY IN CHILDREN

Application of oncolytic viruses in children – conflict between medical and economic necessities

Although the second most frequent cause of death in children after the neonatal period, malignant diseases in childhood are rare compared to the incidences of cancer observed in adults. Only 1% of all malignant diseases are diagnosed in patients under the age of 25 years (2). The small size of pediatric patient groups renders the development of novel therapeutic agents comparatively unattractive for nonpublic funding. This fact is likely to impede the transfer from advanced preclinical evaluation of oncolytic virotherapy approaches to clinical trials focused on children.

Moreover, preclinical and clinical evaluation of oncolytic viruses for application in children needs to answer carefully several specific safety questions. In the case of oncolytic viruses derived from human pathogens, the probability of previous exposure to and immunization against the therapeutic virus used increases with the age of the child. Therefore, differences in the biodistribution of such a virus are

Table IV. *Ongoing clinical trials on oncolytic virotherapy including children and adolescents.*

Virus	Indication	Start	No. of patients	Age (years)	Design
NDV-HUJ	Recurrent glioblastoma multiforme	03/2006	14	11-58	Phase II
NTX-010	Refractory retinoblastoma, neuroblastoma	09/2009	30	3-21	Phase I/IIa
HSV-1716	Refractory non-CNS solid tumors	03/2010	18	13-30	Phase I
JX-594	Refractory neuroblastoma, rhabdomyosarcoma, lymphoma, nephroblastoma, Ewing's sarcoma	08/2010	15	2-21	Phase I

likely to occur and to result in different toxicity profiles compared to adults. In the case of replicating oncolytic viruses, the course of an infection may significantly differ from that observed in adult patients, e.g., it may result in a prolonged or even persistent infection. The incompleteness of distinct tissue development in young children may provide oncolytic viruses with cellular targets, which are not or much less present in adults. This can be exemplified by the developing brain, in which myelinization continues until the end of the second year of life. Therefore, topical application of oncolytic viruses in brain tumors in infants or young children is a particular challenge. After being cured of their disease, children still have a life expectancy of several decades. Accordingly, the time interval to be considered for delayed side effects is obviously longer for patients treated during childhood. Therefore, careful toxicological assessment is a prerequisite for translating preclinical virotherapy studies into clinical trials in children. These additional, specific pediatric safety issues rising ethical as well as economic concerns may have deferred oncolytic virotherapy trials in children and adolescents.

Ongoing oncolytic virotherapy trials involving children or adolescents

At present (November 9, 2010), 24 clinical trials on oncolytic virotherapy have been performed, and 15 studies are still recruiting patients (http://clinicaltrials.gov, Word Health Organization's International Clinical Trials Registry Platform). So far, only a few clinical trials completed or ongoing in adults concern neoplastic diseases of pediatric relevance. In particular, the results of several studies on malignant glioma have been documented, showing the safety of the oncolytic viruses applied and providing initial evidence of antitumor activity (50). As to the clinical application of oncolytic viruses to pediatric glioma patients, only single case reports are available concerning the application of Newcastle disease virus MTH 68-H strain (87, 88), for which a clinical phase I trial (NCT00348842) was started in 2006. In addition, one 11-year-old patient has been included in an adult phase I/II trial with the oncolytic NDV-HUJ strain (9) (see Table IV). Taking together all five pediatric patients from the different publications on Newcastle disease virus, promising results with single cases of long-term therapy response have been documented. Therefore, another phase I/II trial intended to recruit 30 patients between the ages of 3 and 75 years with progression of neuroblastoma, sarcoma or glioblastoma has been conceived (NCT01174537), but has not yet started to recruit patients.

To date, three clinical trials enrolling patients under the age of 18 years have been initiated and are still in progress (see Table IV), and were recently reviewed in detail (8). The first clinical trial exclusively addressed to pediatric patients started in September 2009 (NCT01048892). This phase I trial includes children with refractory extracranial solid tumors with neuroendocrine features (e.g., neuroblastoma, retinoblastoma, carcinoid tumors) treated with NTX-010 (Neotropics, Inc.), a strain of the Seneca Valley virus. The second, still-recruiting pediatric study (NCT01169584) includes patients with relapsed or refractory extracranial solid tumors eligible for intratumoral virus application. In this study, JX-594, a vaccinia virus engineered to express GM-CSF, is administered to 15 patients. The third trial (NCT00931931) is a phase I study that includes adolescents over the age of 13 years with extracranial solid tumors refractory to treatment. This trial is evaluating HSV-1716, an ICP34.5-null mutant that is conditionally replication-competent. In adult cancer patients, clinical safety and selectivity of replication have been demonstrated for intratumoral injection of HSV-1716 into high-grade glioma, squamous carcinoma of the head and neck, and melanoma (89-91). The current trial covers single as well as repeated intratumoral application.

Thus, in addition to single case reports of glioma treatment by Newcastle disease virus, only three clinical trials have started to recruit pediatric patients. However, after more than a decade of clinical research on oncolytic virotherapy for adult cancer patients, the first steps towards oncolytic virotherapy for children and adolescents are now being taken.

DISCLOSURES

The authors state no conflicts of interest.

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