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Determinants of the efficacy of viro-immunotherapy: A review

J.F. de Graaf, M. Huberts, R.A.M. Fouchier, B.G. van den Hoogen*

Viroscience Department, Erasmus Medical Centrum, Rotterdam, The Netherlands

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ABSTRACT

Oncolytic virus immunotherapy is rapidly gaining interest in the field of immunotherapy against cancer. The minimal toxicity upon treatment and the dual activity of direct oncolysis and immune activation make therapy with oncolytic viruses (OVs) an interesting treatment modality. The safety and efficacy of several OVs have been assessed in clinical trials and, so far, the Food and Drug Administration (FDA) has approved one OV. Unfortunately, most treatments with OVs have shown suboptimal responses in clinical trials, while they appeared more promising in preclinical studies, with tumours reducing after immune cell influx. In several clinical trials with OVs, parameters such as virus replication, virus-specific antibodies, systemic immune responses, immune cell influx into tumours and tumour-specific antibodies have been studied as predictors or correlates of therapy efficacy. In this review, these studies are summarized to improve our understanding of the determinants of the efficacy of OV therapies in humans and to provide insights for future developments in the viro-immunotherapy treatment field.

1. Introduction

The development of cancer treatment has been, and still is, a priority in the biomedical research community. The first effective cancer treatments developed included surgery, radiotherapy and chemotherapy, but given their limited use against some cancers, new strategies are still being explored. Immunotherapy is one of the relatively new strategies, in which the immune system is (re-) activated to target and kill malignant cells. The use of immunotherapy to treat cancer has gained substantial attention in the past decade and different approaches have been developed to induce strong anti-tumour immune responses, such as the use of cancer vaccines, adoptive T-cell transfer, monoclonal antibodies against tumour antigens, checkpoint inhibitors and oncolytic viruses (OVs). [1,2] Initially, OVs were explored to induce direct oncolysis through virus replication in tumour cells or activation of the apoptosis pathway resulting in cell death, but more recently it has become clear that activation of the immune system to induce tumour cell death (indirect oncolysis), in which the viral infection works as a kick-start to activate the immune system, may be even more important. [3–5]

Numerous clinical and preclinical studies have reported promising anti-tumour potential for viro-immunotherapy. Compared to chemotherapies, less toxicities and adverse events were reported in clinical trials with viro-immunotherapy, demonstrating the applicability of the therapy. The reported mild adverse effects are often described as flu-like symptoms. [6–8] Talimogene Laherparepvec (T-VEC/IMLYGIC), a genetically modified herpes simplex virus expressing granulocyte macrophage colony stimulating factor (GM-CSF), is the only OV that has been successfully tested in phase III trials. The results from the clinical trial resulted in the application of the therapy in the USA, EU and Australia [9–11]. This application illustrates that viro-immunotherapy is suitable for implementation in daily clinical practice [12,13].

However, while preclinical studies reported positive results, including enhanced immunological anti-tumour responses, tumour shrinkage and even complete clearance, viro-immunotherapy often resulted in a poor anti-tumour efficacy in clinical trials. The approval for only one OV to be used in viro-immunotherapy suggests that efficacy is lost in translation from preclinical trials to the clinic. Determinants of anti-tumour efficacy in murine models are often limited to increased infiltration of T cells into the tumour. However, virological or immunological parameters to predict a positive response to treatment in

Abbreviations: AdV, adenovirus; CR, complete responder; FDA, USA food and drug administration; GM-CSF, Granulocyte-macrophage colony-stimulating factor; HSV, herpes simplex virus; IL, interleukin; IFN, interferon; I.P., intraperitoneal; I.T., intratumoral; I.V., intravenous; MV, measles virus; Nabs, neutralizing antibodies; NDV, Newcastle disease virus; OS, overall survival; OVs, Oncolytic viruses; PD, progressing disease; PR, partial responder; SD, stable disease; TILs, tumor infiltrating lymphocytes; TNF, tumor necrosis factor; Tregs, regulatory T cells Vabs, anti-virus antibodies; VV, vaccinia virus

* Corresponding author at: dr.Molewaterplein 40, 3015 GD, Rotterdam, The Netherlands.
E-mail address: b.vandenhoogen@erasmusmc.nl (B.G. van den Hoogen).

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Table 1
Reported oncolytic virus clinical trials.

Virus	Disease	Regiment		Parameters				Clinical
		Route	Schedule	Adverse events (grade)	Cytokine elevation serum	Viral shedding and replication	Immune responses	Outcome
AdV (CGTG-102) [25]	Advanced solid tumours	I.T.	Single Serial	1 – 2 & 4 = Single	↑Il6, Il10 > Single	Viremia Viremia	↑ TILs > Single	OS: 111 days (n = 72) OS: 277 days (n = 51)
AdV (CGTG-602) [58]	Advanced metastatic tumours	I.T.	Serial	1 – 3	N.A.	Viremia	↑ NAbs, ↓TAA-Abs, ↑TILs	OS: 80 – 135 days (n = 13)
AdV (Ad5/3-Δ24) [61]	Recurrent ovarian cancer	I.P.	Serial	1 – 2	N.A.	Viremia, viruria, saliva, replication	↑ NAbs	SD: 6/8
AdV (Ad5/3-Δ24) [62]	Advanced recurrent refractory solid tumours	I.T.	Dose-escalaion	1 – 3	↑GM-CSF, = TNF-α, IL-6	Viremia	↑ NAbs	PD: 7/7
AdV (Ad5/3-Δ24 + GM-CSF) [62]					↑GM-CSF, = TNF-α, IL-6	Viremia	↑ NAbs, ↑ T-cells	SD: 4/7
AdV (Ad5/3-Δ24 + GM-CSF) [53]	Advanced solid tumours	I.T.	Single Single + CP	1 – 3 1 – 3	N.A. N.A.	Replication Replication	↑ NAbs ↑ NAbs, ↓Tregs	OS: 120 days (n = 8) OS: 376 days (n = 7)
AdV (Temolysin) [63]	Advanced solid tumours	I.T.	Single	1 – 4	↑Il6, Il7 Il10	Viremia	↑ NAbs	OS: 10 months (n = 16) SD: 7/10
AdV (H103) [64]	Advanced solid tumours	I.T.	Dose-escalation	1 – 4	N.A.	N.A.	↑ T-cells, ↑ NAbs	ORR: 11.1 % (n = 3/27)
AdV (Ad5/3-Δ24 + GM-CSF) [65]	Advanced solid tumours	I.T.	Dose-escalation	1 – 3	–	Viremia	↑ T-cells, ↑ NAbs	OS: 200 – 400 days (n = 21)
AdV (ICOVIR-7) [29]	Advanced solid tumours	I.T.	Dose-escalation	1 – 3	↑Il6, Il8 Il10, TNF-a	Viremia	↑ NAbs	PR + SD: 9/17
AdV (Ad5-Δ24-RGD) [66]	Recurrent gynaecologic tumours	I.P.	Dose-escalation	1 – 3	N.A.	Viremia, viruria, saliva, replication	↑ NAbs	SD: 15/21
AdV (DNX-2401) [67]	Recurrent malignant glioma	I.T. (brain)	Dose-escalation	1 – 4	N.A.	Replication	↑TILs	OS: 9.5 months (n = 25) CR: 12 % (3/25)
AdV (Enadenotucirev) [14]	Resectable primary tumours	I.T. I.V.	Single	1 – 2	–	Faecal shedding, replication Replication, viremia	↑ NAbs, ↑TILs	N.A.
AdV (ICOVIR-5) [22]	Cutaneous and uveal melanoma	I.V.	Serial (low) Serial (high)	1 – 3	↑IL-6, IL-10	– Shedding, replication, viremia	↑ NAbs	SD: 2/7 SD: 5/6, OS: 73 – 271 days (n = 13)
HSV (T-VEC) [12,13]	Stage IIIB-IV melanoma	I.T. I.T.	Serial Serial	1 – 4	N.A. N.A.	N.A. Replication	N.A. N.A.	OS: 23.3 months (n = 295) Control (GM-CSF): OS 18.9 months (n = 141)
HSV (NV1020) [42,68]	Metastatic colorectal cancer	I.A.	Single	1 – 2	= IL-1, IL-2, TNF-α, IFN-γ	Shedding and replication	N.A.	OS: 25 months (n = 12) SD: 7/10
HSV (NV1020) [28,41]	Metastatic colorectal cancer	I.A.	Single	1 – 2	↑Il6, IFN-y, TNF-a	–	↑ NAbs	OS: 11.8 – 12.4 months (n = 32)
HSV (G207) [69]	Recurrent malignant glioma	I.T.	Single + radiation	1 – 3	N.A.	Saliva	↑ CD8 + / CD4 + T cells, ↑ NAbs	OS: 7.5 months (n = 9) PR + SD: 6/9
HSV (HF10) [36]	Non-resectable pancreatic cancer	I.T.	Dose-escalation	–	–	Replication	↑ NAbs, ↑NK cells, ↑TILs	OS: 180 days (n = 6) PR + SD: 4/6

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Table 1 (*continued*)

Virus	Disease	Regiment		Parameters				Clinical
		Route	Schedule	Adverse events (grade)	Cytokine elevation serum	Viral shedding and replication	Immune responses	Outcome
HSV (T-Vec) [51]	Stage IIIB-IV melanoma	I.T.	Serial + Ipilimumab	1 – 4	N.A.	N.A.	↑ CD8 + T cells	DRR: 8 months (n = 8/18) CR + PR + SD: 13/18
HSV (T-Vec) [50]	Stage IIIB-IV melanoma	I.T.	Serial + Pembrolizumab	1 – 2	↑IFN-y	N.A.	↑ CD8 + T cells, ↑TILs	CR + PR + SD: 16/21
HSV (Oncovex) [21]	Squamous Cell Cancer of the Head and Neck	I.T.	Serial + Cisplatin + radiation	1 – 4	N.A.	Shedding, viremia	↑ NAbs	OS: 82.4 % up to 29 months (n = 14/17)
HSV (Oncovex) [24]	Refractory metastases from breast or GI cancer, melanoma, or epithelial cancer of the head and neck	I.T.	Single	1 – 2	–	Viremia, viruria, replication	↑ NAbs, ↑TILs	SD: 1/13
			Serial	= single	–	Viremia	↑ NAbs	SD: 2/17
HSV (HSV1716) [47]	Relapsed or refractory extracranial cancers	I.T.	Single (low) Single (high)	1 – 3	N.A.	Viremia	↑ VAbs	OS: 2.25 months (n = 6) OS: 7 months (n = 3)
HSV (Oncovex) [70]	Stage IIIc-IVM1c melanoma	I.T.	Serial	1 – 3	N.A.	–	↑ VAbs	OS: 52 % up to 24 months (n = 19)
MV (MV-CEA) [44]	Recurrent ovarian cancer	I.P.	Single	1 – 3	N.A.	Viruria, saliva	= Vabs	OS: 12.15 months (n = 21)
MV (MV-EZ) [45]	Cutaneous T-cell lymphoma	I.T.	Dose-escalation	1	↑IFN-y, Il12, Il2	Syncytia formation	TILs: ↑ CD8 +, ↓ CD4 +	CR + PR + SD: 5/6
MV (MV-NIS) [71]	Refractory myeloma	I.V.	Dose-escalation	1 – 4	N.A.	Viruria, saliva, viremia	↑ NAbs	CR: 1/32
Parvovirus (ParvOryx) [15]	Progressive primary or recurrent glioblastoma multiforme	I.T. I.V.	Dose-escalation	1 – 4	↑ Il12, Il2	Viremia, shedding Replication, shedding	↑TILs, ↑ a-viral T cells	OS: 464 days (n = 18)
Reovirus (Reolysin) [18]	Metastatic Colorectal cancer	I.V.	Serial	1 – 2	↑ IFN type I	Replication	↑ Nabs, ↑ NK cells	N.A.
Reovirus (Reolysin) [72]	Metastatic breast cancer	I.V.	Serial + paclitaxel	1 – 3	N.A.	N.A.	N.A.	OS: 17.4 months (n = 36)
			Paclitaxel					OS: 10.4 months (n = 38)
Reovirus (Reolysin) [73]	Recurrent ovarian, tubal or peritoneal cancer	I.V.	Serial + Docetaxel or Pemetrexed	1 – 4	N.A.	N.A.	N.A.	OS: 7.8 months (n = 77) Control (chemotherapy): 7.4 months (n = 75)
Reovirus (Reolysin) [74]	Advanced solid tumours	I.V.	Serial + Paclitaxel	1 – 4	N.A.	N.A.	N.A.	OS: 12.6 months (n = 54)
			Paclitaxel					OS: 13.1 months (n = 54)
Reovirus (Reolysin) [33]	Advanced solid tumours	I.V.	Single + Docetaxel	1 – 3	N.A.	Viruria, viremia, saliva, replication	↑ NAbs	CR + PR + SD: 14/16
Reovirus [75]	Recurrent malignant gliomas	I.T.	Single	1 – 2	N.A.	Saliva, feaces	↑ NAbs	OS: 21 weeks (n = 12)
Reovirus (Reolysin) [48]	Recurrent ovarian cancer	I.V.	Serial	1 – 4	N.A.	Replication	↑ NAbs	OS: 165 days (n = 21)
Reovirus (RT3D/Reolysin) [7]	Squamous Cell Carcinoma of the Head and Neck	I.T.	Serial + radiation	1 – 2	N.A.	No shedding, replication	↑ NAbs	PR: 7/14, SD: 7/14
Reovirus (RT3D/Reolysin) [26]	Advanced solid tumours	I.V.	Serial + carboplatin & paclitaxel	1 – 4	N.A.	Shedding	↑ NAbs	OS: 7.1 months (n = 31)
Reovirus (RT3D/Reolysin) [76]	Recurrent malignant glioma	I.T.	Single	1 – 3	N.A.	Viruria, viremia, saliva	N.A.	OS: 140 days (n = 15)
Reovirus (Reolysin) [37]	Relapsed extracranial solid tumors	I.V.	Single Single + CP	2 – 3	N.A.	Viremia	↑ NAbs	SD: 3/24

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Table 1 (*continued*)

Virus	Disease	Regiment		Parameters				Clinical
		Route	Schedule	Adverse events (grade)	Cytokine elevation serum	Viral shedding and replication	Immune responses	Outcome
Reovirus (Reolysin) [77]	Metastatic melanoma	I.V.	Single + carboplatin & paclitaxel	1 – 3	N.A.	N.A.	N.A.	OS: 10.9 months (n = 14)
Reovirus (REO-001) [78]	Advanced solid tumours	I.T.	Single Serial	1 – 4	N.A.	Viremia	↑ NAbs	CR + PR + SD: 7/17
Reovirus (RT3D/Reolysin) [38]	Advanced solid tumours	I.V.	Serial	1 – 3	N.A.	Viremia	↑ Nabs	N.A.
Reovirus (Reolysin) [19]	Relapsed myeloma	I.V.	Single Serial	1 – 3	N.A.	Replication	↑ NAbs	SD: 3/12
SVV (SVV-001) [20]	Neuro-endocrine based tumours	I.V.	Single	1 – 3	N.A.	Replication	↑ NAbs	OS: 780 days (n = 30)
SVV (NTX-010) [27]	Neuro-related tumours	I.V.	Single Serial	1 – 3 1 – 4	N.A.	Viremia, Feacal shedding	↑ NAbs	SD: 6/12 SD: 4/6
VV (PexaVec/JX-594) [16]	Advanced solid tumours	I.V.	Single	1 – 2	↑IFN-y, TNF-a, Il6, Il10, =Il1	Replication	↑ NAbs	PR + SD: 12/ 20
VV (PexaVec/JX-594) [79]	Hepatocellular carcinoma, neuroblastoma and Ewing sarcoma	I.T.	Single	1 – 4	↑IFN-y	Pustules vorm van replication?	↑ α-viral T cells	SD: 4/6
vvDD (JX-929) [17]	Advanced solid cancers	I.V.	Single	1 – 3	↑IFN-y, TNF-a, Il6, Il10, Il7, Il8, GM-CSF	Saliva, replication, viremia	↑ Nabs	OS: 4.8 months (n = 11)
VV (PexaVec/JX-594) [23]	Liver tumours	I.V.	Single (low) Single (high)	1 – 2 1 – 4	↑GM-CSF	Viremia	↑ Nabs, ↑ Neutrophils + Eosinophils, ↑ a-viral T cells	OS: 6.7 months (n = 14) OS: 14.1 months (n = 16)
VV (TG4023) [80]	Primary or metastatic liver tumours	I.T.	Single + F-FC/5-FU	1 – 3	N.A.	N.A.	↑ Nabs	SD: 8/15
VV (PexaVec/JX-594) [54]	Stage IV melanoma	I.T.	Serial	1 – 3	N.A.	Viremia	↑Nabs, ↑Neutrophils + Eosinophils	OS: 7.1 months (n = 10)
VV (GL-ONC1) [81]	Advanced head and neck cancer	I.V.	Single + Cisplatin Serial + Cisplatin	1 – 4	N.A.	Skin rash, Replication	N.A.	2 year OS: 69.2 % (13/ 19)
VV (Pexa-Vec/JX-594) [40]	Refractory primary or metastatic liver cancers	I.T.	Single	I.T.	↑TNF-a, Il6, Il10	Replication, Viremia	↑Eosinophils, ↑ Nabs	OS: 9 months (n = 14) PR + SD: 9/10
VV (Pexa-Vec/JX-594) [55]	Refractory metastatic Colorectal cancer	I.V.	Serial	1 – 3	↑TNF-a, Il6, Il, Il8, MIP-1a/b, MCP-1, ↑↑ Il2, Il10, IFN-y	Viremia, Saliva	↑Neutrophils	OS: 10.3 months (n = 15)
VV (PVSRIPO) [82]	Glioblastoma multiforme	I.T. (brain)	Dose-escalation	1 – 5	–	N.A.	–	OS: 12.5 months (n = 61)
VV (vvDD) [82]	Advanced solid tumours	I.T.	Single	1 – 2	↑ CCL5, CXCL9 & CXCL10	Viremia, replication	↑T cells, =TILs	–
NDV (NDV-HUJ) [49]	Glioblastoma Multiforme	I.V.	Serial	1 – 3	N.A.	Viruria, viremia, replication	↑ Nabs	OS: 32 weeks (n = 14)
NDV (PV701) [32]	Advanced solid tumours	I.V.	Serial (desensitization)	1 – 3	N.A.	Viruria	↑ Nabs	SD: 5/8
NDV (PV701) [30]	Advanced solid tumours	I.V.	Serial (desensitization)	1 – 4	↑ IFN type 1, IFN-y, Il-6, TNF-a	Saliva, Viruria	↑ Nabs, ↑TILs	CR + PR: 2/62
NDV (PV701) [83]	Advanced solid tumours	I.V.	Serial	1 – 3	↑ IFN type 1, TNF-a	Viruria	↑ Nabs	CR + PR: 6/18 SD: 9/18

OS: overall survival, PD: progressing disease, CR: complete responder, PR: partial responder, SD: stable disease, Nabs: neutralizing antibodies, Vab: anti-virus antibodies, TILs: tumour infiltrating lymphocytes.
Used mesh terms: (-Desjarinds and Lang) ("oncolytic viruses"[MeSH Terms] OR ("oncolytic"[All Fields] AND "viruses"[All Fields]) OR "oncolytic viruses"[All Fields] OR ("oncolytic"[All Fields] AND "virus"[All Fields]) OR "oncolytic virus"[All Fields]) NOT ("review"[Publication Type] OR "review literature as topic"[MeSH Terms] OR "review"[All Fields]) AND (Clinical Trial[ptyp] AND ("2008/12/01″[PDAT] : "2018/12/01″[PDAT]).

clinical trials are scarcely evaluated and vary between studies. Differences in administration strategies and clinical observations between studies make it difficult to draw conclusions on the efficacy of different viro-immunotherapies. This review summarizes clinical outcomes of several studies in relation to their corresponding administration strategies and reported parameters to improve our understanding of the underlying determinants of an effective viro-immunotherapy.

2. Administration strategies

The safety and efficacy of several viro-immunotherapies have been assessed in a number of Phase I clinical trials, including those using adenovirus (AdV), herpes simplex virus (HSV), vaccina virus (VV), measles virus (MV), parvovirus, Newcastle disease virus (NDV), reovirus, and Seneca Valley virus (SVV) (summarized in Table 1). Results from these studies demonstrated that the chosen strategies for administration influenced the virological and immunologic parameters and even the safety and efficacy of the therapy.

2.1. Route, dosage and schedule of treatment affecting efficacy

Administration strategies vary in administration route, dosage and schedule. In clinical trials, primarily intratumoral (I.T.) and intravenous (I.V.) injections have been applied. I.T. administration has often been preferred based on the assumption that I.T. administration, in contrast to I.V. injection, provided a better control of viral distribution, increased virus concentrations within the tumour and hence a better therapeutic effect. In two cohort studies with AdV (Enadenotucirev) [14] and Parvovirus (ParvOryx) [15], for treatment of resectable primary tumours or primary glioblastoma multiforme, the I.T. route and I.V. route were directly compared. In these studies, viral DNA was found in the tumours independent of the administration route, suggesting that I.V. administration can result in successful targeting of primary and even metastatic tumour tissues. This suggestion is further supported by similar observations in other I.V. injection based studies using VV (Pexa-Vec, vvDD) [16,17], reovirus (Reolysin) [18,19] and SVV (SVV-001) [16,19,20]. While I.V. injected viruses infected primary tumours as effective as metastatic tumours, I.T. administrated HSV (Oncovex) also infected metastatic lesion, indicating subsequent systemic spread of the virus upon I.T. administration [21]. The results of these studies suggest that differences in administration routes do not substantially affect viral spreading. However, no direct comparison of overall survival between I.V. and I.T. administration has been made yet in clinical trials to show the effect of different administration routes on treatment efficacy. In addition to the administration routes, different dosages and schedule options have been investigated in phase I and II trials. Studies using oncolytic AdV (ICOVIR-5) or VV (Pexa-Vec) demonstrated that the use of a higher dosage improved the overall response rate significantly compared to low dosages. [22,23] Similar results were reported in two studies, respectively with AdV and HSV, in which treatment with serial dosages was compared to single treatment [24,25]. Thus, the use of higher dosages and/or serial treatment schedules improved efficacy compared to a single low dosage without affecting adverse events in the patients of these studies.

2.2. Administration strategy affecting adverse events

The route, dosage or schedule of treatments do not only influence the efficacy, but are also expected to have an effect on adverse effects and thus patient's health. Most clinical trials reported adverse events ranging from grade I to III and occasionally grade IV (Table 1), but there was no direct evidence that the administration strategy directly affected these events. Initially, the I.T. route was considered the safest route, as this route would provide more control over viral distribution, reducing systemic viral recognition and hence should result in less adverse events. However, this assumption did not always hold true for every OV. The two cohort studies using AdV (Enadenotucirev) [14] and Parvovirus (ParvOryx) [15], directly comparing the I.T. and I.V. route, reported no additional toxicities due to I.V administration compared to I.T administration [24,25]. Similar to I.V. administration, higher dosages were thought to result in more adverse events compared to using low dosage. However, this correlation was not found in studies in which patients with melanoma or liver tumours were treated with AdV (ICOVIR-5) and VV (Pexa- Vec), where treatment with high and low dosages were compared [22,23]. In addition, dose-escalation studies with OVs such as SVV (NTX010) and reovirus (Reolysin), have demonstrated that for those OVs adverse effects were not dose dependent [26,27]. Although these studies suggest that the use of high dosage or serial dosages does not necessarily result in increased severe adverse effects, these parameters need to be evaluated for each OV, to improve therapy efficacy and prevent adverse events.

Short-term elevation in cytokine levels, such as IL-6, TNF-α and IFN-γ, upon viro-immunotherapy correlated with adverse events and the administration regimen could affect this type of adverse effects. [16,22,28,29] For example, one patient had high levels of IL-6 (200 pg/mL) upon I.V. injection with AdV (ICOVIR-5), which was found to account for the occurrence of the grade III adverse events that this person accrued. [27] In another study, grade I-II acute flu-like symptoms were associated with post-treatment elevation of IL-6 and IL-8 levels, which rose after I.V. inoculation of poxvirus (vvDD) [17]. To lower these severe adverse effects, longer infusion times or desensitization steps have been evaluated in different studies. In a study with NDV (PV701), the use of bolus dosing resulted in severely elevated cytokine levels and high frequency of adverse effects [30]. In contrast, in a different phase I trial, one hour infusion with the same virus resulted in only minor elevations of TNF and IFN-α expression levels [31]. Similarly, the use of a two-step desensitization strategy, with a smaller dosage followed by an higher dosage, reduced the occurrence of high-grade adverse events [32]. The reduction of adverse events after multiple cycles was also observed in patients treated with oncolytic reovirus [33]. The results of these studies suggest that adverse events are, at least in these clinical trials, temporary and linked to the start of therapy. Unfortunately, none of the studies using the different strategies with NDV (PV701) investigated the effect of the reduced induction of cytokine production on efficacy of the therapy. Several other studies have shown a beneficial effect of increased cytokine levels on therapy efficacy [34]. Future studies should evaluate the effect of administration strategies on cytokine responses, adverse effects and therapy efficacy.

3. Determinants of efficacy: virological and immunological parameters

The direct oncolysis induced by OVs and the indirect effects of the activated immune system influence various virological and immunological parameters. In clinical trials, these parameters consist of anti-viral antibodies, virus replication, systemic immune responses, immune cell influx and anti-tumour antibodies.

3.1. Anti-viral antibodies

Upon OV therapy, the immune system responds to the virus by producing antiviral antibodies, including neutralizing antibodies, which could affect the efficacy of viro-immunotherapy. In a study using AdV (Onyx-015), pre-existing neutralizing antibodies against AdV were associated with lower efficacy. [35] To avoid this neutralizing effect, animal viruses, such as SVV or NDV, were considered more beneficial as treatment modality in viro-immunotherapy. However, the presence of neutralizing antibodies did not seem to effect viral replication in clinical trials using oncolytic Reovirus and HSV [36,37]. Adair et al. and Roulstone et al. demonstrated that reovirus used virus neutralizing antibodies bound by monocytes to target tumours, indicating that these neutralizing antibodies did not necessarily limit viral distribution

[18,38]. In addition, in a dose-escalation study with oncolytic HSV (OncoVEX) pre-existing antibodies reduced adverse events, as in seronegative patients, toxicities seemed to be more frequent compared to patients with pre-existing immunity [24]. The induction of an early antibody response was associated with a complete response of one patient who was treated with oncolytic NDV (HUJ) [39]. A similar observation was made in a study with oncolytic VV (Pexa-Vec) in which responding patients had an increased antibody response compared to non-responders [40]. These studies suggest that an anti-viral immune response could be a predictive parameter for treatment efficacy. However, in studies using the FDA approved HSV (T-VEC) [24], NDV (PV701) [32] or HSV (NV2010) [41], no correlation was observed between increased antiviral antibody titers and efficacy. Therefore, further investigations are necessary to understand the effects of the induction of antiviral antibodies and the potential correlation with efficacy of the therapies.

3.2. Virus replication

In principle, the efficacy of viro-immunotherapy is based on virus induced oncolysis and stimulation of the anti-tumour immune response. Direct oncolysis is a result of virus replication in tumour cells, which thus could be an important determinant of efficacy. Several studies have reported the presence of viral genomes, proteins or even infectious virus in tumour tissues. [15,17,48,22,33,42–47] In some studies, infectious virus was even detected in the tumour of patients 130 days after treatment with NDV (HUJ) and viral antigens 318 days after treatment with HSV (HF10) after the start of therapy [36,49]. Also in studies with the FDA approved HSV (T-VEC) [13], virus replication in tumour tissues was observed. However, the contribution of virus replication in the tumour to therapeutic efficacy is uncertain due to the lack of correlative evidence on efficacy in clinical studies. For instance, no correlation was observed between virus detection in tumours and the response to treatments with MV (MV-CEA) [44] or VV (JX-594) [46]. Therefore, the contribution of the direct oncolytic effect by virus replication and the indirect oncolytic effects induced by the immune system still needs to be established.

3.3. Immune cell influx

Induction of an antitumour immune response is one of the most important objectives in clinical trials with immune therapies and can be divided in local and systemic responses. Viro-immunotherapy studies have shown that increased infiltration of cytotoxic T- and B-cells into the tumour is indicative for positive patient responses. For example, tumour influx of immune cells was observed in both a responding and a non-responding patient after treatment with NDV (PV701). [30] The tumour tissue of the responding patient contained lymphoid follicles with germinal centers consisting of infiltrated immune cells. However, the tumour tissue of the non-responding patient had multiple areas of necrosis and inflammatory mononuclear infiltrating cells. Furthermore, in a study comparing administration of serial versus single dosage of AdV (CGT-102) in a cohort of patients with different types of tumours, an increased T-cell infiltration was found after serial treatment, which was not observed after single treatment [25]. This serial treatment resulted in a median overall survival of 269 days versus 128 days in the single treatment group and suggested the beneficial effects of the tumour infiltration of immune cells. Similar observations were made in a study with the FDA approved HSV (T-VEC) in which low counts of T helper and cytotoxic T cells in blood and tumours correlated with disease progression [50,51]. Therefore, tumour infiltration of T cells remains one of the most important objectives of viro-immunotherapy, which has often been demonstrated in preclinical models, but is not always achieved in clinical trials.

In addition to inducing T cell infiltrations, the reduced presence of immune suppressive regulatory T cells (Tregs) in tumours is considered a prognostic value for survival of cancer patients in general. [52] For instance, in a phase I study with HSV (T-Vec) for patients with melonama, reduced Tregs infiltration was observed into the tumours [50]. In addition, in a study using AdV (Ad5/3-D24-GMCSF) in combination with cyclophosphamide, patients treated with only the virus or only with cyclophosphamide had less Tregs in their tumours compared to patients treated with combination therapy [53]. Improved clinical outcomes were reported for the patients receiving the combination therapy, but a correlation with the decreased Tregs levels was not mentioned. Thus, the importance of reduced amounts of Tregs in tumour tissues as an objective in clinical trials should be further investigated.

3.4. Systemic immune responses

The potential prognostic determinants of efficacy upon treatment could perhaps most easily be determined by evaluation of the systemic immune responses upon treatment. Studies conducting blood analyses often demonstrated that patients did respond systemically to the therapy by having short-term and/or long-term elevated cytokine levels and/or increased immune cell counts (Table 1). In case of short-term elevated cytokine levels, such as those of IL-6, IL-8 and TNF-α, it was already mentioned that this increased the prevalence of adverse effects, but little is known about the influence on treatment efficacy. In addition to short-term elevated cytokine levels, long-term elevated cytokines levels, such as those of IL-2, IL-10 and IFN-γ, are also often observed. For instance, in patients treated with serial dosages of AdV (CGTG-102), long-term elevation of IL-10 levels was observed and an improved overall survival was reported in patients treated with a serial dosage in contrast to patients treated with a single dosage. However, a correlation between increased long-term cytokine levels and survival was not investigated in detail. [25] Similarly, increased counts of granulocytes, such as neutrophils and eosinophils, and cytotoxic ($CD8^+$) and helper T ($CD4^+$) cells upon viro-immunotherapy are often observed, but potential correlations have not been investigated. [43,50,51,54,55] Thus, the effect of systemic immune responses on overall survival remains to be evaluated as prognostic value for treatment efficacy.

3.5. Tumour-associated antigen specific antibodies

Another important systemic immune response is the production of tumour-associated antigen specific antibodies (TAA-Ab), which are produced by B cells and can induce antibody dependent cellular cytotoxicity by NK cells. However, B cells themselves are often negatively associated with tumour development, because they secrete pro-tumourigenic factors and immune suppressive cytokines. [56] In addition, the expression of TAA-Abs often correlates with decreased overall survival in cancer patients [57]. For example, patients that responded to treatment with ADV (CGTC-602) displayed decreased antibody titers against tumour antigens CEA, NY-ESO-1, survivin and MUC-1, whereas anti-tumour T cell responses were increased [58]. These results suggest that inhibition of B cell responses could be important for an effective therapy and reduced TAA production upon treatment could be a good prognostic value for the efficacy of viro-immunotherapy.

4. Future perspectives

Viro-immunotherapy therapy is establishing itself as an immunotherapy. However, in clinical trials, the overall responses have often been limited, whereas in preclinical trials the therapy looked promising. Apparently, the efficacy of the new therapy is lost in translation from murine to human studies in which observations made in preclinical trials, such as T cell infiltrations, are lacking in clinical trials. To improve our understanding about prognostic parameters of effective therapies in humans, we summarized different study outcomes

and their findings.

Several virological and immunological parameters have been reported, including cytokine levels, virus replication, (pre-existing) virus neutralizing antibodies, TAA-Abs and influx of immune cells. While some of these parameters have been infrequently monitored, others were extensively examined but without reporting any clear correlation with efficacy of the therapy. Opinions on the value of some of these observations as determinants of efficacy have been subject to change, such as the presence of pre-existing virus neutralizing antibodies. Against expectations, these antibodies were shown to improve rather than limit virus distribution in most treatments. Slowly, preclinical studies are showing benefits of pre-existing anti-viral antibodies. [59] The increased knowledge on the potential positive role of pre-existing antibodies resulted in exploiting this in viro-immunotherapy through vaccinating patients before treatment [60].

This newly gained insight in the role of virus neutralizing antibodies emphasises the need to obtain more in-depth knowledge on distinct immunologic parameters in order to characterize different determinants of efficacy to improve the successful translation of preclinical studies to clinical trials of viro-immunotherapy.

Declaration of Competing Interest

All authors declare that they don't have a conflict of interest.

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References

[1] J. Lohmueller, O.J. Finn, Current modalities in Cancer immunotherapy: immunomodulatory antibodies, CARs and vaccines, Pharmacol. Ther. 178 (2017) 31–47.
[2] P.K. Bommareddy, M. Shettigar, H.L. Kaufman, Integrating oncolytic viruses in combination Cancer immunotherapy, Nat. Rev. Immunol. 18 (8) (2018) 498–513.
[3] C. Heise, T. Hermiston, L. Johnson, G. Brooks, A. Sampson-Johannes, A. Williams, L. Hawkins, D. Kirn, An adenovirus E1A mutant that demonstrates potent and selective systemic anti-tumoral efficacy, Nat. Med. 6 (10) (2000) 1134–1139.
[4] H. Fukuhara, Y. Ino, T. Todo, Oncolytic virus therapy: a new era of Cancer treatment at dawn, Cancer Sci. 107 (10) (2016) 1373–1379.
[5] M.E. Davola, K.L. Mossman, Oncolytic viruses: how "Lytic" must they Be for therapeutic efficacy? Oncoimmunology 8 (6) (2019) e1581528–e1581528.
[6] M.R. Duffy, K.D. Fisher, L.W. Seymour, Making oncolytic virotherapy a clinical reality: the european contribution, Hum. Gene Ther. 28 (11) (2017) 1033–1046.
[7] K.J. Harrington, E.M. Karapanagiotou, V. Roulstone, K.R. Twigger, C.L. White, L. Vidal, D. Beirne, R. Prestwich, K. Newbold, M. Ahmed, et al., Two-Stage Phase I Dose-Escalation Study of Intratumoral Reovirus Type 3 Dearing and Palliative Radiotherapy in Patients with Advanced Cancers, Clin. Cancer Res. 16 (11) (2010) 3067–3077.
[8] W.D. Tap, R.L. Jones, B.A. Tine, B. Van; Chmielowski, A.D. Elias, D. Adkins, M. Agulnik, M.M. Cooney, M.B. Livingston, G. Pennock, et al., Olaratumab and doxorubicin versus doxorubicin alone in Soft tissue sarcoma, Lancet 388 (10043) (2016) 488–497.
[9] https://www.cancer.gov/news-events/cancer-currents-blog/2015/t-vec-melanoma (accessed May 22, 2020).
[10] https://www.nps.org.au/australian-prescriber/articles/talimogene-laherparepvec (accessed May 22, 2020).
[11] https://www.ema.europa.eu/en/medicines/human/EPAR/imlygic (accessed May 22, 2020).
[12] R.H.I. Andtbacka, H.L. Kaufman, F. Collichio, T. Amatruda, N. Senzer, J. Chesney, K.A. Delman, L.E. Spitler, I. Puzanov, S.S. Agarwala, et al., Talimogene laherparepvec improves durable response rate in patients with advanced melanoma, J. Clin. Oncol. 33 (25) (2015) 2780–2788.
[13] J. Chesney, S. Awasthi, B. Curti, L. Hutchins, G. Linette, P. Triozzi, M.C.B. Tan, R.E. Brown, J. Nemunaitis, E. Whitman, et al., Phase IIIb safety results from an expanded-access protocol of Talimogene Laherparepvec for patients with unresected, stage IIIB-IVM1c melanoma, Melanoma Res. 28 (1) (2018) 44–51.
[14] R. Garcia-Carbonero, R. Salazar, I. Duran, I. Osman-Garcia, L. Paz-Ares, J.M. Bozada, V. Boni, C. Blanc, L. Seymour, J. Beadle, et al., Phase 1 study of intravenous administration of the chimeric adenovirus enadenotucirev in patients undergoing primary tumor resection, J. Immunother. Cancer 5 (1) (2017) 1–13.
[15] K. Geletneky, J. Hajda, A.L. Angelova, B. Leuchs, D. Capper, A.J. Bartsch, J.O. Neumann, T. Schöning, J. Hüsing, B. Beelte, et al., Oncolytic H-1 parvovirus shows safety and signs of immunogenic activity in a first phase I/IIa glioblastoma trial, Mol. Ther. 25 (12) (2017) 2620–2634.
[16] C.J. Breitbach, J. Burke, D. Jonker, J. Stephenson, A.R. Haas, L.Q.M. Chow, J. Nieva, T.H. Hwang, A. Moon, R. Patt, et al., Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans, Nature 477 (7362) (2011) 99–104.
[17] S. Downs-Canner, Z.S. Guo, R. Ravindranathan, C.J. Breitbach, M.E. O'Malley, H.L. Jones, A. Moon, J.A. McCart, Y. Shuai, H.J. Zeh, et al., Phase 1 Study of Intravenous Oncolytic Poxvirus (VvDD) in Patients with Advanced Solid Cancers, Mol. Ther. 24 (8) (2016) 1492–1501.
[18] R.A. Adair, V. Roulstone, K.J. Scott, R. Morgan, G.J. Nuovo, M. Fuller, Cell carriage, delivery, and selective replication of an oncolytic virus in tumor in patients, Sci. Transl. Med. 4 (138) (2012) 39–46.
[19] D.W. Sborov, G.J. Nuovo, A. Stiff, T. Mace, G.B. Lesinski, A phase 1 trial of single agent reolysin in patients with relapsed multiple myeloma, Clin. Cancer Res. 20 (23) (2014) 5946–5955.
[20] C.M. Rudin, J.T. Poirier, N.N. Senzer, J.S. Jr, K.D. Burroughs, P.S. Reddy, C.L. Hann, L. Paul, Phase I clinical study of Seneca Valley virus (SVV-001), a replication-competent picornavirus, in advanced solid tumors with neuroendocrine features charles, HHS Public Access 17 (4) (2017) 888–895.
[21] K.J. Harrington, M. Hingorani, M.A. Tanay, J. Hickey, S.A. Bhide, P.M. Clarke, L.C. Renouf, K. Thway, A. Sibtain, I.A. McNeish, et al., Phase I/II study of oncolytic HSVGM-CSFin combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell Cancer of the head and neck, Clin. Cancer Res. 16 (15) (2010) 4005–4015.
[22] M. García, R. Moreno, M. Gil-Martin, M. Cascallò, M.O. de Olza, C. Cuadra, J.M. Piulats, V. Navarro, M. Domenech, R. Alemany, et al., A phase 1 trial of oncolytic adenovirus ICOVIR-5 administered intravenously to cutaneous and uveal melanoma patients, Hum. Gene Ther. 00 (00) (2018) hum.2018.107..
[23] J. Heo, T. Reid, L. Ruo, C.J. Breitbach, S. Rose, M. Bloomston, M. Cho, H.Y. Lim, H.C. Chung, C.W. Kim, et al., Randomized dose-finding clinical trial of oncolytic immunotherapeutic vaccinia JX-594 in liver Cancer, Nat. Med. 19 (3) (2013) 329–336.
[24] J.C. Hu, R.S. Coffin, C.J. Davis, N.J. Graham, N. Groves, P.J. Guest, K.J. Harrington, N.D. James, C.A. Love, I. McNeish, A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor, Clin. Cancer Res. (2006) 12.
[25] A. Kanerva, P. Nokisalmi, I. Diaconu, A. Koski, V. Cerullo, I. Liikanen, S. Tähtinen, M. Oksanen, R. Heiskanen, S. Pesonen, et al., Antiviral and antitumor T-Cell immunity in patients treated with GM-CSF-Coding oncolytic adenovirus, Clin. Cancer Res. 19 (10) (2013) 2734–2744.
[26] E.M. Karapanagiotou, V. Roulstone, K. Twigger, M. Ball, C. Nutting, K. Newbold, M.E. Gore, J. Larkin, N. Konstantinos, M. Coffey, et al., Phase I/II trial of carboplatin and paclitaxel chemotherapy in combination with intravenous oncolytic reovirus in patients with advanced malignancies, Clin. Cancer Res. 18 (7) (2012) 2080–2089.
[27] M.J. Burke, C. Ahern, B.J. Weigel, J.T. Poirier, C.M. Rudin, Y. Chen, T.P. Cripe, B.M. Bernhardt, S.M. Blaney, Phase I trial of Seneca Valley virus (NTX-010) in children with relapsed / refractory solid tumors: a report of the children's oncology group, Pediatr. Blood Cancer 62 (5) (2015) 743–750.
[28] S.K. Geevarghese, D. Geller, H.A. A.; de Haan, M. Hörer, A.E. Knoll, A. Mescheder, J. Nemunaitis, T.R. Reid, D.Y. Sze, K.K. Tanabe, et al., Phase I/II study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory colorectal Cancer Metastatic to the liver, Hum. Gene Ther. 21 (9) (2010) 1119–1128.
[29] P. Nokisalmi, S. Pesonen, S. Escutenaire, M. Särkioja, M. Raki, V. Cerullo, L. Laasonen, R. Alemany, J. Rojas, M. Cascallo, et al., Oncolytic adenovirus ICOVIR-7 in patients with advanced and refractory solid tumors, Clin. Cancer Res. 16 (11) (2010) 3035–3043.
[30] A.L. Pecora, N. Rizvi, G.I. Cohen, N.J. Meropol, D. Sterman, J.L. Marshall, S. Goldberg, P. Gross, J.D. O'Neil, W.S. Groene, et al., Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers, J. Clin. Oncol. 20 (9) (2002) 2251–2266.
[31] S.J. Hotte, R.M. Lorence, H.W. Hirte, S.R. Polawski, M.K. Bamat, J.D. O'Neil, M.S. Roberts, W.S. Groene, P.P. Major, An optimized clinical regimen for the oncolytic virus PV701, Clin. Cancer Res. 13 (3) (2007) 977–985.
[32] S.A. Laurie, J.C. Bell, H.L. Atkins, J. Roach, M.K. Bamat, J.D. O'Neil, M.S. Roberts, W.S. Groene, R.M. Lorence, A Phase 1 Clinical Study of Intravenous Administration of PV701, an Oncolytic Virus, Using Two-Step Desensitization, Clin. Cancer Res. 12 (8) (2006) 2555–2562.
[33] C. Comins, J. Spicer, A. Protheroe, V. Roulstone, K. Twigger, C.M. White, R. Vile, A.A. Melcher, M. Phelps, K.J. Harrington, et al., REO-10: a phase I study of intravenous reovirus and docetaxel in patients with advanced Cancer, Clin. Cancer Res. 16 (22) (2010) 5546–5572.
[34] K.C. Conlon, M.D. Miljkovic, T.A. Waldmann, Cytokines in the treatment of Cancer, J. Interferon Cytokine Res. 39 (1) (2018) 6–21.
[35] D. Kirn, Oncolytic virotherapy for Cancer with the adenovirus Dl1520 (Onyx-015): results of phase I and II trials, Expert Opin. Biol. Ther. 1 (3) (2001) 525–538.
[36] A. Nakao, H. Kasuya, T.T. Sahin, N. Nomura, A. Kanzaki, M. Misawa, T. Shirota, S. Yamada, T. Fujii, H. Sugimoto, et al., A phase i dose-escalation clinical trial of intraoperative direct intratumoral injection of HF10 oncolytic virus in non-resectable patients with advanced pancreatic Cancer, Cancer Gene Ther. 18 (3) (2011) 167–175.
[37] E. Anders Kolb, V. Sampson, D. Stabley, A. Walter, K. Sol-Church, A Phase I Trial

and Viral Clearance Study of Reovirus (Reolysin) in Children with Relapsed or Refractory Extra-Cranial Solid Tumors: A Children's Oncology Group Phase I Consortium Report, Pedriatric Blood Cancer 62 (5) (2015) 751–758.
[38] V. Roulstone, K. Khan, H.S. Pandha, S. Rudman, M. Coffey, G.M. Gill, A.A. Melcher, R. Vile, K.J. Harrington, Jde Bono, et al., Phase I trial of cyclophosphamide as an immune modulator for optimizing oncolytic reovirus delivery to solid tumors, Clin. Cancer Res. 21 (6) (2015) 1305–1312.
[39] A. Freeman, Z. Zakay-Rones, J. Gomoril, E. Linetsky, L. Rasooly, E. Greenbaum, S. Rozenman-Yair, a Panet, E. Libson, C. Irving, et al., Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme, Mol. Ther. 13 (1) (2006) 221–228.
[40] B.H. Park, T. Hwang, T.C. Liu, D.Y. Sze, J.S. Kim, H.C. Kwon, S.Y. Oh, S.Y. Han, J.H. Yoon, S.H. Hong, et al., Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver Cancer: a phase I trial, Lancet Oncol. 9 (6) (2008) 533–542.
[41] D.Y. Sze, A.H. Iagaru, S.S. Gambhir, H.A. De Haan, T.R. Reid, Response to intra-arterial oncolytic virotherapy with the herpes virus NV1020 evaluated by [^{18} F] Fluorodeoxyglucose positron emission tomography and computed tomography, Hum. Gene Ther. 23 (1) (2012) 91–97.
[42] Y. Fong, T. Kim, A. Bhargava, L. Schwartz, K. Brown, L. Brody, A. Covey, M. Karrasch, G. Getrajdman, A. Mescheder, et al., A herpes oncolytic virus can Be delivered via the vasculature to produce biologic changes in human colorectal Cancer, Mol. Ther. 17 (2) (2009) 389–394.
[43] M.K. Kim, C.J. Breitbach, A. Moon, J. Heo, Y.K. Lee, M. Cho, J.W. Lee, S. Kim, D.H. Kang, J.C. Bell, et al., Oncolytic and immunotherapeutic vaccinia induces Cancer cell lysis in humans, Cancer Immunol. Immunother. 5 (185) (2013).
[44] E. Galanis, L.C. Hartmann, W.A. Cliby, H.J. Long, P. Prema, B.A. Barrette, J.S. Kaur, P.J.H. Jr, I. Aderca, P.J. Zollman, et al., Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian Cancer, Cancer Res. 70 (3) (2010) 875–882.
[45] L. Heinzerling, V. Künzi, P.A. Oberholzer, T. Kündig, H. Naim, R. Dummer, Oncolytic measles virus in cutaneous T-Cell lymphomas mounts antitumor immune responses in Vivo and targets interferon-resistant tumor cells, Blood 106 (7) (2005) 2287–2294.
[46] C.J. Breitbach, R. Arulanandam, N. De Silva, S.H. Thorne, R. Patt, M. Daneshmand, A. Moon, C. Ilkow, J. Burke, T.H. Hwang, et al., Oncolytic vaccinia virus disrupts tumor-associated vasculature in humans, Cancer Res. 73 (4) (2013) 1265–1275.
[47] K.A. Streby, J.I. Geller, M.A. Currier, P.S. Warren, J.M. Racadio, A.J. Towbin, M.R. Vaughan, M. Triplet, K. Ott-Napier, D.J. Dishman, et al., Intratumoral injection of HSV1716, an oncolytic herpes virus, is safe and shows evidence of immune response and viral replication in young Cancer patients, Clin. Cancer Res. 23 (14) (2017) 3566–3574.
[48] E. Galanis, S.N. Markovic, V.J. Suman, G.J. Nuovo, R.G. Vile, T.J. Kottke, W.K. Nevala, M.A. Thompson, J.E. Lewis, K.M. Rumilla, et al., Phase II trial of intravenous administration of reolysin ® (Reovirus Serotype-3-Dearing strain) in patients with metastatic melanoma, Mol. Ther. 20 (10) (2012) 1998–2003.
[49] A.I. Freeman, Z. Zakay-Rones, J.M. Gomori, E. Linetsky, L. Rasooly, E. Greenbaum, S. Rozenman-Yair, A. Panet, E. Libson, C.S. Irving, et al., Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme, Mol. Ther. 13 (1) (2006) 221–228.
[50] A. Ribas, R. Dummer, I. Puzanov, A. VanderWalde, R.H.I. Andtbacka, O. Michielin, A.J. Olszanski, J. Malvehy, J. Cebon, E. Fernandez, et al., Oncolytic virotherapy promotes intratumoral t cell infiltration and improves Anti-PD-1 immunotherapy, Cell 170 (6) (2017) 1109-1119.e10.
[51] I. Puzanov, M.M. Milhem, D. Minor, O. Hamid, A. Li, L. Chen, M. Chastain, K.S. Gorski, A. Anderson, J. Chou, et al., Talimogene Laherparepvec in combination with ipilimumab in previously untreated, unresectable stage IIIB-IV melanoma, J. Clin. Oncol. 34 (22) (2016) 2619–2626.
[52] B. Shang, Y. Liu, S.J. Jiang, Y. Liu, Prognostic value of tumor-infiltrating FoxP3+ regulatory t cells in cancers: a systematic review and meta-analysis, Sci. Rep. (2015) 5.
[53] V. Cerullo, I. Diaconu, L. Kangasniemi, M. Rajecki, S. Escutenaire, A. Koski, V. Romano, N. Rouvinen, T. Tuuminen, L. Laasonen, et al., Immunological effects of low-dose cyclophosphamide in Cancer patients treated with oncolytic adenovirus, Mol. Ther. 19 (9) (2011) 1737–1746.
[54] T.H. Hwang, A. Moon, J. Burke, A. Ribas, J. Stephenson, C.J. Breitbach, M. Daneshmand, N. De Silva, K. Parato, J.S. Diallo, et al., A mechanistic proof-of-Concept clinical trial with JX-594, a targeted multi-mechanistic oncolytic poxvirus, in patients with metastatic melanoma, Mol. Ther. 19 (10) (2011) 1913–1922.
[55] S.H. Park, C.J. Breitbach, J. Lee, J.O. Park, H.Y. Lim, W.K. Kang, A. Moon, J.-H. Mun, E.M. Sommermann, L. Maruri Avidal, et al., Phase 1b trial of biweekly intravenous pexa-vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus in colorectal Cancer, Mol. Ther. 23 (9) (2015) 1532–1540.
[56] G.J. Yuen, E. Demissie, S.B. Pillai, Lymphocytes and Cancer: a love-hate relationship, Trends Cancer 2 (12) (2016) 747–757.
[57] C.-K. Heo, Y.Y. Bahk, E.-W. Cho, Tumor-associated autoantibodies as diagnostic and prognostic biomarkers, BMB Rep. 45 (12) (2012) 677–685.
[58] O. Hemminki, S. Parviainen, J. Juhila, R. Turkki, N. Linder, J. Lundin, M. Kankainen, A. Ristimäki, A. Koski, I. Liikanen, et al., Immunological data from Cancer patients treated with Ad5/3-E2F-Δ24-GMCSF suggests utility for tumor immunotherapy, Oncotarget 6 (6) (2015) 4467.
[59] J.M. Ricca, A. Oseledchyk, T. Walther, C. Liu, L. Mangarin, T. Merghoub, J.D. Wolchok, D. Zamarin, Pre-existing immunity to oncolytic virus potentiates its immunotherapeutic efficacy, Mol. Ther. 26 (4) (2018) 1008–1019.
[60] R.A. Berkeley, L.P. Steele, A.A. Mulder, D.J.M. van den Wollenberg, T.J. Kottke, J. Thompson, M. Coffey, R.C. Hoeben, R.G. Vile, A. Melcher, et al., Antibody-neutralized reovirus is effective in oncolytic virotherapy, Cancer Immunol. Res. 6 (10) (2018) 1161 LP – 1173.
[61] K.H. Kim, I.P. Dmitriev, S. Saddekni, E.A. Kashentseva, R.D. Harris, R. Aurigemma, S. Bae, K.P. Singh, G.P. Siegal, D.T. Curiel, et al., A phase I clinical trial of Ad5/3-Δ24, a novel serotype-chimeric, infectivity-enhanced, conditionally-replicative adenovirus (CRAd), in patients with recurrent ovarian Cancer, Gynecol. Oncol. 130 (3) (2013) 518–524.
[62] S. Pesonen, I. Diaconu, V. Cerullo, S. Escutenaire, M. Raki, L. Kangasniemi, P. Nokisalmi, G. Dotti, K. Guse, L. Laasonen, et al., Integrin targeted oncolytic adenoviruses Ad5-D24-RGD and Ad5-RGD-D24-GMCSF for treatment of patients with advanced chemotherapy refractory solid tumors, Int. J. Cancer 130 (8) (2012) 1937–1947.
[63] J. Nemunaitis, A.W. Tong, M. Nemunaitis, N. Senzer, A.P. Phadke, C. Bedell, N. Adams, Y.A. Zhang, P.B. Maples, S. Chen, et al., A phase i study of telomerase-specific replication competent oncolytic adenovirus (Telomelysin) for various solid tumors, Mol. Ther. 18 (2) (2010) 429–434.
[64] J.L. Li, H.L. Liu, X.R. Zhang, J.P. Xu, W.K. Hu, M. Liang, S.Y. Chen, F. Hu, D.T. Chu, A phase I trial of intratumoral administration of recombinant oncolytic adenovirus overexpressing HSP70 in advanced solid tumor patients, Gene Ther. 16 (2009).
[65] A. Koski, L. Kangasniemi, S. Escutenaire, S. Pesonen, V. Cerullo, I. Diaconu, P. Nokisalmi, M. Raki, M. Rajecki, K. Guse, Treatment of Cancer patients with a serotype 5/3 chimeric oncolytic adenovirus expressing GMCSF, Mol. Ther. 18 (2010).
[66] K.J. Kimball, M.A. Preuss, M.N. Barnes, M. Wang, P. Gene, W. Wan, H. Kuo, S. Saddekni, C.R. Stockard, E. William, et al., A Phase I Study of a Tropism Modified Conditionally Replicative Adenovirus (CRAd) for Recurrent Gynecologic Malignancies, Clin. Cancer Res. 16 (21) (2010) 5277–5287.
[67] F.F. Lang, C. Conrad, C. Gomez-Manzano, W.K.A. Yung, R. Sawaya, J.S. Weinberg, S.S. Prabhu, G. Rao, G.N. Fuller, K.D. Aldape, et al., Phase I study of DNX-2401 (Delta-24-RGD) oncolytic adenovirus: replication and immunotherapeutic effects in recurrent malignant glioma, J. Clin. Oncol. 36 (14) (2018) 1419–1427.
[68] N. Kemeny, K. Brown, A. Covey, T. Kim, A. Bhargava, L. Brody, B. Guilfoyle, N.P. Haag, M. Karrasch, B. Glasschroeder, et al., Phase I, Open-Label, Dose-Escalating Study of a Genetically Engineered Herpes Simplex Virus, NV1020, in Subjects with Metastatic Colorectal Carcinoma to the Liver, Hum. Gene Ther. 17 (12) (2006) 1214–1224.
[69] J.M. Markert, S.N. Razdan, H.C. Kuo, A. Cantor, A. Knoll, M. Karrasch, L.B. Nabors, M. Markiewicz, B.S. Agee, J.M. Coleman, et al., A phase 1 trial of oncolytic HSV-1, G207, given in combination with radiation for recurrent GBM demonstrates safety and radiographic responses, Mol. Ther. 22 (5) (2014) 1048–1055.
[70] N.N. Senzer, H.L. Kaufman, T. Amatruda, M. Nemunaitis, T. Reid, G. Daniels, R. Gonzalez, J. Glaspy, E. Whitman, K. Harrington, Phase II Clinical Trial of a Granulocyte-Macrophage Colony-Stimulating Factor-Encoding, Second-Generation Oncolytic Herpesvirus in Patients with Unresectable Metastatic Melanoma, J. Clin. Oncol. 27 (2009).
[71] A. Dispenzieri, C. Tong, B. LaPlant, M.Q. Lacy, K. Laumann, D. Dingli, Y. Zhou, M.J. Federspiel, M.A. Gertz, S. Hayman, et al., Phase I trial of systemic administration of edmonston strain of measles virus, genetically engineered to express the sodium iodide symporter in patients with recurrent or refractory multiple myeloma, Leukemia 31 (12) (2017) 2791–2798.
[72] V. Bernstein, S.L. Ellard, S.F. Dent, D. Tu, M. Mates, S.K. Dhesy-Thind, L. Panasci, K.A. Gelmon, M. Salim, X. Song, et al., A randomized phase II study of weekly paclitaxel with or without Pelareorep in patients with metastatic breast Cancer: final analysis of canadian Cancer trials group IND.213, Breast Cancer Res. Treat. 167 (2) (2018) 485–493.
[73] C. Robert, G.V. Long, B. Brady, C. Dutriaux, M. Maio, L. Mortier, J.C. Hassel, P. Rutkowski, C. McNeil, E. Kalinka-Warzocha, et al., Nivolumab in previously untreated melanoma without *BRAF* mutation, N. Engl. J. Med. 372 (4) (2015) 320–330.
[74] D.E. Cohn, M.W. Sill, J.L. Walker, D. O'Malley, C.I. Nagel, T. Rutledge, W. Bradley, D. Richardson, K. Moxley, C. Aghajanian, Randomized phase IIB evaluation of weekly paclitaxel versus weekly paclitaxel with oncolytic reovirus (Reolysin®) in recurrent ovarian, tubal, or peritoneal Cancer: an NRG Oncology/Gynecologic oncology group study, Gynecol. Oncol. 146 (3) (2018) 477–483.
[75] P. Forsyth, G. Roldán, D. George, C. Wallace, C.A. Palmer, D. Morris, G. Cairncross, M.V. Matthews, J. Markert, Y. Gillespie, et al., A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas, Mol. Ther. 16 (3) (2008) 627–632.
[76] K.P. Kicielinski, E.A. Chiocca, J.S. Yu, G.M. Gill, M. Coffey, J.M. Markert, Phase 1 Clinical Trial of Intratumoral Reovirus Infusion for the Treatment of Recurrent Malignant Gliomas in Adults, Mol. Ther. 22 (5) (2014) 1056–1062.
[77] D. Mahalingam, C. Fountzilas, J. Moseley, N. Noronha, H. Tran, R. Chakrabarty, G. Selvaggi, M. Coffey, B. Thompson, J. Sarantopoulos, A phase II study of REOLYSIN®(Pelareorep) in combination with carboplatin and paclitaxel for patients with advanced malignant melanoma, Cancer Chemother. Pharmacol. 79 (4) (2017) 697–703.
[78] D.G. Morris, X. Feng, L.M. Difrancesco, K. Fonseca, P.A. Forsyth, A.H. Paterson, M.C. Coffey, B. Thompson, REO-001: A Phase i Trial of Percutaneous Intralesional Administration of Reovirus Type 3 Dearing (Reolysin®) in Patients with Advanced Solid Tumors, Invest. New Drugs 31 (3) (2013) 696–706.
[79] T.P. Cripe, M.C. Ngo, J.I. Geller, C.U. Louis, M.A. Currier, J.M. Racadio, A.J. Towbin, C.M. Rooney, A. Pelusio, A. Moon, et al., Phase 1 study of intratumoral pexa-vec (JX-594), an oncolytic and immunotherapeutic vaccinia virus, in pediatric Cancer patients, Mol. Ther. 23 (3) (2014) 602–608.
[80] F. Husseini, J.P. Delord, C. Fournel-Federico, J. Guitton, P. Erbs, M. Homerin, C. Halluard, C. Jemming, C. Orange, J.M. Limacher, et al., Vectorized Gene Therapy

of Liver Tumors: Proof-of-Concept of TG4023 (MVA-FCU1) in Combination with Flucytosine, Ann. Oncol. 28 (1) (2017) 169–174.
[81] L.K. Mell, K.T. Brumund, G.A. Daniels, S.J. Advani, K. Zakeri, M.E. Wright, S.J. Onyeama, R.A. Weisman, P.R. Sanghvi, P.J. Martin, et al., Phase I trial of intravenous oncolytic vaccinia virus (GL-ONC1) with cisplatin and radiotherapy in patients with locoregionally advanced head and neck carcinoma, Clin. Cancer Res. 23 (19) (2017) 5696–5702.
[82] A. Desjardins, M. Gromeier, J.E. Herndon, N. Beaubier, D.P. Bolognesi, A.H. Friedman, H.S. Friedman, F. McSherry, A.M. Muscat, S. Nair, et al., Recurrent glioblastoma treated with recombinant poliovirus, N. Engl. J. Med. 379 (2) (2018) 150–161.
[83] S.J. Hotte, R.M. Lorence, H.W. Hirte, S.R. Polawski, M.K. Bamat, J.D. O'Neil, M.S. Roberts, W.S. Groene, P.P. Major, An optimized clinical regimen for the oncolytic virus PV701, Clin. Cancer Res. 13 (3) (2007) 977–985.

Ms. J. Fréderique de Graaf obtained her Master's degree in Infection and Immunity at the university of Utrecht, The Netherlands, during which she obtained research training at the Laboratory of Translational Immunology of Utrecht Medical Institute, The Netherlands and the Biochemistry Department of the University of Ottawa, Canada. At the Erasmus Medical Institute, her PhD studies involve avian paramyxoviruses and their application as oncolytic therapy in pancreatic cancer patients.

Mr. Marco Huberts acquired his Master's degree in Oncology at the Vrije Universiteit in Amsterdam, The Netherlands. He received research training at the Medical Oncology department at the Vrije Universiteit Medical Centre in Amsterdam, The Netherlands and the Cancer Cell Plasticity laboratory at the Garvan Institute of Medical Research in Sydney, Australia. His PhD studies in the department of Viroscience at the Erasmus Medical Centre, Rotterdam, The Netherlands aim to determine the efficacy of various viro-immunotherapies as a treatment of pancreatic, prostate and brain cancer.

Prof. Ron A.M. Fouchier is professor in Molecular Virology at Erasmus MC Rotterdam. He studied Microbiology in Wageningen and obtained a PhD for HIV/AIDS research at the University of Amsterdam in 1995, followed by 4 years of postdoctoral research at the University of Pennsylvania. His current research is focused on respiratory viruses of humans and animals, antigenic drift, zoonoses and pandemics, virus transmission and molecular virology. Fouchier is elected member of the Royal Dutch Academy of Sciences, the Royal Holland Society of Sciences and Humanities and Academia Europe. In 2006 he received the Heine-Medin award of the European Society for Clinical Virology and in 2013 the Huibregtsen award for top innovative science with societal impact.

Dr. Bernadette G. van den Hoogen is assistant professor at Erasmus MC Rotterdam. She obtained a PhD at Erasmus MC on "the Discovery and Characterization of the Human Metapneumovirus (HMPV)". Her postdoctoral and current research is focused on all aspects of the interaction between RNA viruses and the immune system, which has been funded by the Netherlands Organisation for Scientific Research (NWO) and by the EU's Horizon2020 research and innovation programme. Her research on oncolytic viruses has been focusing on the use of NDV as oncolytic virus for treatment pancreatic adenocarcinoma's. This research is funded by (NWO) and the foundation "Overleven met alvleesklier kanker' (OAK) to establish the safety of these oncolytic viruses for humans and poultry.