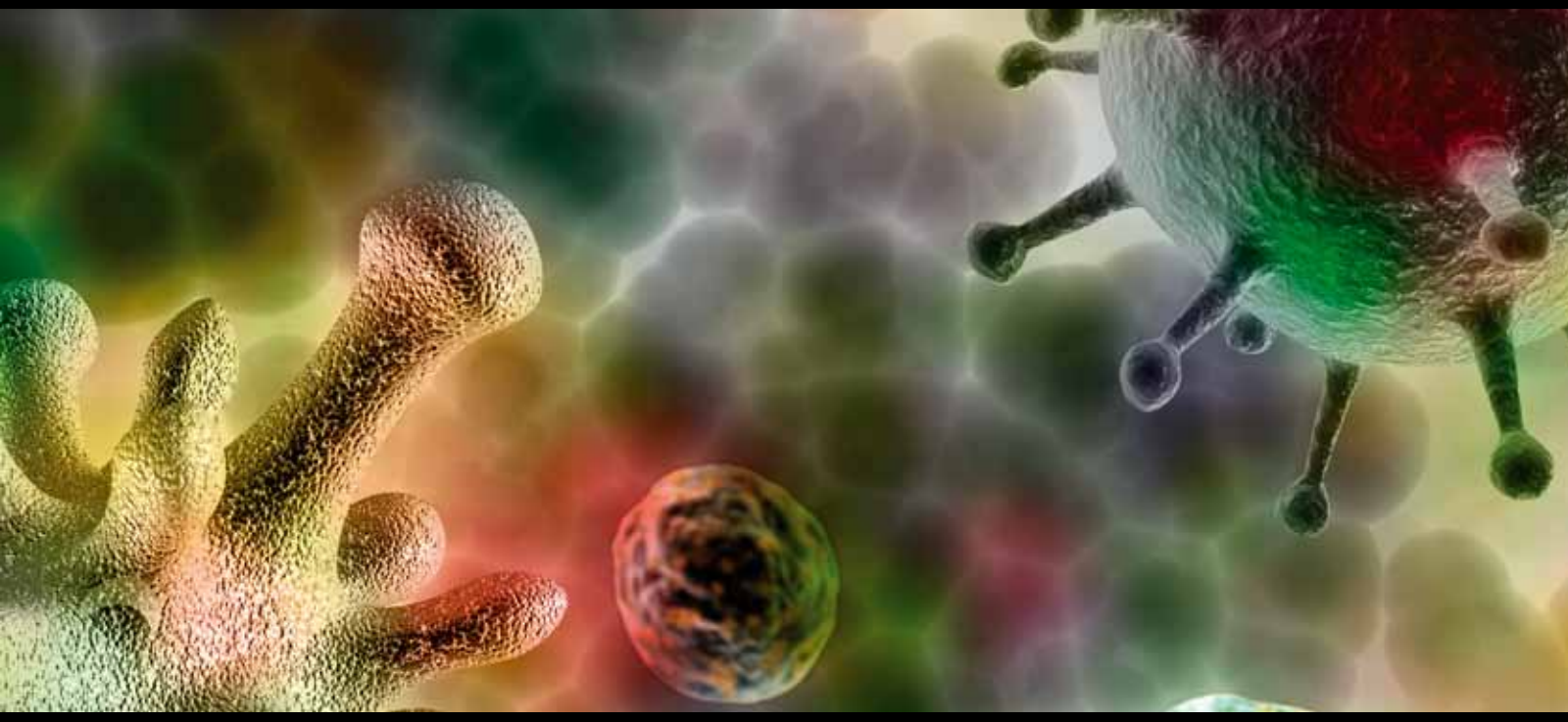
Advances in Virology

Oncolytic Viruses

Guest Editors: Nanhai G. Chen, Aladar A. Szalay, R. Mark L. Buller, and Ulrich M. Lauer



Oncolytic Viruses

Advances in Virology

Oncolytic Viruses

Guest Editors: Nanhai G. Chen, Aladar A. Szalay,
R. Mark L. Buller, and Ulrich M. Lauer

Editorial Board

Contents

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 320206, 2 pages
doi:10.1155/2012/320206

Editorial
Oncolytic Viruses

Nanhai G. Chen,[1,2] Aladar A. Szalay,[1,2,3,4,5] R. Mark L. Buller,[6] and Ulrich M. Lauer[7]

[1] *Genelux Corporation, San Diego Science Center, San Diego, CA 92019, USA*
[2] *Department of Radiation Oncology, Rebecca and John Moores Comprehensive Cancer Center, University of California, San Diego, CA 92093, USA*
[3] *Department of Biochemistry, University of Wuerzburg, 97074 Wuerzburg, Germany*
[4] *Rudolf Virchow Center for Experimental Biomedicine, University of Wuerzburg, 97078 Wuerzburg, Germany*
[5] *Institute for Molecular Infection Biology, University of Wuerzburg, 97078 Wuerzburg, Germany*
[6] *Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, St. Louis, MO 63104, USA*
[7] *Department of Gastroenterology and Hepatology, Medical University Hospital, 72076 Tübingen, Germany*

Correspondence should be addressed to Nanhai G. Chen, nchen@genelux.com

Received 7 March 2012; Accepted 7 March 2012

Oncolytic viruses, by definition, are viruses that are capable of infecting and selectively replicating in cancer cells, eventually leading to cell death without harming healthy cells. The concept of using viruses to treat cancer dates back over a century. There was renewed interest in oncolytic virotherapy in the 1990s after the first genetically engineered oncolytic virus, a herpes simplex virus-1 thymidine kinase mutant, was reported in 1991. Over the last two decades great progress has been made in this field, and several oncolytic viruses have entered into clinical trials. This special issue on oncolytic viruses addresses some of the challenges that need to be overcome in order to achieve success in the clinic.

Oncolytic viruses can be delivered into cancer patients or preclinical animal models by a number of routes including intratumoral, intravenous, intrapleural, intraperitoneal delivery and hepatic artery infusion. Although intratumoral injection has been widely studied both preclinically and clinically, systemic delivery is the preferred method for treatment of the patient populations in greatest need of new efficacious therapies those with metastatic or inaccessible disease. The paper by M. S. Ferguson et al. summarizes the data from clinical trials using systemically delivered oncolytic viruses and then identifies barriers to this delivery approach and proposes solutions.

The host immune response is a double-edged sword to the success of oncolytic virotherapy. The paper by C. A. Alvarez-Breckenridge et al. discusses the multifaceted relationship between oncolytic viruses and natural killer (NK) cells, a key component of innate immunity. The NK response to oncolytic virotherapy results in premature viral clearance while also mediating downstream antitumor immunity. Thus, it is critical to find an optimal approach to finely balance host antiviral and antitumoral immune responses.

The paper by M. H. Verheije and P. J. M. Rottier reviews the transduction targeting strategies currently employed to generate new oncolytic viruses with improved tumor specificity. The authors discuss advantages and limitations of each strategy by examples, and special attention is given to viruses expressing a bispecific adaptor protein consisting of two domains, one binding to the virion, the other to a cell surface protein of interest.

Hematopoietic stem cell transplant (HCT) is used to treat hematological malignancies; however, the contamination of healthy hematopoietic stem and progenitor cells with cancer cells presents a major challenge. The paper by S. Bais et al. provides a detailed review of using oncolytic viruses to treat hematological malignancies, and in particular, the use of oncolytic viruses to purge contaminating cancer cells for HCT.

Many new oncolytic viruses have been generated through genetic engineering based on rational design. The paper by M. Bauzon and T. Hermiston presents an alternative method, that is, directed evolution that involves passaging diverse viruses under conditions that mimic the human cancer microenvironment. Using ColoAd1, the first directed evolution-derived oncolytic virus as an example, the authors

underline the benefits of directed evolution and propose methods to "arm" these novel viruses and control their replication.

This special issue highlights some important recent findings in the field of oncolytic virotherapy and addresses challenges that the field is currently facing. Several oncolytic viruses have already entered, or will be entering randomized phase III trials. Amgen, the largest independent biotechnology company, has recently joined the field through acquisition of Biovex, highlighting the promising future of this therapeutic approach.

Nanhai G. Chen
Aladar A. Szalay
R. Mark L. Buller
Ulrich M. Lauer

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 805629, 14 pages
doi:10.1155/2012/805629

Review Article

Systemic Delivery of Oncolytic Viruses: Hopes and Hurdles

Mark S. Ferguson,[1] Nicholas R. Lemoine,[1,2] and Yaohe Wang[1,2]

[1] *Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, London EC1M 6BQ, UK*
[2] *Sino-British Research Centre for Molecular Oncology, Zhengzhou University, Zhengzhou 450052, China*

Correspondence should be addressed to Yaohe Wang, yaohe.wang@qmul.ac.uk

Received 22 August 2011; Accepted 18 October 2011

Academic Editor: Ulrich M. Lauer

Despite recent advances in both surgery and chemoradiotherapy, mortality rates for advanced cancer remain high. There is a pressing need for novel therapeutic strategies; one option is systemic oncolytic viral therapy. Intravenous administration affords the opportunity to treat both the primary tumour and any metastatic deposits simultaneously. Data from clinical trials have shown that oncolytic viruses can be systemically delivered safely with limited toxicity but the results are equivocal in terms of efficacy, particularly when delivered with adjuvant chemotherapy. A key reason for this is the rapid clearance of the viruses from the circulation before they reach their targets. This phenomenon is mainly mediated through neutralising antibodies, complement activation, antiviral cytokines, and tissue-resident macrophages, as well as nonspecific uptake by other tissues such as the lung, liver and spleen, and suboptimal viral escape from the vascular compartment. A range of methods have been reported in the literature, which are designed to overcome these hurdles in preclinical models. In this paper, the potential advantages of, and obstacles to, successful systemic delivery of oncolytic viruses are discussed. The next stage of development will be the commencement of clinical trials combining these novel approaches for overcoming the barriers with systemically delivered oncolytic viruses.

1. Introduction

Cancer remains a major health problem and is the 5th leading cause of death worldwide [1]. There have been many advances in the last few decades both in surgical care and chemoradiotherapy regimes. Certainly this has contributed to improved survival rates for commonly occurring cancers. However, relapse and disease progression are still all too common occurrences in modern medical practice. A variety of novel adjuvant therapies have been developed over the last decade, and oncolytic viruses have been particularly promising members of this cohort.

Oncolytic viruses came to medical prominence in the 19th century when coincidental viral infections were observed to cause regression of some forms of haematological malignancies. Rabies inoculation was also demonstrated to regress a patient's advanced cervical carcinoma [2]. A succession of studies in the 1950s and 60s were unable to establish oncolytic viral therapy as a viable anti-cancer modality. As a result, the field remained a medical curiosity until the advent of genetic engineering in the late 1980s. In the last decade, there have been rapid advancements in the oncolytic viral therapy field. Naturally occurring oncolytic viruses have been identified such as Vaccinia virus, Reovirus, and Newcastle disease virus. These viruses naturally preferentially infect tumour cells whilst sparing normal tissue. However, other viruses have been identified that once attenuated are also successful oncolytic agents such as herpes simplex virus type 1 and Adenovirus. These viruses have then been engineered to be more tumour specific and less pathogenic to normal tissues [2]. This has been achieved by a variety of modifications [3]. Herpes simplex virus has had two of its latency genes deleted (ICP0 & ICP4) and only has one copy of its virulence factor, γ134.5, remaining. As a further level of safety its thymidine kinase gene has been deleted. Deletion of the thymidine kinase gene means that viruses can only replicate efficiently in cells with upregulation of the EGFR/Ras signalling pathway, which is commonly the case in tumour cells [4, 5]. This approach has been widely employed successfully with Vaccinia virus developed for clinical trial use. Adenoviruses used in clinical trials have E1B 55 K gene deleted, which is involved in late viral RNA export and restricts E1B 55 K-deleted adenovirus replication in normal primary cells [6]. All of these modifications are designed

to make the oncolytic viruses more tumour specific since these gene deletions do not hamper their ability to replicate in the dysregulated tumour environment; however, they prevent replication in adjacent or distant normal tissue.

A yet more exciting development over the last decade has been the incorporation of transgenes into these viruses allowing expression of a variety of exogenous agents in the tumour microenvironment raising the very real prospect of truly immunomodulatory oncolytic viral therapy, and now the discussion has moved onto which transgenes might be the most effective [7].

A range of delivery methods have been employed for these novel agents, chief amongst them has been intratumoural delivery. A broad range of oncolytic viruses have been delivered via intratumoural injection with a measure of success in treating easily reachable solid tumours [8, 9]. However, death from cancer is often the result of inaccessible or metastatic disease. In this context, oncolytic viruses delivered intratumourally rely on viral replication at the tumour site and then systemic dissemination to the distant sites. However, this is transient and often ineffective due to the development of immune responses to the viral infection.

Systemic delivery of oncolytic viruses (OVs) affords the opportunity to treat both the primary tumour and any overt or undiagnosed metastatic deposits simultaneously. As a result, this method of delivery is a very attractive option for the treatment of patients with advanced/metastatic disease or patients with inaccessible disease such as those with pancreatic cancer or brain cancer due to physiological barriers, such as blood-brain barrier.

2. Clinical Trials

There have been many clinical trials of a variety of OVs delivered systemically, as summarised in Table 1. Oncolytic adenovirus was one of the first oncolytic viruses to be developed and licensed for treatment of cancer [8, 24]. The first generation of oncolytic adenovirus, ONYX-015 (also known as *dl*1520, H101 in China), is a genetically modified adenovirus with deletion of the 55 kD gene in the E1B region. Nemunaitis et al. [10] in 2001 performed a dose escalation study using this agent in patients with advanced carcinoma with lung metastases. They demonstrated that ONYX-015 was safe to deliver systemically with no toxicity up to doses of 2×10^{13} particles, but the study was not designed for objective tumour responses. Also commencing in 2001, a succession of studies delivered ONYX-015 via hepatic artery infusion for the treatment of metastatic colorectal carcinoma with liver deposits [11–13]. In the first of these trials, a phase I dose escalation study, one patient (9%) responded after combination therapy with conventional chemotherapy and two patients (18%) had stable disease lasting several months [11]. In a larger phase II follow-up trial, three patients (11%) had partial responses, nine (33%) had stable disease, and eleven (41%) patients had progressive disease [12]. A final phase II trial by this group demonstrated similar results to the previous studies with overall median survival of 10.7 months with two patients (8%) having a partial response and a further eleven (46%) having stable disease [13]. Of those with stable disease the median survival was prolonged to nineteen months. In a different study, Small et al. [14] treated patients with hormone-refractory metastatic prostate cancer using a single intravenous infusion. Unlike ONYX-015, the adenovirus (CG7870) in this trial was modified so that E1A was under the control of the rat probasin promoter and E1B was under the control of the PSA promoter-enhancer, thus making it prostate specific. Results from this trial were disappointing with no complete nor partial responses, although five patients (22%) did have a 25% to 49% reduction in their serum PSA values.

PV701 is a naturally attenuated Newcastle disease virus, which has been used systemically in a number of clinical trials between 2002 and 2007 [15–18]. Three of these trials were phase I studies in patients with a variety of advanced/metastatic solid tumours [15, 16, 18]. In the Pecora et al. [15] study in 2002, 62 patients were assessed for a tumour response and two patients (3%) had a major response and 14 patients (23%) had stable disease for 4–30 months. Hotte et al. [18] performed a small phase I study and although not designed to assess efficacy, four major (22%) and two minor (11%) responses to the treatment were observed. A similarly sized trial by Laurie et al. [16] in 2006 reported stable disease in four patients (25%) for greater than six months. Freeman et al. [17] investigated the safety of using Newcastle disease virus in patients with recurrent glioblastoma multiforme and as with the other studies the treatment was well tolerated but the efficacy was again disappointing with only one patient (7%) having a complete response.

NV1020 is a Herpes Simplex virus type 1 with deletions of the latency factors ICPO and ICP4, and only one copy of its virulence factor y134.5. Another element of safety is the insertion of the α4 promoter to control the HSV-1 TK gene expression, which sensitises the virus to antiviral drugs such as acyclovir. One phase I trial [19, 20] delivering NV1020 via hepatic artery infusion in patients with hepatic metastases from colorectal primaries refractory to first-line treatment reported seven patients (58%) with stable disease and two patients showing a partial response. Median survival in this group was 25 months. Another trial by Geevarghese et al. [21] in 2010 again delivered NV1020 by hepatic artery infusion in patients with advanced metastatic colorectal carcinoma but this time followed by conventional chemotherapy. After completion of the combined approach, there was a 68% response rate, with one patient with a partial response and fourteen patients with stable disease. Median survival in this study was 11.8 months.

Interrogation of the various clinical trial registration sites (http://clinicaltrials.gov/, WHO trials register, https://www.clinicaltrialsregister.eu/, http://www.controlled-trials.com/) reveals that there are no ongoing nor pending trials systemically delivering Adenovirus, Newcastle disease virus, or Herpes Simplex virus type 1.

Reolysin is a type 3 Dearing Reovirus in its wild-type form. Vidal et al. [23] completed the only trial using systemic delivery in 2008. They performed a phase I dose escalation study assessing the safety of a variety of doses.

Table 1: Completed clinical trials using systemically delivered oncolytic viruses for the treatment of solid tumours.

Viral agent	Virus species	Modifications	Cancer type	Pt no.	Treatment regime	Toxicity	Results	References
ONYX-015	Adenovirus	E1B-55 kD-ve	Advanced carcinoma with lung metastasis	10	Phase I, dose-escalation study	No dose-limiting toxicity was observed. Grade 2-3 toxicities were common (fever, rigors and fatigue).	IV administration was well tolerated up to 2×10^{13} particles. Not designed for objective tumour responses.	Nemunaitis et al., 2001 [10]
ONYX-015	Adenovirus	E1B-55 kD-ve	Liver metastases from gastrointestinal carcinoma	11	Phase I, dose-escalation study hepatic artery infusion	No dose-limiting toxicity. Commonly mild to moderate fever, rigors and fatigue.	Objective response with chemotherapy + virus in a patient who was refractory to both 5-FU and ONYX-015 as single agents. 2 high dose patients had stable disease on combination therapy lasting from 7 to 17 months.	Reid et al., 2001 [11]
Onyx-015	Adenovirus	E1B-55 kD-ve	Metastatic liver deposits from gastrointestinal primaries	27	Phase II, hepatic artery infusion in combination with 5 FU and leucovorin	2 patients had reversible grade 3/4 hyperbilirubinemia and 1 patient had a reversible severe systemic inflammatory response.	3 patients with partial responses, 4 with minimal responses, 9 with stable disease, and 11 with progressive disease. Unclear from this study whether observed responses were due to viral treatment or chemotherapy or combination.	Reid et al., 2002 [12]
ONYX-015	Adenovirus	E1B-55 kD-ve	Metastatic colorectal cancer refractory to conventional therapy	24	Phase I/II, hepatic artery infusion	No dose-limiting toxicity was observed.	Overall median survival was 10.7 months. 2 patients had partial responses (tumur vol reductions of 66% and 72%). 11 patients had stable disease after viral treatment and median survival in this group was 19 months.	Reid et al., 2005 [13]
CG7870	Adenovirus	E1A under control of the rat probasin promoter E1B under control of the PSA promoter-enhancer	Hormone-refractory metastatic prostate cancer	23	Phase I, single intravenous infusion	Mostly grade 1 or 2 flulike symptoms were observed. There were 8 grade 3 events (fever or fatigue). At higher doses, asymptomatic grade 1 or 2 transaminitis were reported.	No partial or complete tumour responses were observed. 5 patients had a decrease in serum PSA of 25% to 49% following a single treatment.	Small et al., 2006 [14]

Table 1: Continued.

Viral agent	Virus species	Modifications	Cancer type	Pt no.	Treatment regime	Toxicity	Results	References
PV701	Newcastle disease virus	Naturally attenuated	Advanced or metastatic solid cancers that were refractory to standard treatment	79	Patients were recruited into 4 different IV dosing regimes.	Grade 3 fever in 11% flu-like symptoms	62 patients assessed for response, 2 major responses, 14 had no disease progression for 4–30 months.	Pecora et al., 2002 [15]
PV701	Newcastle disease virus	Naturally attenuated	Pallitive solid tumors	16	Phase 1	No dose-limiting toxicities. Mild flu-like symptoms were common.	Four patients had stable disease for ≥6 months.	Laurie et al., 2006 [16]
NDV-HUJ	Newcastle disease virus	Naturally attenuated	Recurrent glioblastoma multiforme	14	Phase I/II	5 patients had grade I/II constitutional fever.	One patient achieved a complete response.	Freeman et al., 2006 [17]
PV701	Newcastle disease virus	Naturally attenuated	Advanced chemorefractory cancer	18	Phase 1	Mild or moderate in severity, and self-limiting	Not design for assessment of response but 4 major and 2 minor tumour responses were observed.	Hotte et al., 2007 [18]
NV1020	Herpes simplex virus type 1	ICP0 & ICP4-ve Only 1 copy of γ134.5 Transgene inserted HSV-1 TK gene (α4)	Hepatic colorectal metastases refractory to first-line chemotherapy	12	Phase I, dose-escalation study, hepatic artery infusion	Mild or moderate in severity, and self-limiting	Tumour response assessed at 28 days after viral delivery. 7 patients had stable disease. 2 patients had a partial response (tumour vol reductions of 39% and 20%). Median survival for this group was 25 months.	Kemeny et al., 2006 [19] Fong et al., 2009 [20]
NV1020	Herpes simplex virus type 1	ICP0 & ICP4-ve Only 1 copy of γ134.5 Transgene inserted HSV-1 TK gene (α4)	Advanced metastatic colorectal	Phase 1 = 13, Phase 2 = 19	Phase I/II study, hepatic artery infusion treatment followed by two or more cycles of conventional chemotherapy	Mild-to-moderate febrile reactions after each NV1020 infusion. Grade 3/4 transient lymphopenia in two patients	After completion of NV1020 administration, 50% showed stable disease. Best overall tumor control rate after completion of combined therapy was 68% (1 partial response, 14 stable disease). Median time to progression was 6.4 months. Median overall survival was 11.8 months. One-year survival was 47.2%.	Geevarghese et al., 2010 [21]

Table 1: Continued.

Viral agent	Virus species	Modifications	Cancer type	Pt no.	Treatment regime	Toxicity	Results	References
JX-594	Vaccinia—Wyeth	TK-ve Transgene inserted in TK region hGM-CSF (pE/L) and Lac-Z (p7.5)	Unresectable primary hepatocellular carcinoma	9	Phase 2, pilot safety study IV then IT injection followed by sorafenib treatment	They assert viral treatment was well tolerated and sorafenib side effects were consistent with previously reported.	7 out of 9 were able to be assessed for response. Of these, 6 achieved necrotic responses. 5 had stable disease and 1 had a partial response.	http://www.clinicaltrials.gov/ Jennerex Biotherapeutics, Inc. NCT01171651 http://www.jennerex.com/pr_091310.html
JX-594	Vaccinia—Wyeth	TK-ve Transgene inserted in TK region hGM-CSF (pE/L) and Lac-Z (p7.5)	Metastatic solid tumour disease which is refractory to conventional therapy specifically: Melanoma, Lung Cancer, Renal cell cancer, SCC of head and neck	23	Phase I, Dose Escalation Study	No dose-limiting toxicities. Mild flu-like symptoms were common.	Demonstrated that at the higher doses used (1×10^7 to 3×10^7 PFU/kg) JX-594 can selectively infect, replicate and express transgene products in target tumour tissue whilst sparing normal tissue. Although not designed for efficacy, one patient had partial response.	http://www.clinicaltrials.gov/ Jennerex Biotherapeutics, Inc. NCT00625456 Breitbach et al., 2011 [22]
Reolysin	Reovirus—type 3 Dearing	Wild-type	Advanced or metastatic solid cancers that were refractory to standard treatment	33	Phase 1, Dose Escalation study	No dose-limiting toxicity was observed. Grade 1-2 toxicities were common (fever, fatigue and headache).	Antitumor activity was observed radiologically and by tumor markers.	Vidal et al., 2008 [23]

As such they observed no dose-limiting toxicity, and they further comment that antitumour activity was observed both radiologically and by tumour markers. However, no objective radiologic responses were observed in terms of Response Evaluation Criteria in Solid Tumours. Despite this they did report that eight patients showed disease stabilisation. There are also two phase II trials and one phase III trial that have been registered (NCT01166542, NCT01199263, NCT01280058). The phase III trial is in patients with metastatic or recurrent squamous cell carcinoma of the head and neck, whereas the two phase II trials are in recurrent ovarian/fallopian tube cancer and recurrent pancreatic cancer, respectively. All these trials are still recruiting (Table 2).

JX-594 is a Vaccinia virus based on the Wyeth strain with a thymidine kinase (TK) deletion and the insertion of human granulocyte macrophage colony stimulating factor (hGM-CSF) gene and Lac-Z into the TK-deleted region. These transgenes are under the control of pE/L and p7.5 promoters, respectively. Jennerex Biotherapeutics Inc. has reported the results of one trial using this agent systemically in patients with unresectable primary hepatocellular carcinoma. They performed a phase I safety study delivering JX-594 initially systemically then intratumourally with subsequent sorafenib treatment. Seven out of nine of their patients were suitable to be assessed: in six patients (67%), the tumours necrosed, and of these five patients (56%) had stable disease and one patient (11%) had a partial response. They have recently reported the results of another dose escalation study using JX-594 in patients with metastatic solid tumour disease, which was refractory to conventional therapy. The treatment was well tolerated and at higher doses of virus (1×10^7 to 3×10^7 PFU/kg), and they demonstrated that JX-594 can selectively infect, replicate, and express transgene products in target tumour tissue whilst sparing normal tissue. Although the study was not designed for efficacy, one patient had partial response [22]. Jennerex Biotherapeutics Inc. has two trials pending with respect to this agent, the details of which are illustrated in Table 2.

In general these clinical trials have shown that oncolytic viruses can be delivered systemically with limited toxicity and latency. However, what they have not shown, and indeed were not powered to show, is that these agents are efficacious at treating either the primary tumour or metastatic disease. There is a complete lack of appropriately powered phase IIb or phase III trials using OVs delivered systemically, although there are a few pending for Reovirus. The data that are available demonstrate that systemically delivered oncolytic viruses offer only modest improvements, if at all, over and above conventional second-line therapy. Clearly if intravenously delivered OVs are to play a part in the future treatment of advanced cancer, there needs to be dramatic improvement.

3. Barriers to Systemic Delivery of Oncolytic Viruses

There are many obstacles to successful systemic delivery of viruses; host defences limit most oncolytic viruses' ability to infect tumours after systemic administration. Blood cells, complement, antibodies, and antiviral cytokines [25], as well as nonspecific uptake by other tissues such as the lung, liver and spleen, tissue-resident macrophages, and additionally poor virus escape from the vascular compartment [3] are the main barriers to systemic delivery of oncolytic viruses (Figure 1). Clearly, in order for this method to be effective, the virus must persist in the circulation without depletion or degradation while selectively infecting tumour cells.

4. Neutralising Antibodies

Preexisting immunity is a major problem for systemically delivered viruses whether this has developed due to the ubiquitous nature of the virus, previous immunization, or prior oncolytic viral therapy. Vaccinia virus was used in the worldwide immunisation program for the eradication of smallpox and so many people who are now developing cancer have a preexisting immunity to this OV. Reovirus is universally present within the environment and as a result many people have immunity to it [26, 27]. Furthermore, White et al. [28] have demonstrated that the antibody titre to Reovirus increases dramatically after systemic delivery and others have shown that the presence of these antibodies significantly impairs effective intravenous administration [29, 30]. One simple strategy for overcoming this problem has been to sequentially deliver related viruses with different serotypes or chimeric viruses [31].

Nature has already provided several solutions when considering the significant hurdles to effective systemic delivery with regards to Vaccinia virus, which can potentially be delivered systemically [32] since the Extracellular Enveloped Virus (EEV) form shrouds itself in a host cell-derived envelope and thus can evade both complement and neutralising antibodies [33–36]. Indeed, strains of Vaccinia virus can be engineered that produce more of this immune-evasive form [37]. However, in the clinical setting, it is the intracellular mature virion (IMV) form of the virus that will potentially be injected systemically, and it is this form that must successfully reach the target tissue before any EEV form can be produced. IMV—unlike EEV—is highly immunogenic and is rapidly cleared from the organism if intravenously delivered.

Clearly methods need to be developed that can overcome this acquired immunity. One such strategy is the so-called "Trojan Horse" technique, where cells are taken from the model organism infected with the OV *ex vivo* and then reinfused. Yotnda et al. created transgenic cytotoxic T lymphocytes (CTL), which were transduced with the adenoviral E1 gene under the control of the cell activation-dependent CD40 ligand promoter. The CTLs were transduced *ex vivo* with a conditionally replicating chimera of Adenovirus 5 with the fiber protein of Ad35. This was added as the Ad35 fiber protein can infect cells through a coxsackie and adenovirus receptor-(CAR-) independent method this is required as there is low expression of CAR on CTLs. The transgenic CTL was specifically targeted, and upon binding and subsequent activation, Adenovirus was produced.

Table 2: Ongoing or pending clinical trials using systemically delivered oncolytic viruses for the treatment of solid tumours.

Viral agent	Virus species	Modifications	Cancer type	Patient No.	Treatment regime	Status	References
MV-NIS	oncolytic measles virus—Edmonston vaccine strain	Transgene: thyroidal sodium iodide symporter	Recurrent or refractory multiple myeloma	Est 54 Enrolment closes June 2012	Phase I trial Safe and dose escalation study of vaccine therapy when given with or without cyclophosphamide	Recruiting	http://www.clinicaltrials.gov/ Mayo Clinic NCT00450814
JX-594	Vaccinia—Wyeth	TK-ve Transgene inserted in TK region hGM-CSF (pE/L) and Lac-Z (p7.5)	Metastatic colorectal carcinoma	Est 20 Not open for enrolment yet	Neoadjuvant Phase 2a trial IV or IT injection followed by surgical resection of metastatic hepatic deposits	Not yet open	http://www.clinicaltrials.gov/ Jennerex Biotherapeutics, Inc. NCT01329809
JX-594	Vaccinia—Wyeth	TK-ve Transgene inserted in TK region hGM-CSF (pE/L) and Lac-Z (p7.5)	Metastatic colorectal carcinoma refractory to or intolerant of oxaliplatin, irinotecan, and Erbitux	Est 15 Enrolment closes June 2012	Phase 1b dose escalation study IV injection biweekly (to evaluate the safety and tolerability of JX-594)	Results pending	http://www.clinicaltrials.gov/ Jennerex Biotherapeutics, Inc. NCT01380600
Reolysin	Reovirus—type 3 Dearing	Wild-type	Metastatic or recurrent SCC of H&N	Est 280 enrolment closes Dec 2012	Phase 3 IV administration in combination with paclitaxel and carboplatin versus chemotherapy treatment alone	Recruiting	http://www.clinicaltrials.gov/ Oncolytics Biotech NCT01166542
Reolysin	Reovirus—type 3 Dearing	Wild-type	Recurrent or persistent ovarian, fallopian Tube, or primary peritoneal cancer	Est 110 enrolment closes December 2012	Phase II weekly paclitaxel versus weekly paclitaxel with IV Reovirus	Recruiting	http://www.clinicaltrials.gov/ Gynecologic Oncology GroupNCT01199263
Reolysin	Reovirus—type 3 Dearing	Wild-type	Recurrent or metastatic pancreatic cancer	Est 70 enrolment closes December 2013	Phase II carboplatin, paclitaxel plus Reovirus versus carboplatin and paclitaxel	Recruiting	http://www.clinicaltrials.gov/ Arthur G. James Cancer Hospital & Richard J. Solove Research Institute NCT01280058

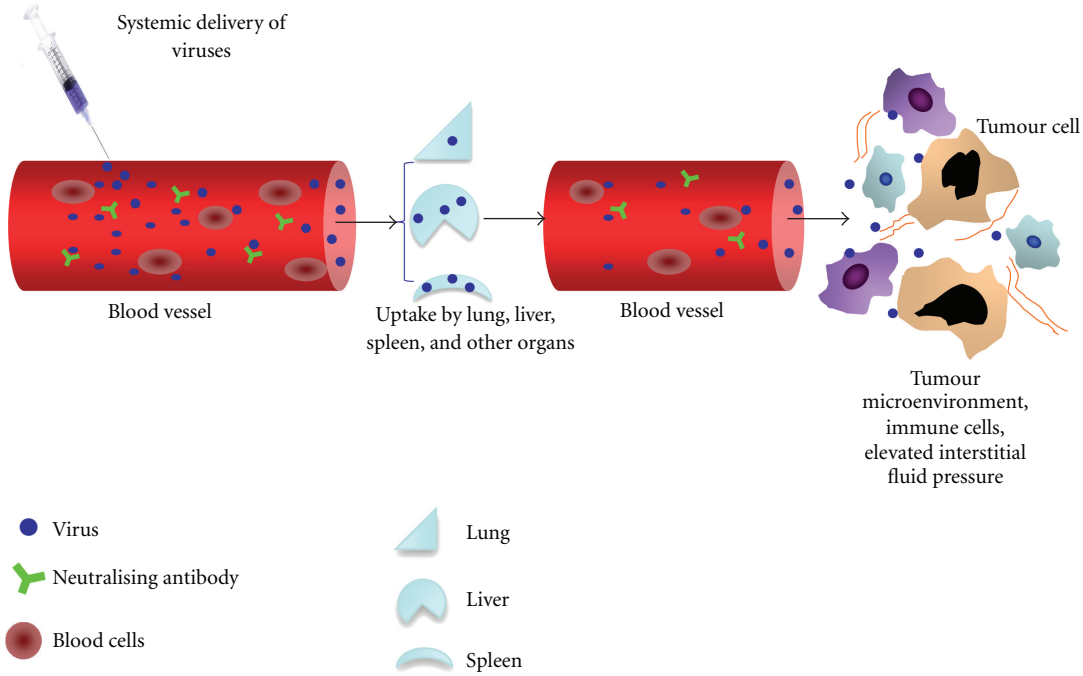


Figure 1: Hurdles of systemic delivery of oncolytic viruses to tumour cells. After intravenous injection, viruses are neutralised by pre-existing antibodies and complement activation. Oncolytic viruses also interact with blood cells. Sequestration into other organs and the reticuloendothelial system is a particular problem, often with resulting toxicities. Macrophages in the lung, liver (aka kupffer cells), and spleen are major players to clear oncolytic viruses after systemic delivery. From the blood stream, viruses have to pass through a mixture of extracellular matrix and cells (including normal and immune cells) before reaching the tumour. The connective tissue of the tumour matrix is important in the regulation and creation of the tumour vasculature; the tumour vasculature itself and interstitial pressures are also key factors involved in the ability of the virus to penetrate the tumour mass.

This occurred since upon activation of the CTL by its specific antigen, the AKNA transcription factor is transiently expressed driving CD40 and E1A expression. Thus by this mechanism, Adenovirus production is tightly linked to CTL activation by its specific tumour-associated antigen resulting in a tumour-specific delivery of Adenovirus [38]. Work by Ilett et al. [39, 40] has shown that dendritic cells loaded *in vitro* with Reovirus will "deliver" the virus successfully to melanoma cells in the presence of neutralising serum. Furthermore, they have shown that Reoviruses loaded into mature dendritic cells are able to infect tumour sites effectively *in vivo* despite preexisting viral immunity. Other cells have been used as potential viral carriers in preclinical models such as cytokine-induced killer (CIK) cells [41], monocytes [42], endothelial cells [42], mesenchymal stem cells [43–45], T-cells [40, 46, 47], dendritic cells [40], and tumour cells [48–50]. Also, stimulated peripheral blood cells, infected with oncolytic Measles virus, have successfully infected Raji lymphomas or hepatocellular carcinoma in the presence of neutralizing antibodies [42]. However, the "Trojan Horse" strategy may not be effective for brain tumours, for which some carrier cells are not able to pass physiological barriers, such as the blood-brain.

Another interesting approach has been developed by Yotnda et al. [51] in which they encapsulated a conditionally replicating competent plasmid based on ONYX-015 in a liposome. They showed that despite circulating Adenovirus antibodies, the liposome-coated viruses were able to infect subcutaneous tumours in mice.

Fontanellas et al. [52] have attempted to overcome the host immunity which develops after repeated administration of Adenovirus by inhibition of T cells and depletion of B-cells with anti-CD20 antibody. Although this study was not targeted at cancer therapy, they demonstrated that this immunosuppressive regime was successful in facilitating gene transfer to hepatocytes despite preexisting Adenoviral immunity.

Another immunosuppressive strategy is to use cyclophosphamide to modulate antiviral immunity in combination with intravenous Reovirus. This has been evaluated in a preclinical murine model by Qiao et al. [53], in which they reported delivery of 1×10^7 plaque-forming units per milligram of tumour with this regime with only mild toxicity to the mice, whereas without cyclophosphamide, effective seeding of the tumour was not achieved. For this particular regime, cyclophosphamide is often used at a lower dose and would not result in significant side effects while it is combined with oncolytic viruses.

5. Complement Activation

Complement activation is an important antiviral mechanism. Vaccinia virus in its EEV form incorporates host

proteins within its membrane that may well prevent complement activation [36]. Furthermore, it has long been established that Vaccinia virus secretes a variety of immune-modulating molecules. One of the major secreted proteins is Vaccinia complement control protein (VCP), which binds and inactivates C4b and C3b [54–56] thus inhibiting the classic and alternative pathways of complement activation. Furthermore, there is compelling evidence from a variety of viral infection models that complement activation induces various elements within the adaptive immune system [57–62]. Recent work has suggested that VCP dampens viral antibody responses and reduces the accumulation of CD4+ and CD8+ cells at the site of infection in a complement-dependent manner [63]. This has led to at least one group using VCP to perturb complement activation outside the context of a Vaccinia infection [64] and raises the possibility of using it in combination with other OVs to block complement activation.

Herpes simplex virus type 1 has also evolved strategies to prevent complement activation. HSV-1 secretes glycoprotein E that acts as an IgG Fc receptor and effectively blocks both IgG Fc-mediated complement activation and antibody-dependent cellular cytotoxicity [65]. Also HSV-1 produces glycoprotein C that binds C3b and is also critical in preventing C5 activation [66].

Adenovirus activates the complement system by various mechanisms but recent *in vivo* pre-clinical data suggests that this activation can be effectively reduced by shielding Adenovirus with polyethylene glycol [67]. Another approach to ameliorate complement activation, undertaken in Adenovirus, is to make the virus express soluble CD59 [68] and thus prevent deposition of the membrane attack complex.

6. Antiviral Cytokines

Viral infections stimulate a variety of cytokines to be produced (for review see Randall 2008 [69]). These include type 1 interferons (IFN), type 2 IFN, and type 3 IFN [70, 71]. Although these molecules have pleiotropic functions, the main effects are to promote apoptosis in virus-infected cells and induce cellular resistance to viral infection in noninfected cells [72]. Additionally, they recruit elements of the adaptive immune system, such as dendritic cells, leading to potentially lasting immunity [73]. Most oncolytic viruses express proteins that block these IFNs [74–76], or their downstream targets, but the anti-viral response is often still sufficient to prevent intra-tumoral spread of the OV.

As has been mentioned earlier, the "Trojan horse" strategy is a potentially powerful technique for delivering oncolytic viruses systemically. Ahmed et al. demonstrated that mesenchymal stem cells infected *ex vivo* with Adenovirus and then subsequently reinfused had great advantages in terms of delivery particularly with respect to attenuating the IFN-gamma response at the tumour site since mesenchymal stem cells suppress activated T-cells [77]. Another strategy to overcome the antiviral cytokines is to pretreat with histone deacetylase inhibitors, which induce epigenetic changes that blunt antiviral cytokine responses at the tumour sites and have been shown to greatly improve the effectiveness of OV therapy [78, 79].

7. Nonspecific Uptake by Other Tissues Such as the Liver and Spleen

It is known that many viruses are either filtered or taken up by the lung, liver, or spleen thus reducing systemic availability. Our group has demonstrated that the spleen is pivotal in the early clearance of systemically delivered Vaccinia virus (unpublished data by James Tysome et al.). Furthermore, up to 90% of Adenovirus type 5 is sequestered from the blood by Kupffer cells [80] and as a result this acts as a major obstacle for the systemic delivery of Adenovirus.

With respect to Adenovirus, several lines of investigation have developed strategies for improving its systemic availability. Shashkova et al. [25] demonstrated in a pre-clinical model that pretreatment with warfarin followed by multiple doses of replication-defective Adenovirus successfully depleted Kupffer cells and prevented hepatocyte binding, thus improving the antitumour efficacy of a subsequent single dose of oncolytic Adenovirus. Another important factor involved in liver sequestration of Adenovirus 5 is the binding of its hexon with blood coagulation factor X. Zhang et al. [31] have developed a hexon-chimeric oncolytic Adenovirus type 5 that has Adenovirus type 48's hexon, which only weakly binds factor X. They have demonstrated that this chimera has a significantly reduced liver uptake.

8. Suboptimal Viral Escape from the Vascular Compartment

Adenovirus is known to bind to human erythrocytes [81, 82], and this reduces its therapeutic availability when delivered systemically. Furthermore, it is well known that the neovasculature within solid tumours is very chaotic and abnormally leaky with often markedly raised interstitial pressures leading to reduced viral penetration of the tumour mass. Oncolytic viruses are known to stabilize tumour vasculature directly improving tumour penetrance [83]. Interestingly, other work has shown that the addition of antiangiogenic agents with oncolytic viruses can further normalise the vasculature and improve viral delivery in preclinical models [84, 85]. There is also emerging evidence that blockade of the Hedgehog signaling pathway can affect tumour vasculature [86]. Thus a Hedgehog antagonist may prove to be an effective treatment in combination with a systemically delivered oncolytic virus or indeed incorporated within one as a transgene. Another potential agent that could be incorporated into an OV as a transgene is histidine-rich glycoprotein (HRG) particularly in the context of repeated systemic administrations of OV. This protein has been shown to normalise tumour vasculature through its ability to polarize macrophages from M2-like TAM phenotype to M1-like tumour inhibitory phenotype [87].

9. Other Physical Methods to Enhance Systemic Delivery

Microbubbles have been developed as a potential method for enhancing the systemic delivery of a variety of agents including oncolytic viruses. They were first developed to help deliver small molecules to target tissues [88–91]. Microbubbles are ultrasound contrast agents that contain high-molecular weight gases which are less soluble and do not diffuse easily, and as a result the microbubbles persist in the circulation for a few minutes passing through the microcirculation several times [88]. Ultrasound-targeted destruction of the microbubbles allows focused release of the oncolytic virus at the tumour site, and a secondary effect is transient and localised increased cellular permeability which potentially can improve viral infection of the cancer cells [92]. This technique has been used *in vivo* with Adenovirus successfully delivering the virus to the tumour site in mice [93, 94]. The technique has not yet been used with other oncolytic viruses.

10. Tissue-Resident Macrophages

To date, most pre-clinical studies examining systemic delivery of Vaccinia virus have used nude mice bearing xenograft tumours. It is now clear that there is a need to assess systemic delivery in an immune-competent model as host immunity is a major barrier. Indeed, results from our group have demonstrated that while Vaccinia virus can effectively infect tumour cells in nude mice after systemic delivery, infection of tumour cells cannot be achieved at similar levels in the immunocompetent model. Concurrently, work in our group revealed that depletion of macrophages by clodronate liposomes dramatically enhanced Vaccinia virus infection of tumours in immunocompetent mice after systemic delivery (unpublished data by James Tysome et al.). This almost completely restored the antitumour potency to the level seen in nude mice. However, clodronate liposomes nonselectively deplete macrophages and therefore potentially diminish any beneficial activity in the tumour microenvironment unrelated to viral clearance. Consequently, this necessitates a search for a novel, more selective agent that could interfere transiently with macrophage function and thus enhance the systemic delivery of Vaccinia virus.

In general, it should be possible to perturb macrophage function at a variety of stages such as their development, recruitment/migration, or blocking their phagocytic function. Several lines of evidence have highlighted an important role for phosphatidylinositol 3-kinases (PI3K) [95–98] in macrophage phagocytosis. These observations imply that PI3K inhibitors may be potential therapeutic agents for enhancement of systemic delivery of Vaccinia virus, and other OVs, by blocking macrophage uptake/clearance of the viruses. One caveat to this is that therapeutic interference in the PI3K pathway may have to be targeted at individual or groups of PI3K isoforms [99]. It is known that mammals have eight isoforms of PI3K, but the specific isoforms of PI3K involved in macrophage phagocytosis and Vaccinia, or other OV, uptake have yet to be elucidated.

11. Conclusions

To date, the systemic delivery of oncolytic viruses has been shown to be safe but not efficacious mainly due to immunological factors that facilitate rapid clearance of these agents. There is a range of novel methods that are being developed at a pre-clinical level to overcome these hurdles which have been reported to be successful *in vivo* mainly in murine models.

However, we need to remember that mouse models are just that—they are models, which offer opportunities to investigate the effect of host factors on systemic delivery of oncolytic virus *in vivo*. The major problem is that the host immune responses to some oncolytic viruses in mice are completely different from those in humans reflecting their mutual genetic divergence 65 million years ago. Most importantly, for some oncolytic viruses such as oncolytic adenovirus, murine models of cancer are suboptimal as murine tissue and cells do not support adenovirus replication. Therefore, the information derived from these models about the host immune response to oncolytic adenovirus is certainly different and nonrepresentative of the situation in humans. Given these limitations, the next step will be the commencement of clinical trials combining these methods with systemically delivered oncolytic viruses, investigating whether these strategies work in humans. Several agents that can enhance the systemic delivery of oncolytic viruses have been separately used or tested in clinical trials. It is conceivable that a combination strategy to enhance the systemic delivery of oncolytic viruses should and will be employed in the near future. This strategy may provide an effective therapeutic approach for treatment of primary tumours, the metastatic deposits, and tumour entities, which are not easily accessible for conventional therapeutic agents because of physiological barriers. The blood-brain barrier is one such obstacle, which it has been demonstrated that several oncolytic viruses have been able to pass identifying them as potential candidates in the treatment of brain tumours.

In conclusion, if an optimal approach to enhance the systemic delivery of oncolytic viruses can be achieved by rationally targeting different factors, the outcome for treatment of advanced cancers would be dramatically improved.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

The authors' work is supported by Royal College of Surgeons of England, Natural Sciences Foundation of China, and the National Institute for Health Research/Cancer Research UK (Experimental Cancer Medicine Centres).

References

[1] W. H. Organisation, "Disease and injury country estimates," 2008, http://www.who.int/healthinfo/global_burden_disease/estimates_country/en/index.html.

[2] E. Kelly and S. J. Russell, "History of oncolytic viruses: genesis to genetic engineering," *Molecular Therapy*, vol. 15, no. 4, pp. 651–659, 2007.

[3] H. H. Wong, N. R. Lemoine, and Y. Wang, "Oncolytic viruses for cancer therapy: overcoming the obstacles," *Viruses*, vol. 2, no. 1, pp. 78–106, 2010.

[4] G. C. Katsafanas and B. Moss, "Vaccinia virus intermediate stage transcription is complemented by Ras-GTPase-activating protein SH3 domain-binding protein (G3BP) and cytoplasmic activation/proliferation-associated protein (p137) individually or as a heterodimer," *Journal of Biological Chemistry*, vol. 279, no. 50, pp. 52210–52217, 2004.

[5] H. Yang, S. K. Kim, M. Kim et al., "Antiviral chemotherapy facilitates control of poxvirus infections through inhibition of cellular signal transduction," *Journal of Clinical Investigation*, vol. 115, no. 2, pp. 379–387, 2005.

[6] C. C. O'Shea, L. Johnson, B. Bagus et al., "Late viral RNA export, rather than p53 inactivation, determines ONYX-015 tumor selectivity," *Cancer Cell*, vol. 6, no. 6, pp. 611–623, 2004.

[7] M. A. Mac Cheever, "Twelve immunotherapy drugs that could cure cancers," *Immunological Reviews*, vol. 222, no. 1, pp. 357–368, 2008.

[8] F. R. Khuri, J. Nemunaitis, I. Ganly et al., "A controlled trial of intratumoral ONYX-015, a selectively-replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer," *Nature Medicine*, vol. 6, no. 8, pp. 879–885, 2000.

[9] J. R. Hecht, R. Bedford, J. L. Abbruzzese et al., "A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma," *Clinical Cancer Research*, vol. 9, no. 2, pp. 555–561, 2003.

[10] J. Nemunaitis, C. Cunningham, A. Buchanan et al., "Intravenous infusion of a replication-selective adenovirus (ONYX-015) in cancer patients: safety, feasibility and biological activity," *Gene Therapy*, vol. 8, no. 10, pp. 746–759, 2001.

[11] T. Reid, E. Galanis, J. Abbruzzese et al., "Intra-arterial administration of a replication-selective adenovirus (dl1520) in patients with colorectal carcinoma metastatic to the liver: a phase I trial," *Gene Therapy*, vol. 8, no. 21, pp. 1618–1626, 2001.

[12] T. Reid, E. Galanis, J. Abbruzzese et al., "Hepatic arterial infusion of a replication-selective oncolytic adenovirus (dl1520): phase II viral, immunologic, and clinical endpoints," *Cancer Research*, vol. 62, no. 21, pp. 6070–6079, 2002.

[13] T. R. Reid, S. Freeman, L. Post, F. McCormick, and D. Y. Sze, "Effects of Onyx-015 among metastatic colorectal cancer patients that have failed prior treatment with 5-FU/leucovorin," *Cancer Gene Therapy*, vol. 12, no. 8, pp. 673–681, 2005.

[14] E. J. Small, M. A. Carducci, J. M. Burke et al., "A phase I trial of intravenous CG7870, a replication-selective, prostate-specific antigen-targeted oncolytic adenovirus, for the treatment of hormone-refractory, metastatic prostate cancer," *Molecular Therapy*, vol. 14, no. 1, pp. 107–117, 2006.

[15] A. L. Pecora, N. Rizvi, G. I. Cohen et al., "Phase I trial of intravenous administration of PV701, an oncolytic virus, in patients with advanced solid cancers," *Journal of Clinical Oncology*, vol. 20, no. 9, pp. 2251–2266, 2002.

[16] S. A. Laurie, J. C. Bell, H. L. Atkins et al., "A phase 1 clinical study of intravenous administration of PV701, an oncolytic virus, using two-step desensitization," *Clinical Cancer Research*, vol. 12, no. 8, pp. 2555–2562, 2006.

[17] A. I. Freeman, Z. Zakay-Rones, J. M. Gomori et al., "Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme," *Molecular Therapy*, vol. 13, no. 1, pp. 221–228, 2006.

[18] S. J. Hotte, R. M. Lorence, H. W. Hirte et al., "An optimized clinical regimen for the oncolytic virus PV701," *Clinical Cancer Research*, vol. 13, no. 3, pp. 977–985, 2007.

[19] N. Kemeny, K. Brown, A. Covey 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," *Human Gene Therapy*, vol. 17, no. 12, pp. 1214–1224, 2006.

[20] Y. Fong, T. Kim, A. Bhargava et al., "A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal cancer," *Molecular Therapy*, vol. 17, no. 2, pp. 389–394, 2009.

[21] S. K. Geevarghese, D. A. Geller, H. A. De Haan et al., "Phase I/II study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory colorectal cancer metastatic to the liver," *Human Gene Therapy*, vol. 21, no. 9, pp. 1119–1128, 2010.

[22] C. J. Breitbach, J. Burke, D. Jonker et al., "Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans," *Nature*, vol. 477, no. 7362, pp. 99–102, 2011.

[23] L. Vidal, H. S. Pandha, T. A. Yap et al., "A phase I study of intravenous oncolytic reovirus type 3 dearing in patients with advanced cancer," *Clinical Cancer Research*, vol. 14, no. 21, pp. 7127–7137, 2008.

[24] J. Nemunaitis, I. Ganly, F. Khuri et al., "Selective replication and oncolysis in p53 mutant tumors with ONYX-015, an E1B-55kD gene-deleted adenovirus, in patients with advanced head and neck cancer: a phase II trial," *Cancer Research*, vol. 60, no. 22, pp. 6359–6366, 2000.

[25] E. V. Shashkova, K. Doronin, J. S. Senac, and M. A. Barry, "Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus," *Cancer Research*, vol. 68, no. 14, pp. 5896–5904, 2008.

[26] G. Y. Minuk, R. W. Paul, and P. W. K. Lee, "The prevalence of antibodies to Reovirus type 3 in adults with idiopathic cholestatic liver disease," *Journal of Medical Virology*, vol. 16, no. 1, pp. 55–60, 1985.

[27] J. H. Tai, J. V. Williams, K. M. Edwards, P. F. Wright, J. E. Crowe, and T. S. Dermody, "Prevalence of reovirus-specific antibodies in young children in Nashville, Tennessee," *Journal of Infectious Diseases*, vol. 191, no. 8, pp. 1221–1224, 2005.

[28] C. L. White, K. R. Twigger, L. Vidal et al., "Characterization of the adaptive and innate immune response to intravenous oncolytic reovirus (Dearing type 3) during a phase I clinical trial," *Gene Therapy*, vol. 15, no. 12, pp. 911–920, 2008.

[29] Y. Chen, D. C. Yu, D. Charlton, and D. R. Henderson, "Pre-existent adenovirus antibody inhibits systemic toxicity and antitumor activity of CN706 in the nude mouse LNCaP xenograft model: Implications and proposals for human therapy," *Human Gene Therapy*, vol. 11, no. 11, pp. 1553–1567, 2000.

[30] V. Tsai, D. E. Johnson, A. Rahman et al., "Impact of human neutralizing antibodies on antitumor efficacy of an oncolytic adenovirus in a murine model," *Clinical Cancer Research*, vol. 10, no. 21, pp. 7199–7206, 2004.

[31] Z. Zhang, J. Krimmel, Z. Zhang, Z. Hu, and P. Seth, "Systemic delivery of a novel liver-detargeted oncolytic adenovirus causes reduced liver toxicity but maintains the antitumor response in a breast cancer bone metastasis model," *Human Gene Therapy*, vol. 22, no. 9, pp. 1137–1142, 2011.

[32] K. Flanagan, R. T. Glover, H. Hörig, W. Yang, and H. L. Kaufman, "Local delivery of recombinant vaccinia virus expressing secondary lymphoid chemokine (SLC) results in a CD4 T-cell dependent antitumor response," *Vaccine*, vol. 22, no. 21-22, pp. 2894–2903, 2004.

[33] Y. Ichihashi, "Extracellular enveloped vaccinia virus escapes neutralization," *Virology*, vol. 217, no. 2, pp. 478–485, 1996.

[34] M. Law, R. Hollinshead, and G. L. Smith, "Antibody-sensitive and antibody-resistant cell-to-cell spread by vaccinia virus: Role of the A33R protein in antibody-resistant spread," *Journal of General Virology*, vol. 83, no. 1, pp. 209–222, 2002.

[35] G. L. Smith, J. A. Symons, A. Khanna, A. Vanderplasschen, and A. Alcamí, "Vaccinia virus immune evasion," *Immunological Reviews*, vol. 159, pp. 137–154, 1997.

[36] A. Vanderplasschen, E. Mathew, M. Hollinshead, R. B. Sim, and G. L. Smith, "Extracellular enveloped vaccinia virus is resistant to complement because of incorporation of host complement control proteins into its envelope," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7544–7549, 1998.

[37] D. H. Kirn, Y. Wang, W. Liang, C. H. Contag, and S. H. Thorne, "Enhancing poxvirus oncolytic effects through increased spread and immune evasion," *Cancer Research*, vol. 68, no. 7, pp. 2071–2075, 2008.

[38] P. Yotnda, B. Savoldo, N. Charlet-Berguerand, C. Rooney, and M. Brenner, "Targeted delivery of adenoviral vectors by cytotoxic T cells," *Blood*, vol. 104, no. 8, pp. 2272–2280, 2004.

[39] E. J. Ilett, M. Bárcena, F. Errington-Mais et al., "Internalization of oncolytic reovirus by human dendritic cell carriers protects the virus from neutralization," *Clinical Cancer Research*, vol. 17, no. 9, pp. 2767–2776, 2011.

[40] E. J. Ilett, R. J. Prestwich, T. Kottke et al., "Dendritic cells and T cells deliver oncolytic reovirus for tumour killing despite pre-existing anti-viral immunity," *Gene Therapy*, vol. 16, no. 5, pp. 689–699, 2009.

[41] S. H. Thorne, R. S. Negrin, and C. H. Contag, "Synergistic antitumor effects of immune cell-viral biotherapy," *Science*, vol. 311, no. 5768, pp. 1780–1784, 2006.

[42] I. D. Iankov, B. Blechacz, C. Liu et al., "Infected cell carriers: a new strategy for systemic delivery of oncolytic measles viruses in cancer virotherapy," *Molecular Therapy*, vol. 15, no. 1, pp. 114–122, 2007.

[43] T. Hakkarainen, M. Sarkioja, P. Lehenkari et al., "Human mesenchymal stem cells lack tumor tropism but enhance the antitumor activity of oncolytic adenoviruses in orthotopic lung and breast tumors," *Human Gene Therapy*, vol. 18, no. 7, pp. 627–641, 2007.

[44] S. Komarova, Y. Kawakami, M. A. Stoff-Khalili, D. T. Curiel, and L. Pereboeva, "Mesenchymal progenitor cells as cellular vehicles for delivery of oncolytic adenoviruses," *Molecular Cancer Therapeutics*, vol. 5, no. 3, pp. 755–766, 2006.

[45] A. M. Sonabend, I. V. Ulasov, M. A. Tyler, A. A. Rivera, J. M. Mathis, and M. S. Lesniak, "Mesenchymal stem cells effectively deliver an oncolytic adenovirus to intracranial glioma," *Stem Cells*, vol. 26, no. 3, pp. 831–841, 2008.

[46] C. Pfirschke and V. Schirrmacher, "Cross-infection of tumor cells by contact with T lymphocytes loaded with Newcastle disease virus," *International Journal of Oncology*, vol. 34, no. 4, pp. 951–962, 2009.

[47] J. Qiao, T. Kottke, C. Willmon et al., "Purging metastases in lymphoid organs using a combination of antigen-nonspecific adoptive T cell therapy, oncolytic virotherapy and immunotherapy," *Nature Medicine*, vol. 14, no. 1, pp. 37–44, 2008.

[48] G. Coukos, A. Makrigiannakis, E. H. Kang et al., "Use of carrier cells to deliver a replication-selective herpes simplex virus-1 mutant for the intraperitoneal therapy of epithelial ovarian cancer," *Clinical Cancer Research*, vol. 5, no. 6, pp. 1523–1537, 1999.

[49] A. Munguia, T. Ota, T. Miest, and S. J. Russell, "Cell carriers to deliver oncolytic viruses to sites of myeloma tumor growth," *Gene Therapy*, vol. 15, no. 10, pp. 797–806, 2008.

[50] Z. Raykov, G. Balboni, M. Aprahamian, and J. Rommelaere, "Carrier cell-mediated delivery of oncolytic parvoviruses for targeting metastases," *International Journal of Cancer*, vol. 109, no. 5, pp. 742–749, 2004.

[51] P. Yotnda, A. R. Davis, M. J. Hicks, N. S. Templeton, and M. K. Benner, "Liposomal enhancement of the antitumor activity of conditionally replication-competent adenoviral plasmids," *Molecular Therapy*, vol. 9, no. 4, pp. 489–495, 2004.

[52] A. Fontanellas, S. Hervás-Stubbs, I. Mauleón et al., "Intensive pharmacological immunosuppression allows for repetitive liver gene transfer with recombinant adenovirus in nonhuman primates," *Molecular Therapy*, vol. 18, no. 4, pp. 754–765, 2010.

[53] J. Qiao, H. Wang, T. Kottke et al., "Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus," *Clinical Cancer Research*, vol. 14, no. 1, pp. 259–269, 2008.

[54] N. M. Girgis, B. C. DeHaven, X. Fan, K. M. Viner, M. Shamim, and S. N. Isaacs, "Cell surface expression of the vaccinia virus complement control protein is mediated by interaction with the viral A56 protein and protects infected cells from complement attack," *Journal of Virology*, vol. 82, no. 9, pp. 4205–4214, 2008.

[55] G. J. Kotwal, S. N. Isaacs, R. McKenzie, M. M. Frank, and B. Moss, "Inhibition of the complement cascade by the major secretory protein of vaccinia virus," *Science*, vol. 250, no. 4982, pp. 827–830, 1990.

[56] A. Sahu, S. N. Isaacs, A. M. Soulika, and J. D. Lambris, "Interaction of vaccinia virus complement control protein with human complement proteins: factor I-mediated degradation of C3b to iC3b1 inactivates the alternative complement pathway," *Journal of Immunology*, vol. 160, no. 11, pp. 5596–5604, 1998.

[57] A. H. J. Kim, I. D. Dimitriou, M. C. H. Holland et al., "Complement C5a receptor is essential for the optimal generation of antiviral CD8+ T cell responses," *Journal of Immunology*, vol. 173, no. 4, pp. 2524–2529, 2004.

[58] M. Kopf, B. Abel, A. Gallimore, M. Carroll, and M. F. Bachmann, "Complement component C3 promotes T-cell priming and lung migration to control acute influenza virus infection," *Nature Medicine*, vol. 8, no. 4, pp. 373–378, 2002.

[59] A. F. Ochsenbein, D. D. Pinschewer, B. Odermatt, M. C. Carroll, H. Hengartner, and R. M. Zinkernagel, "Protective T cell-independent antiviral antibody responses are dependent on complement," *Journal of Experimental Medicine*, vol. 190, no. 8, pp. 1165–1174, 1999.

[60] M. Suresh, H. Molina, M. S. Salvato, D. Mastellos, J. D. Lambris, and M. Sandor, "Complement component 3 is required for optimal expansion of CD8 T cells during a systemic viral infection," *Journal of Immunology*, vol. 170, no. 2, pp. 788–794, 2003.

[61] A. Verschoor, M. A. Brockman, M. Gadjeva, D. M. Knipe, and M. C. Carroll, "Myeloid C3 determines induction of humoral responses to peripheral herpes simplex virus infection," *Journal of Immunology*, vol. 171, no. 10, pp. 5363–5371, 2003.

[62] A. Verschoor, M. A. Brockman, D. M. Knipe, and M. C. Carroll, "Cutting edge: myeloid complement C3 enhances the humoral response to peripheral viral infection," *Journal of Immunology*, vol. 167, no. 5, pp. 2446–2451, 2001.

[63] N. M. Girgis, B. C. DeHaven, Y. Xiao, E. Alexander, K. M. Viner, and S. N. Isaacs, "The vaccinia virus complement control protein modulates adaptive immune responses during infection," *Journal of Virology*, vol. 85, no. 6, pp. 2547–2556, 2011.

[64] S. B. Pushpakumar, G. Perez-Abadia, C. Soni et al., "Enhancing complement control on endothelial barrier reduces renal post-ischemia dysfunction," *Journal of Surgical Research*, vol. 170, no. 2, pp. e263–e270, 2011.

[65] J. M. Lubinski, H. M. Lazear, S. Awasthi, F. Wang, and H. M. Friedman, "The herpes simplex virus 1 IgG Fc receptor blocks antibody-mediated complement activation and antibody-dependent cellular cytotoxicity in vivo," *Journal of Virology*, vol. 85, no. 7, pp. 3239–3249, 2011.

[66] L. M. Hook, J. M. Lubinski, M. Jiang, M. K. Pangburn, and H. M. Friedman, "Herpes simplex virus type 1 and 2 glycoprotein C prevents complement-mediated neutralization induced by natural immunoglobulin M antibody," *Journal of Virology*, vol. 80, no. 8, pp. 4038–4046, 2006.

[67] J. Tian, Z. Xu, J. S. Smith, S. E. Hofherr, M. A. Barry, and A. P. Byrnes, "Adenovirus activates complement by distinctly different mechanisms in vitro and in vivo: indirect complement activation by virions in vivo," *Journal of Virology*, vol. 83, no. 11, pp. 5648–5658, 2009.

[68] J. Gandhi, S. M. Cashman, and R. Kumar-Singh, "Soluble CD59 expressed from an adenovirus in vivo is a potent inhibitor of complement deposition on murine liver vascular endothelium," *PLoS One*, vol. 6, no. 6, article e21621, 2011.

[69] R. E. Randall and S. Goodbourn, "Interferons and viruses: an interplay between induction, signalling, antiviral responses and virus countermeasures," *Journal of General Virology*, vol. 89, no. 1, pp. 1–47, 2008.

[70] S. V. Kotenko, G. Gallagher, V. V. Baurin et al., "IFN-λs mediate antiviral protection through a distinct class II cytokine receptor complex," *Nature Immunology*, vol. 4, no. 1, pp. 69–77, 2003.

[71] D. B. Stetson and R. Medzhitov, "Type I interferons in host defense," *Immunity*, vol. 25, no. 3, pp. 373–381, 2006.

[72] C. E. Samuel, "Antiviral actions of interferons," *Clinical Microbiology Reviews*, vol. 14, no. 4, pp. 778–809, 2001.

[73] A. Le Bon and D. F. Tough, "Links between innate and adaptive immunity via type I interferon," *Current Opinion in Immunology*, vol. 14, no. 4, pp. 432–436, 2002.

[74] A. Alcami and G. L. Smith, "Vaccinia, cowpox, and camelpox viruses encode soluble gamma interferon receptors with novel broad species specificity," *Journal of Virology*, vol. 69, no. 8, pp. 4633–4639, 1995.

[75] I. Haralambieva, I. Iankov, K. Hasegawa, M. Harvey, S. J. Russell, and K. W. Peng, "Engineering oncolytic measles virus to circumvent the intracellular innate immune response," *Molecular Therapy*, vol. 15, no. 3, pp. 588–597, 2007.

[76] K. Mossman, C. Upton, R. M. L. Buller, and G. McFadden, "Species specificity of ectromelia virus and vaccinia virus interferon-γ binding proteins," *Virology*, vol. 208, no. 2, pp. 762–769, 1995.

[77] A. U. Ahmed, C. E. Rolle, M. A. Tyler et al., "Bone marrow mesenchymal stem cells loaded with an oncolytic adenovirus suppress the anti-adenoviral immune response in the cotton rat model," *Molecular Therapy*, vol. 18, no. 10, pp. 1846–1856, 2010.

[78] T. L. A. Nguyên, H. Abdelbary, M. Arguello et al., "Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 39, pp. 14981–14986, 2008.

[79] A. Otsuki, A. Patel, K. Kasai et al., "Histone deacetylase inhibitors augment antitumor efficacy of herpes-based oncolytic viruses," *Molecular Therapy*, vol. 16, no. 9, pp. 1546–1555, 2008.

[80] R. Alemany, K. Suzuki, and D. T. Curiel, "Blood clearance rates of adenovirus type 5 in mice," *Journal of General Virology*, vol. 81, no. 11, pp. 2605–2609, 2000.

[81] R. C. Carlisle, Y. Di, A. M. Cerny et al., "Human erythrocytes bind and inactivate type 5 adenovirus by presenting Coxsackie virus-adenovirus receptor and complement receptor 1," *Blood*, vol. 113, no. 9, pp. 1909–1918, 2009.

[82] M. Lyons, D. Onion, N. K. Green et al., "Adenovirus type 5 interactions with human blood cells may compromise systemic delivery," *Molecular Therapy*, vol. 14, no. 1, pp. 118–128, 2006.

[83] M. Aghi, S. D. Rabkin, and R. L. Martuza, "Angiogenic response caused by oncolytic herpes simplex virus-induced reduced thrombospondin expression can be prevented by specific viral mutations or by administering a thrombospondin-derived peptide," *Cancer Research*, vol. 67, no. 2, pp. 440–444, 2007.

[84] R. K. Jain, A. V. Finn, F. D. Kolodgie, H. K. Gold, and R. Virmani, "Antiangiogenic therapy for normalization of atherosclerotic plaque vasculature: a potential strategy for plaque stabilization," *Nature Clinical Practice Cardiovascular Medicine*, vol. 4, no. 9, pp. 491–502, 2007.

[85] K. Kurozumi, J. Hardcastle, R. Thakur et al., "Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy," *Journal of the National Cancer Institute*, vol. 99, no. 23, pp. 1768–1781, 2007.

[86] K. Nakamura, J. Sasajima, Y. Mizukami et al., "Hedgehog promotes neovascularization in pancreatic cancers by regulating Ang-1 and IGF-1 expression in bone-marrow derived pro-angiogenic cells," *PloS One*, vol. 5, no. 1, article e8824, 2010.

[87] C. Rolny, M. Mazzone, S. Tugues et al., "HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF," *Cancer Cell*, vol. 19, no. 1, pp. 31–44, 2011.

[88] B. B. Goldberg, J. B. Liu, and F. Forsberg, "Ultrasound contrast agents: a review," *Ultrasound in Medicine and Biology*, vol. 20, no. 4, pp. 319–333, 1994.

[89] I. V. Larina, B. M. Evers, and R. O. Esenaliev, "Optimal drug and gene delivery in cancer cells by ultrasound-induced cavitation," *Anticancer Research*, vol. 25, no. 1, pp. 149–156, 2005.

[90] A. Lawrie, A. F. Brisken, S. E. Francis, D. C. Cumberland, D. C. Crossman, and C. M. Newman, "Microbubble-enhanced ultrasound for vascular gene delivery," *Gene Therapy*, vol. 7, no. 23, pp. 2023–2027, 2000.

[91] K. Y. Ng and Y. Liu, "Therapeutic ultrasound: Its application in drug delivery," *Medicinal Research Reviews*, vol. 22, no. 2, pp. 204–223, 2002.

[92] W. G. Pitt, G. Husseini, and B. J. Staples, "Ultrasonic drug delivery—a general review," *Expert Opinion on Drug Delivery*, vol. 1, no. 1, pp. 37–56, 2004.

[93] A. Greco, A. Di Benedetto, C. M. Howard et al., "Eradication of therapy-resistant human prostate tumors using an ultrasound-guided site-specific cancer terminator virus delivery approach," *Molecular Therapy*, vol. 18, no. 2, pp. 296–306, 2010.

[94] C. M. Howard, F. Forsberg, C. Minimo, J. B. Liu, D. A. Merton, and P. P. Claudio, "Ultrasound guided site specific gene delivery system using adenoviral vectors and commercial ultrasound contrast agents," *Journal of Cellular Physiology*, vol. 209, no. 2, pp. 413–421, 2006.

[95] N. Araki, M. T. Johnson, and J. A. Swanson, "A role for phosphoinositide 3-kinase in the completion of macropinocytosis and phagocytosis by macrophages," *Journal of Cell Biology*, vol. 135, no. 5, pp. 1249–1260, 1996.

[96] D. Cox, B. M. Dale, M. Kashiwada, C. D. Helgason, and S. Greenberg, "A regulatory role for Src homology 2 domain-containing inositol 5′-phosphatase (SHIP) in phagocytosis mediated by Fcγ receptors and complement receptor 3 (αMβ2; CD11b/CD18)," *Journal of Experimental Medicine*, vol. 193, no. 1, pp. 61–71, 2001.

[97] D. Cox, C. C. Tseng, G. Bjekic, and S. Greenberg, "A requirement for phosphatidylinositol 3-kinase in pseudopod extension," *Journal of Biological Chemistry*, vol. 274, no. 3, pp. 1240–1247, 1999.

[98] T. H. Sulahian, A. Imrich, G. Deloid, A. R. Winkler, and L. Kobzik, "Signaling pathways required for macrophage scavenger receptor-mediated phagocytosis: analysis by scanning cytometry," *Respiratory Research*, vol. 9, p. 59, 2008.

[99] S. Wee, D. Wiederschain, S. M. Maira et al., "PTEN-deficient cancers depend on PIK3CB," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 35, pp. 13057–13062, 2008.

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 702839, 14 pages
doi:10.1155/2012/702839

Review Article

Deciphering the Multifaceted Relationship between Oncolytic Viruses and Natural Killer Cells

Christopher A. Alvarez-Breckenridge,[1,2] Jianhua Yu,[3] Balveen Kaur,[2,3] Michael A. Caligiuri,[3] and E. Antonio Chiocca[2,3]

[1] *Medical Scientist Training Program, The Ohio State University, Columbus, OH 43210, USA*
[2] *Dardinger Laboratory for Neuro-Oncology and Neurosciences, Department of Neurological Surgery, The Ohio State University, Columbus, OH 43210, USA*
[3] *The Ohio State University Comprehensive Cancer Center, The Ohio State University, Wiseman Hall, 400 West 12th Avenue, Columbus, OH 43210, USA*

Correspondence should be addressed to E. Antonio Chiocca, ea.chiocca@osumc.edu

Received 28 June 2011; Accepted 14 September 2011

Academic Editor: Ulrich M. Lauer

Despite active research in virotherapy, this apparently safe modality has not achieved widespread success. The immune response to viral infection appears to be an essential factor that determines the efficacy of oncolytic viral therapy. The challenge is determining whether the viral-elicited immune response is a hindrance or a tool for viral treatment. NK cells are a key component of innate immunity that mediates antiviral immunity while also coordinating tumor clearance. Various reports have suggested that the NK response to oncolytic viral therapy is a critical factor in premature viral clearance while also mediating downstream antitumor immunity. As a result, particular attention should be given to the NK cell response to various oncolytic viral vectors and how their antiviral properties can be suppressed while maintaining tumor clearance. In this review we discuss the current literature on the NK response to oncolytic viral infection and how future studies clarify this intricate response.

1. Introduction

The field of oncolytic viral therapy is currently at a crossroads. With over twenty years of attention directed towards oncolytic viruses (OV), clinical trials have been encouraging, but have left investigators with the task of identifying barriers that can be circumvented to achieve more successful virotherapy. Some of the most prevalent obstacles include the antiviral host response to OV, the angiogenic response to viral infection, extracellular barriers to viral spread, and inefficient/nonspecific receptor-ligand interactions on target cells [1]. Interestingly, various groups have also demonstrated that an inability to achieve adequate antitumor immunity also represents a significant barrier to tumor clearance [2]. In order to optimize virotherapy for clinical success, the relevance of these barriers, along with the conflicting roles of antiviral and antitumor immunity, must be clarified. While various groups have studied the host response to OV, the natural killer (NK) cell response to various oncolytic viruses has been less thoroughly investigated.

In order to appreciate both the current literature surrounding the NK response to OV therapy and understand how these cells can be targeted in future studies, it is essential to understand the role of these cells in viral clearance and tumor immunology. Interestingly, profound human NK cell deficiencies have led to overwhelming herpes viral infections, supporting the notion that this innate immune effector cell has specific recognition of, and control over, viral infection [3–5]. Additionally, multiple reports have associated NK cell levels with tumor regression [6–9]. Taken together, these findings highlight potentially conflicting roles for NK cells in oncolytic virotherapy. On the one hand, the antiviral properties of these cells may be detrimental to viral propagation and viral mediated tumor clearance. Conversely, an activated NK response following OV infection of tumors may stimulate NK-mediated antitumor immunity

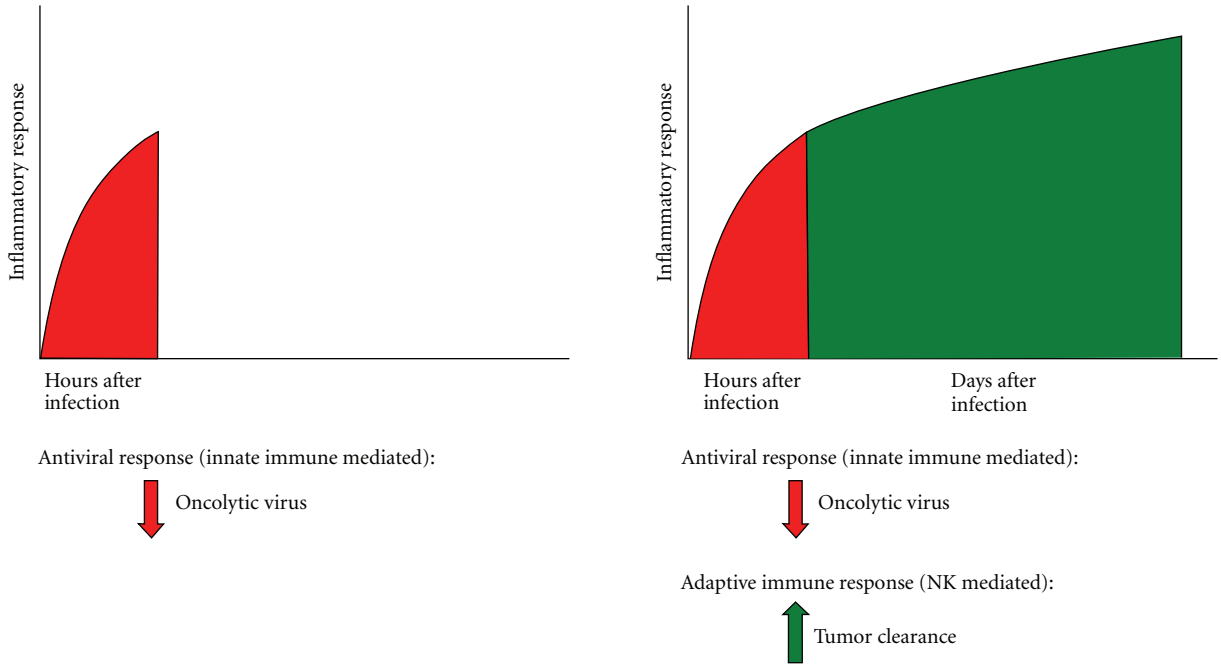


Figure 1: The immune reaction to oncolytic viral infection is two-phased response. Within hours after infection, the innate immune response consisting of NK cells, macrophages, and neutrophils is recruited to the site of infection and mediates initial viral clearance. Following this response to infection, innate immune mediators, particularly NK cells, mediate the downstream adaptive immune response that is a critical antitumor mediator. In order to reconcile this biphasic response, initial immune suppression targeting NK cells may be required initially after viral infection followed by a period of immune stimulation to elicit antitumor immunity.

(Figure 1). While most studies to date have focused on the dichotomous nature of the NK response, it is likely that a more nuanced approach will be needed in which the antiviral response to infection is initially suppressed while antitumor immunity is selectively stimulated.

Investigators frequently attempt to correlate the success of their oncolytic viral therapy with immune cell infiltration following infection. Using this metric, NK cells have been highlighted as a relevant factor in response to OV infection. However, significantly less attention has been directed towards the nature and relevance of this viral-induced NK response. For example, what role do NK cells have in recruiting activated macrophages following OV therapy? Does OV administration induce a different NK activation profile compared to infection with its wild-type counterpart? Does OV infection of tumor lead to the preferential NK-mediated clearance of these virally infected cells compared to uninfected tumor and therefore impeded viral oncolysis? Are there discrepancies between activated NK cells that are recruited in mice bearing xenograft tumors versus syngeneic tumors? Lastly, is it possible to temporarily pharmacologically modulate the NK immune response to OV-infected cells in order to enhance OV therapeutic efficacy? These are just a few of the questions that should be considered as investigators move beyond just determining that these cells are recruited following infection. In this review, we will discuss the nature of the host response to OV infection, highlight the clinical relevance of NK cells in antiviral defense, consider the literature surrounding the NK response to OV therapy, and suggest areas for future investigation.

2. Placing NK Cell Biology in the OV Context

Although a corpus of evidence has delineated both the role of NK cells in tumor clearance for various tumor models and their role in viral eradication, the importance of NK cells in response to OV therapy is just beginning to be appreciated. In order to decipher their role in the OV context, it is important to first understand the basic properties of NK cell biology. Human NK cells are divided into separate $CD56^{bright}$ and $CD56^{dim}$ populations that differ in their functional capacity and localization [10]. Approximately 90% of circulating and splenic NK cells are $CD56^{dim}CD16^{+}$, express perforin, and possess cytotoxic capacity when interacting with target cells [11]. In contrast, $CD56^{bright}$CD16-NK cells are detected in lymph nodes and tonsils, lack perforin, and readily produce cytokines such as IFN-γ in response to stimulation with IL-12, -15, and -18 [12, 13]. In mice, NK cells have been differentiated into three subsets according to CD11b and CD27 expression [14]. NK cell differentiation in mice occurs from a relatively immature $C11b^{dull}CD27^{+}$ state to the double positive $CD11b^{+}CD27^{+}$ and ultimately to the senescent $CD11b^{+}CD27^{dull}$. Notably, both the double positive and senescent NK cells have been demonstrated to secrete IFN-γ and carry out cell-mediated cytotoxicity. While investigators have identified differential anatomical distribution

for each type of NK cell in wild type mice, there have been no examinations into the maturation state of NK cells within the tumor microenvironment either in the absence or presence of OV. Despite the differences in developmental and activation markers on NK cells in mice and humans [15], a basic understanding of the role of NK cells in response to OV therapy in mice establishes a framework for future studies in clinical trials.

While certain NK cells are sufficiently mature to produce both cytotoxic and cytokine responses, these two functions are products of the cytokine microenvironment. For instance, type I interferon, IL-12, and IL-18 are critical for the induction of NK activation [16]. Moreover, much like T cells require "priming" for full activation, IL-15 has been elucidated as a cytokine that is critical for the priming function of murine NK cells [15, 17]. While initial efforts have examined the role of certain activating cytokines within the tumor microenvironment following OV therapy [18, 19], additional work is needed to understand their roles in OV clearance and tumor killing.

NK cells are able to carry out their diverse repertoire of activities through a detection system that relies on the engagement of a variety of cell surface activating and inhibitory receptors on NK cells (Figure 2). Through the binding of these receptors, a dynamic equilibrium is achieved that differentiates the recognition of "self" cells from transformed target cells. The activating NK cell receptors detect the presence of ligands on cells that are in a "distressed" state. These include stress ligands in mice (e.g., RAE1, H60, and MULT1) and humans (e.g., ULBP1-3 and MICA/B) that bind to the NKG2D activating receptor on NK cells. Natural cytotoxicity receptors (NCRs) are a family of activating receptors critical for mediating NK killing. They consist of NKp46 which is endogenously expressed on NK cells and NKT-like cells in both mice and humans [20]; NKp44 which is solely expressed on human NK cells, with constitutive expression only after cytokine stimulation, and plasmacytoid dendritic cells [21]; NKp30 which is exclusively expressed on resting and activated human NK cells [22]. While the identity of ligands for NCRs is a field of intense investigation [23], recent discoveries have identified influenza hemagglutinin (HA) as an activating ligand for NKp46 and NKp44 [24]. Besides mediating the eradication of tumor and virally infected cells, NKp30 interacts with immature dendritic cells (imDCs). Following NKp30 binding to an unknown ligand on imDCs [25], the imDCs are subsequently either killed or develop into mature DCs that can mediate a Th1 response [26] culminating in tumor/viral eradication. To counteract this process, human cytomegalovirus tegument protein pp65 impedes NKp30 activation through NKp30-CD3ζ receptor dissociation and the concomitant circumvention of NKp30-mediated maturation of dendritic cells [27, 28]. Known activating ligands for NKp30 include two activating cellular proteins. These include B7-H6 [29], a cell surface protein associated with tumor formation and Bat3 [30], a released cellular stress protein. In addition to their activating receptors, NK cells also possess an array of inhibitory receptors that are used to survey cells for the absence of constitutively expressed self-ligands. For instance, MHC-I expression is recognized by the inhibitory receptor killer cell immunoglobulin-like receptors in humans, lectin-like Ly49 dimers in mice, and CD94-NKG2A heterodimer in both species [15].

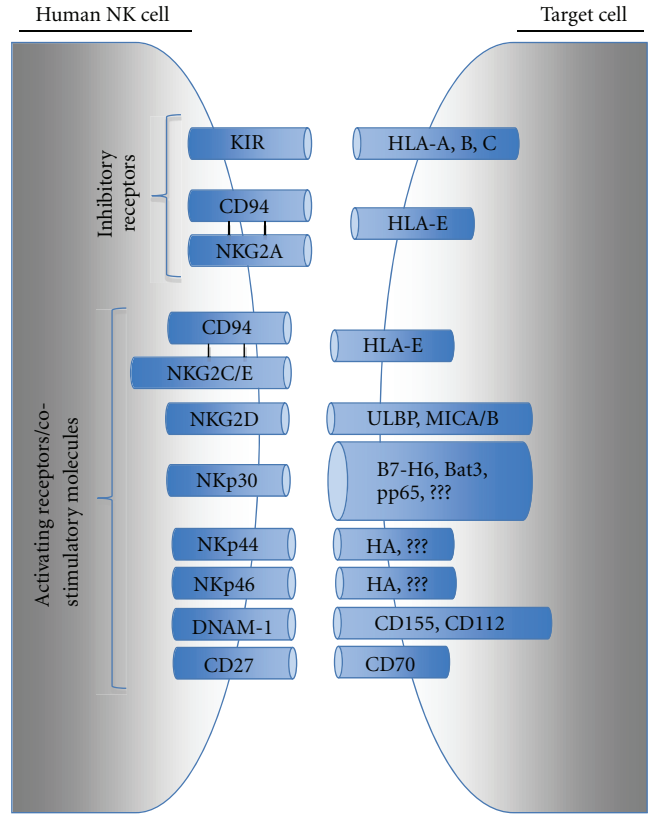


Figure 2: NK cell activation is mediated by a variety of cell surface inhibitory and activating receptors that recognize cell surface ligands on target cells. Viral infection, oncogenic transformation, and cellular stressors result in the downregulation in ligands for NK inhibitory receptors while concomitantly increasing the expression of NK activating ligands. Despite the presence of both activating and inhibitory signals on target cells, the overall balance of these signals dictates NK activation and target cell clearance.

Taken together, by identifying cells that have absent MHC-I expression and/or upregulation of stress or virally encoded ligands for NK activating receptors, a target cell can be susceptible to NK-mediated lysis. By characterizing the NK ligand signature following OV infection and deciphering the receptor-ligand interactions that are responsible for NK recognition of virally infected cells, a mechanistic understanding can potentially guide the development of therapeutic approaches to selectively target the NK receptors implicated in either the anti-OV or antitumor response.

3. The Host Response to OV Therapy

The rapid response of innate immunity, consisting of NK cells, monocytes, macrophages, and neutrophils provides an initial and potent line of defense for the host and limits initial viral infection, replication, and spread; facilitates

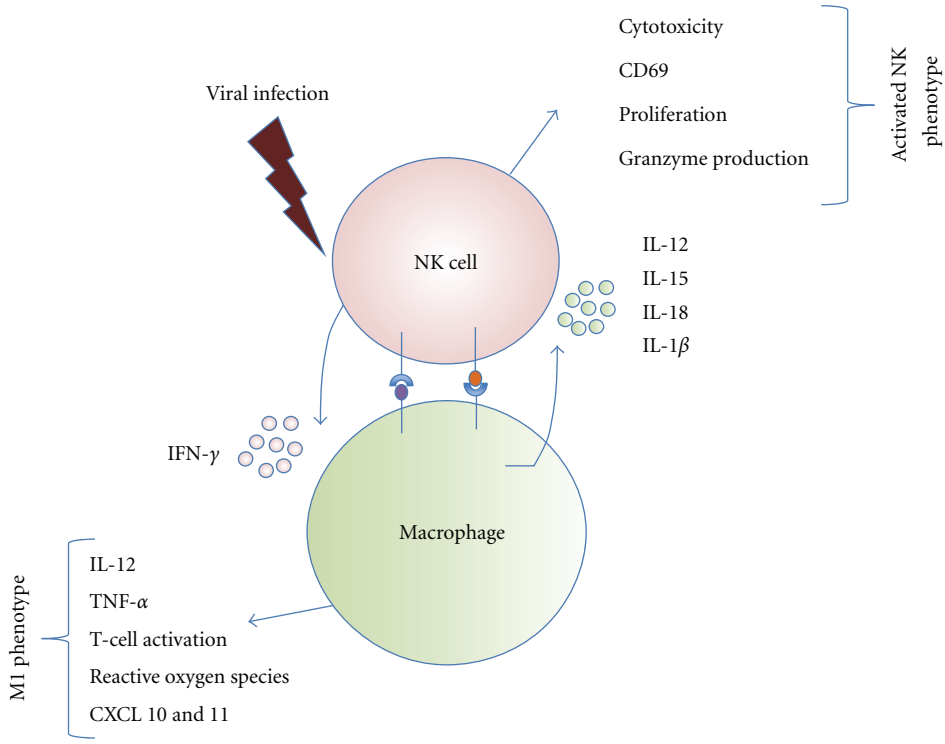


Figure 3: Innate immune effector cells, including NK cells and macrophages, represent an initial barrier to viral infection, replication, and spread. Following infection, NK cells are recruited to the site of infection and adopt an activated phenotype. Through their IFN-γ production, they also facilitate the maturation and activation of macrophages which adopt an inflammatory "M1" phenotype. Lastly, macrophages create a feedback loop by producing a variety of inflammatory cytokines that mediate NK activation. This inflammatory response creates a potent antiviral microenvironment while also communicating with the adaptive immunity.

the maturation of antigen presenting cells; communicates with the adaptive arm of the immune system to regulate its response. Due to the rapid decline in viral titers within days of inoculating various oncolytic viruses [31, 32], the innate immune response has been implicated as a critical factor in this response. While neutrophils are the first antiviral responders that are recruited to a site of infection, efficient viral clearance at the cellular level requires both NK cells and monocyte-derived cells. Activated NK cells mediate direct lysis of infected target cells by releasing cytotoxic granules containing lytic enzymes [33] or by binding to apoptosis-inducing receptors on target cells [34]. NK cell-mediated preferential lysis of HSV- or vaccinia-virus-infected cells has been shown to prevent viral dissemination to neighboring cells [35]. While recruitment of NK cells to infected tumor tissue has correlated with reduced viral spread and OV efficacy, IFN-γ production by NK cells has also been shown to set the stage for the subsequent adaptive immune response [36, 37].

Apart from NK cells, macrophages also play a critical role in OV clearance. Upon viral infection, resident or recruited macrophages initially secrete IL-12 to activate NK cells while NK cells complete the feedback loop by secreting IFN-γ—the prototypic macrophage activator, without which macrophages cannot clear microbes [36] (Figure 3). In fact, recruitment of infiltrating monocytic cells has been shown to coincide with clearance of over 80% of HSV-derived oncolytic viral particles [38, 39]. Increased intra-tumoral presence of macrophage/microglia cells has also been reported in human patients treated with adenovirus [40, 41] or HSV-1-derived OV [42] indicating the global significance of macrophages in OV therapy.

Despite their antiviral properties, NK cells have pleiotropic effects that may also be critical in tumor killing. NK cells have been shown to augment the tumoricidal effects in various models. This includes the most well-studied example of a melanoma model in which NK cells have been defined as an essential cellular component for VSV efficacy [43]. In this model, NK cells functioned synergistically with the adaptive antitumor immune response, launched in response to viral antigens expressed by tumor cells. Therefore, it appears that NK cells can serve a dual function—both as potential inhibitors of viral replication and as critical mediators to establish an effective antitumor immunity following viral antigen presentation within the tumor cells. These findings emphasize the impact of variations in tumor models, anatomical location of the tumors, and properties of the viruses that are being tested [2].

In future studies, a refined approach will be needed to manipulate individual cell populations while considering both the timing and nature of the intervention in order to maximize therapeutic regimens. One promising, albeit simplistic, approach will be to combine OV inoculation with transient immunomodulation in order to achieve initial viral

replication, followed by restoration of immune activity to harness the immunotherapeutic potential of virotherapy.

4. NK Deficiencies

NK cell deficiencies, while being a rare phenomenon, provide valuable information about the role of these cells in antimicrobial defense and potentially the NK response to OV. While these deficiencies are relatively uncommon, they indicate the essential nature of these cells in host defense. The most informative group of disorders involves an isolated human NK cell deficiency that is associated with a specific gene mutation [4]. The only known human gene alteration resulting in an isolated NK cell deficiency results from a polymorphism in CD16, the IgG Fc receptor which is activated following binding to IgG. In this polymorphism, the CD16 epitope recognized by mAb B73.1 is changed by a T → A substitution at position 230 resulting in L48 → H [4]. Individuals homozygous for this alteration have phenotypically normal NK cells but are not recognized by mAb B73.1 [44, 45]. Several individuals have been documented to have a homozygous 48H phenotype and they reported to have recurrent viral infections. In particular, a 5-year-old girl was documented to have frequent respiratory infections, recurrent HSV stomatitis, and recurrent herpetic whitlow [45]. This child was deficient in NK cell cytotoxicity against K562 target cells but had normal antibody-dependent cellular cytotoxicity (ADCC). Taken together, this 48H phenotype suggests the importance of this epitope in resistance to viral infections.

A second group of NK cell deficiencies result from unknown gene mutations. The most striking example of human NK cell deficiency is from a female adolescent with an absolute NK cell deficiency based on a lack of lymphocytes expressing CD56 or CD16 and an absence of both NK cell cytotoxicity and ADCC. This patient presented with disseminated, life-threatening varicella infection and subsequently developed both CMV pneumonitis and cutaneous HSV [5]. There have also been reports of individuals with functional NK deficiency in which NK cells are present as a normal percentage of peripheral blood lymphocytes, but are deficient in activity. For example, four patients have been reported with widespread or invasive HSV disease, all with basal NK cell cytotoxicity against HSV-infected fibroblasts [46].

Although isolated NK cell deficiencies present the opportunity to understand NK cell specific genes and NK cell roles in human antimicrobial defense, a variety of other diseases have NK cell deficiency as a component. For instance, in Griscelli syndrome patients have variable immune deficiencies that typically include a marked reduction in NK cell cytotoxicity but an ability to induce cytotoxicity upon IFN-α or IL-2 stimulation [47–49]. Despite this variable responsiveness, patients with this syndrome have a propensity for EBV and HSV infections [47]. In leukocyte adhesion deficiency (LAD), patients have elevated peripheral leukocytes and in some patients, a corresponding recurrence of HSV infection [50, 51]. In these patients, the ability of NK cells to mediate cytotoxicity, ADCC, or kill HSV-infected target cells is severely attenuated. In LAD, there are a variety of mutations in the β2 integrin CD18 [52]. This results in the inappropriate expression of various key adhesion complexes including LFA-1 (CD11a/DC18) and Mac-1 (CD11b/CD18). Notably, LFA-1 associates with the immunoglobulin-like activating receptor DNAM-1 [53]. Taken collectively, the findings from both isolated human NK cell defects and diseases that include NK cell deficiencies suggest that human NK cell activity is especially important in limiting viral infection and may similarly attenuate oncolytic viral propagation.

5. The Interface between NK Cells and Oncolytic Viral Therapy

5.1. NK Cells and Antitumor Immunity. In fully immunocompetent animal models, the variables that ultimately determine clinical success are the amount of viral replication inside the tumor, the antiviral immune response elicited by viral infection, and the stimulation of an antitumor immune response. However, differences in oncolytic viral vectors, tumor models, and the anatomical locations of tumors add a layer of complexity that makes broad conclusions about host immunity difficult to achieve. Innate immune responses have the potential of mediating cytotoxicity directly against tumors while simultaneously mediating downstream immune response [54]. Among the innate immune cell compartment that mediates this response, NK cells stand out as a key cellular factor [55]. While the presence of NK cells within human tumors is associated with a positive prognosis [6–9], their infiltration within many tumors is often sparse [56, 57]. Since NK cells possess both antiviral and antitumor properties, it is not surprising that their involvement is equally controversial. While there are examples of studies that have found no involvement of NK cells in response to oncolytic viral infections [58, 59], the majority of studies find that they are relevant in some capacity. To start, we will focus on studies that both highlight the need of achieving antitumor immunity and determine the essential role of NK cells in mediating this response.

One of the first reports to mechanistically describe the essential nature of antitumor immunity was the work of Diaz et al. in which VSV was administered intratumorally for the treatment of an immunocompetent B16 melanoma model [43]. In this study, depletion experiments were performed to demonstrate that tumor regression was achieved in a CD8 and NK cell-dependent manner. While markers of NK cells and CD8 T-cell activation were not extensively examined, the authors did observe that CD8 priming correlated with increased cell counts in both the tumor and draining lymph nodes; however, NK cell numbers remained unchanged following infection. Corroborating the relevance of these findings, the treatment of prostate cancer with reovirus overrode the prominent immunosuppressant milieu of prostate adenocarcinoma [60, 61] and elicited an antitumor CD8 T-cell response along with prominent NK cell infiltration [62]. Miller and Fraser also found that intratumoral therapy with oHSV for metastatic melanoma was abrogated in syngeneic models lacking NK and T-cell subsets [63]. Lastly,

inoculation of HSV-1716 induced the production of IFN-γ inducible chemokines from human DCs along with the migration of NK and CD8 cells into murine tumors [64]. Taken collectively, these findings from various tumor models treated with VSV, reovirus, and oHSV highlight the apparent relevance of NK and T cells as mediators of antitumor efficacy [2].

Interestingly, a recent Phase I trial examining intravenous administration of oncolytic reovirus found that CD8 and NK cells increased by 33% and 38%, respectively, following OV infection [65]. These findings appear to highlight the relevance of these cellular components in the clinical setting. Despite these observations, increases in immune cell numbers do not necessarily correlate with immune cells activation in response to infection. As a result, future studies are needed to evaluate the phenotypic profile and relevance of each immune cell component following oncolytic viral infection.

In addition to the CD8 and NK response, Diaz et al. examined the significance of regulatory T cells (Tregs) in their model [43]. Following viral inoculation, increased numbers of Tregs were detected within the tumor. Surprisingly, Treg depletion did not increase antitumor efficacy by relieving the suppression of antitumor CD8 cells; rather, the antiviral immune response was significantly enhanced following Treg depletion, resulting in both decreased viral titers and decreased OV efficacy. In this model, these findings suggest that Treg suppression is active at the level of antiviral, rather than antitumor immunity.

The treatment of B16 subcutaneous tumors and lung metastasis can also be treated with intravenous VSV, albeit using a different mechanism. In the study by Kottke et al., vascular leak syndrome (VLS) correlated with enhanced oncolytic VSV localization to subcutaneous and metastatic lung tumors [66]. VLS is induced following IL-2-mediated endothelial cell injury which was exacerbated with Treg depletion [67, 68]. The authors hypothesized that this endothelial damage and concomitant vascular permeability created an environment that facilitated viral access from the circulation to the tumor. Interestingly, NK cells were critical for VLS-mediated localization and spread through the tumor while also allowing for the continued delivery of virus to tumors in the presence of previously vaccinated mice [66].

In contrast to the work by Diaz et al. [43], the induction of VLS through Treg depletion induced a markedly different immune response to OV infection [66]. While IL-2 expands the pool of NK cells *in vivo*, these cells are kept in check by Tregs which are similarly expanded by IL-2. Thus, depletion of Tregs appears to result in hyperactivated NK cells manifesting in enhanced VLS, cytokine production, and cytotoxic effector functions [66]. Moreover, this efficacious response was recapitulated in the same tumor model using i.v. administration of reovirus combined with IL-2 and cyclophosphamide (CPA) [69]. When given at low doses, CPA has been demonstrated to enhance immune responses against tumors through transient depletion of Tregs [70, 71]. Consistent with the findings with VSV combined with IL-2 and Treg depletion [66], reovirus/IL-2/CPA cotherapy achieved an activated NK phenotype that achieved significant tumor regression [69].

Somewhat surprisingly, however, this state of NK cell hyperactivation, which presumably includes antiviral properties, correlates with increased VSV localization, replication, and spread. These findings suggest that in a VLS virotherapy model, the antiviral properties of hyperactivated NK cells are overridden by their ability to localize systemically injected virus to the tumor. These findings were also in contrast to the findings of Diaz et al. [43] in which Treg depletion was detrimental for CD8/NK cell-mediated efficacy of IT administration of VSV. Thus, Treg depletion clearly has positive and negative outcomes and emphasizes the need to consider opposing interactions between antitumor and antiviral immune cells.

While the field of oncolytic viral therapy is built upon the premise that a small initial inoculum will go through progressive rounds of viral replication in order to achieve tumor clearance, the work of Galivo et al. used a mutant form of VSV to modify this fundamental principle [72]. Rather than just relying on viral oncolysis, it is becoming increasingly apparent that antitumor immunity is a critical factor for VSV-mediated efficacy. This was clarified by Galivo et al. in which a single replication cycle VSV vector, but not a replication defective or UV inactivated virus, was found to achieve equal therapeutic efficacy compared to a fully replication competent VSV vector [72]. Thus, the ability of oncolytic VSV to proceed through multiple rounds of viral replication is unnecessary; rather, the immune response to intratumoral injection of a live, viable virus that is able to express its genome is the essential effector mechanism for tumor clearance [72]. Further supporting this premise, both viruses elicited a nearly identical NK and CD8 T-cell immune response, thereby confirming that antiviral immunity and the ability to elicit an acute proinflammatory response is an essential component for achieving antitumor efficacy that is seen in the B16 model [72].

An additional mechanistic component for VSV-mediated clearance of B16 is the role of IL-28 and NK cells in this process. In the study by Wongthida et al., IL-28 expression following VSV infection is identified as a key mediator of antitumor immunity [73]. Both the expression of IL-28 and the presence of its cognate receptor on B16 tumors were required for therapeutic efficacy. Moreover, IL-28-mediated activation of bone marrow cells to induce bystander cytotoxicity against B16 while maintaining an environment conducive for viral replication and spread [73]. Although IL-28 was produced from $GR1^+$ and $Macs3^+$ and not NK cells, depletion of NK1.1 cells eliminated cytotoxicity against B16 cells. It appears that this is coordinated through IL-28 induction of NK ligands on B16 cells and IL-28-mediated activation of IFN-γ production from NK cells (Table 1). Thus, IL-28 was identified as a novel mediator of NK cell activation that is essential for VSV therapeutic efficacy. Further work is needed to extrapolate these findings to other tumor models and oncolytic viral vectors; however, this initial report suggests that screening tumors for IL-28 receptor may represent a useful prognostic marker for predicting therapeutic response [73].

A recent study by Granot et al. has added additional evidence to the relevancy of NK cells in mediating viral

Table 1: Relevant NK receptor-ligand interactions in the field of virotherapy.

Treatment	Altered NK ligand or receptor	Reference
IL-28	↑ RAE-1	[73]
	↑ H60	[73]
	↑ MULT-1	[73]
Valproic acid	↓ NKp30 and NKp46	[75]
	↑ MICA/B	[76]
VSV+UL141	↓ CD155	[77]
HSV	↓ MICA	[78]
	↑ NCR ligands	[79]
Parvovirus	↑ CD155	[80]
ΔNS1 influenza type A	↑ Influenza hemagglutinin	[81]

mediated tumor clearance. Using a replication deficient Sindbis virus to treat SCID mice bearing ES-2 ovarian carcinoma xenografts, the authors found that tumor clearance was dependent upon the presence of functional NK cells [74]. In addition, the efficacy of a recombinant Sindbis/IL-2 expressing virus was dependent upon NK cells and IFN-γ production that ultimately mediated macrophage polarization towards an inflammatory M1 phenotype. These findings highlight the role of NK-mediated tumor clearance in immunodeficient mice treated with a replication defective viruses. It is important to interpret these findings in the context that viral replication was not a goal with this viral vector. Moreover, it is important to note that NK cells were the key antitumor mediator in the SCID mice used in the experiment. As a result, future work will need to discriminate (a) whether NK cell activity is still essential when viral replication is desired, and (b) the relative importance of NK cell coordinated adaptive antitumor immunity in immunocompetent models.

5.2. Pharmacologic Modulation of the Host Response to OV. While antitumor immunity can be a critical tool for OV efficacy, various pharmacologic cotherapies have also been used to counteract this response and synergize with OV therapy. For instance, the complement system is rapidly activated following viral infection and is intended to directly neutralize virus; consequently, abrogating this response could potentially enhance OV efficacy. This question was addressed by using cobra venom factor which depletes the C3 component of the complement system and was subsequently shown to enhance OV infection [82].

Cyclophosphamide (CPA) has also been used to attenuate antibody-mediated activation of the complement system, serum neutralization of virus, and reduce peripheral blood mononuclear counts that are responsible for producing an antiviral cytokine response [19]. Due to the pleiotropic immunosuppressive nature of CPA and its ability to halt the antiviral immune response, *in vivo* CPA treatment significantly reduced viral clearance and increased viral propagation while reducing immune cell filtration [19, 83]. Taken together, these findings suggest that targeting the immune response to OV therapy is a particularly useful modality towards achieving enhanced OV efficacy.

While angiogenesis provides resources to growing tumors, this increased vascularity is also associated with increased immune cell trafficking. As a result, antiangiogenic agents have the potential of reducing not only tumor growth but also the antiviral tumor microenvironment. For example, cilengitide is an antiangiogenic, cyclic RGD (cRGD) peptide that was originally identified as antagonists for the integrins $\alpha v\beta 3$ and $\alpha v\beta 5$ [84]. cRGD also limits leukocyte recruitment to sites of inflammation [85], reduces myeloid cell adhesion and transendothelial cell migration [86, 87]. When combined with OV, cRGD limited both OV-mediated inflammatory gene expression and CD45 leukocyte recruitment [18]. This reduced inflammatory response resulted in increased OV propagation *in vivo* and significantly enhanced therapeutic efficacy of OV in animals with intracranial tumors [18].

An additional pharmacologic approach that has been demonstrated to enhance OV therapy is the use of the histone deacetylase inhibitor valproic acid (VPA). VPA has been shown to enhance the efficacy of oncolytic HSV [88] through the inhibition of IFN-I, STAT-1, PKR, and PML signaling within infected glioblastoma cells. By targeting intracellular mediators that are responsible for creating an antiviral state [89], this therapeutic approach targets the antiviral host response prior to the recruitment of antiviral cellular mediators.

5.3. NK Cells and Antiviral Immunity. Despite a number of studies suggesting that natural killer cells are a critical component for achieving tumor clearance following oncolytic viral therapy, the deleterious nature of innate immunity has also been well documented with immunosuppressive cotherapies that enhance OV efficacy. Mathematical modeling has previously shown that the timing of the antiviral innate immune response to OV can be detrimental to therapy [90]. A variety of important changes associated with antiviral immunity occur in the tumor microenvironment in response to oHSV treatment of malignant GBM. These include profound increases in IFN-γ transcript and protein levels; upregulation of IFN-γ inducible chemokines; increased hyperpermeability with an associated inflammatory cell infiltrate; a rapid rise in an inflammatory transcriptome including type I interferon, TNFα, iNOS, and IL-15 [18, 32, 83, 91].

Studies in multiple tumor models and oncolytic viruses have observed a rapid decline in viral titers that occurs within days of inoculating various oncolytic viruses [32], suggesting that impediments are in place that limits successive rounds of viral replication. Using a GBM model, the clearance of over 80% of oHSV occurs in an IFN-γ dependent manner and corresponds with the rapid recruitment of NK cells and peripheral macrophages into the site of viral infection, suggesting that this response is a potential factor mediating oHSV clearance [64]. Moreover, transient immunomodulation with cyclophosphamide attenuated NK cell and macrophage recruitment to the site of oncolytic HSV-1 infection while resulting in profoundly increased viral titers and

tumor clearance. Apart from HSV-1-derived OV, CPA has also been shown to increase the oncolytic capacity of other OVs derived from HSV-2 [92], adenovirus [39], and reovirus [69, 93]. In fact, based on the promising preclinical results seen with CPA and OV, the combination of CPA with measles virus is currently being evaluated for safety and efficacy in human patients [94]. Similarly, clinical trials of reovirus with CPA (clinicatrials.gov, NCT01240538) and adenovirus with CPA (A. Hemminki, personal communication) are being conducted.

The finding that CPA enhances OV therapy through the suppression of immune cell recruitment was validated through a study demonstrating that macrophage depletion enhanced the efficacy of oHSV therapy in glioblastoma [91]. Clodronate encapsulated in liposomes is engulfed by phagocytic cells resulting in intracellular accumulation of apoptosis inducing clodronate [95]. When combined with OV therapy, CL-mediated depletion of peripheral phagocytic cells resulted in a 5-fold increase in OV titers in intracranial glioblastoma. While these findings partly recapitulated the effect of CPA on OV replication, they were unable to achieve enhanced *in vivo* survival demonstrated with CPA [91]. A potential reason for these findings may relate to the inability of clodronate to cross the blood brain barrier, thereby limiting its ability to deplete phagocytic microglial cells in addition to peripheral macrophages. Alternatively, macrophages may represent just one of multiple barriers present within the innate immune response to OV. In fact, a study by Breitbach et al. found that neutrophil depletion increased oncolytic viral titers and enhanced tumor clearance [96].

These findings suggest that OV replication may be limited by cellular components of the innate immune system shortly after viral infection. While we have previously demonstrated that microglia and macrophages play a critical role in limiting the therapeutic efficacy of OV [91], the role of NK cells has received less attention. In a series of two separate studies, Altomonte et al. detected NK cell infiltration into hepatocellular tumors treated with VSV and demonstrated a critical role for NK cell-mediated viral clearance in this model. First, intratumoral VSV replication and tumor killing was significantly enhanced in mice depleted of NK cells [97]. Second, a recombinant VSV encoding a viral chemokine binding protein (equine herpesvirus-1 glycoprotein G) attenuated NK cell recruitment, enhanced viral titers and tumor necrosis, and dramatically increased the overall survival of tumor-bearing mice compared to mice treated by parental VSV [97]. Due to the broad range of binding partners of the chemokine binding protein, an additional recombinant VSV encoding UL141 was created in order to more specifically inhibit NK cell recruitment and activation [77] (Table 1). UL141 is derived from human CMV that inhibits NK cell activation by blocking cell surface expression of CD155 on infected cells, thereby attenuating DNAM-1 mediating signaling on NK cells. In this study, rVSV-UL141 similarly enhanced virotherapy by specifically targeting the NK response to viral infection [77]. These series of findings suggest that NK cell recruitment and activation, at least in some models, can be detrimental to viral oncolysis and should be circumvented.

Based on findings that oHSV elicits brisk NK recruitment to the site of infection and CPA modulation of innate immunity enhances oHSV-mediated glioblastoma killing [18, 19, 32, 83, 90, 98], the functional relevance of these cells *in vivo* brain tumor models remains unclear and additional studies will be needed to determine this. The most recent clinical trial for oncolytic HSV in recurrent glioblastoma patients attempted to determine evidence of HSV replication and the extent of immune infiltration into the tumor microenvironment following OV administration [42]. While viral replication was observed in a portion of the patients enrolled in the study, there was significant variability in viral levels between patients. These findings are in contrast to the assumption in the field of OV therapy that even small initial inoculums should amplify significantly following successive rounds of viral replication [91, 99–101]. Additionally, immunohistochemical (IHC) analysis of tumor tissue demonstrated that G207 administration elicited a robust increase in CD3, CD8, CD20, and HAM56 staining. Additionally, testing of the genetically engineered mutant adenovirus (ONYX-015) in a phase I trial for glioblastoma provided similar results. Notably, when biopsies were taken from recurrent tumors, a significant inflammatory, mononuclear infiltrate was observed within the tumor microenvironment. Notably, in both of these studies, staining with the pan-NK maker CD56 was not included. These collective IHC findings are consistent with our preclinical findings [18, 32] and suggest that OV infection/replication causes the recruitment of inflammatory infiltrates into the site of viral infection. The nature of this histological feature is uncertain. For example, it is unclear whether these findings correlate with a potential benefit by stimulating an antitumor immune response or a detriment by eliminating OV initially after infection thereby preventing initial rounds of viral replication within the tumor. Taken together, the findings from these clinical trials demonstrate the need for a clearer understanding of the host-based factors and cellular mediators that are responsible for limiting viral infection, replication, and spread. By clarifying the host response to the virus, subsequent clinical trials can be designed to modulate these obstacles to viral propagation and achieve enhanced OV efficacy.

5.4. NK Cell Interactions with OV Infected Cells. Despite the conflicting viewpoints that NK cells are either a benefit or a hindrance to OV efficacy, most investigators would argue that NK cells and their place within innate immunity have a critical role in achieving success with this therapeutic modality [2]. As a result, the knowledge gained from uncovering the mechanistic signals governing NK-mediated recognition of OV infected cells has the potential of benefiting both schools of thought. For instance, in cases where the NK response is deleterious to OV efficacy, it may be necessary to design oncolytic viruses that express decoys or suppressors of NK activating ligands or combine the oncolytic virus with cotherapies that achieve similar NK avoidance of oHSV infected target cells. In tumors where NK-mediated killing is beneficial to OV therapy, the opposite approach could be used so that the oncolytic virus is combined with an

immunostimulatory agent that heightens the expression of critical NK activating ligands. In either case, it will be necessary to uncover the underlying signals that mediate NK recognition of OV infected tumor cells.

NK cells are able to carry out their diverse repertoire of activities through a detection system that relies on the engagement of a variety of cell surface activating and inhibitory receptors on NK cells that bind MHC class I and class I-like molecules (Figure 2). Through the binding of these receptors, a dynamic equilibrium is achieved that differentiates the recognition of "self" cells from malignantly or virally transformed target cells. While a number of viral vectors are being tested for the treatment of an equally diverse array of tumors, studying the NK response to oHSV infection of GBM, for example, can be extended to various virotherapy models. GBMs are readily killed by NK cells *in vitro* [102], and despite an intense immunosuppressive tumor microenvironment as the GBM progresses, a recent report found that peripheral NK numbers were not altered in patients with GBM [103]. NK cells have also been demonstrated to preferentially lyse virally infected target cells through either the elaboration of cytotoxic granules containing lytic enzymes or through the binding of apoptosis-inducing receptors on target cells [33]. Through the expression of both virally and tumor/stress associated ligands for NK activating receptors, a target cell such as an infected GBM can become susceptible to NK-mediated lysis. Countering this is the production of TGF-β and related molecules by the refractory GBM cells that impede both NK cell production of IFN-γ [104] and the ability of NK cells to directly lyse tumor cell targets [105].

Recent discoveries have explored the role of HSV-1 infection in modulating NK activating ligand expression. HSV-1 infection of fibroblast cells, through its immediate early gene product ICP0, resulted in increased susceptibility to NK-mediated lysis in an MHC-I independent fashion [79]. NK activation was achieved independently of the NK activating receptor NKG2D [79]. Rather, NK stimulation was elicited through the expression of an unknown ligand(s) of the NCRs on the cell surface of infected cells. Moreover, HSV infection was subsequently found to downmodulate NKG2D ligand expression due to late viral gene products [78] (Table 1). These findings demonstrate the modulation of ligands for NK activating receptors following wild type HSV infection; however, neither the identity of these ligands nor the nature of their cooperative binding to NK activating receptors is currently resolved.

Beyond cell-mediated viral clearance, NK cells are potent producers of IFN-γ [104]. In the context of oHSV therapy, the detrimental role of this antiviral cytokine has been uncovered through an IFN-γ depletion study that resulted in enhanced intratumoral viral titers [32]. Additionally, IFN-γ is vitally important in the activation of macrophages, thereby facilitating their ability to kill both viral and obligate intracellular pathogens [106]. Collectively, these findings demonstrate the critical nature of NK cells in coordinating the antiviral response to oHSV therapy.

A number of groups have also studied the ability of human glioblastomas to induce NK cell cytotoxicity. Based on their expression profile of ligands for activating NK receptors [102, 107], NK cells have been demonstrated to actively lyse a variety of glioblastoma cells in a NKp46 and DNAM-1 dependent manner [102].

The mechanism of NK-mediated cytotoxicity in other oncolytic viral models is also being explored. Recently, work by Bhat et al. found that oncolytic parvovirus infection of PDAC cell lines resulted in increased IFN-γ, TNFα, and MIP1a/b release from NK cells in coculture [80]. Moreover, parvovirus infection appeared to sensitize virally infected tumors cells to NK-mediated killing through the downregulation of MHC-I, enhanced expression of NK activating ligands such as CD155, and involvement of multiple NK activating receptors including NCRs, DNAM-1, and NKG2D [80]. Based on the findings that target cells infected with wild type influenza A virus (IAV) were exquisitely sensitive to NK-mediated lysis through NKp46 recognition of HA expressed on virally infected targets, an oncolytic IAV was developed. This novel virus lacks the NS1 gene and grows efficiently in IFN-resistant malignant cells with concomitant Ras overexpression [108]. Using the ΔNS1 IAV infected prostate cancer cells, Ogbomo et al. found that NK cells overrode MHC-I inhibition and exhibited rapid ERK activation, increased degranulation, and heightened NKp46-mediated target cell lysis compared to uninfected cells [81]. These findings suggest that second-generation versions of these viruses that coexpress an NK activating cytokine such as IL-2 or IL-12 may be a useful tool in activating NK cells *in vivo*, particularly in cases where the immunosuppressive environment of the tumor results in resistance to NK-mediated lysis.

By deciphering the critical receptor-ligand interactions that are responsible for NK activation following viral infection, this has the potential of paving the way for the development of novel therapeutic approaches. This can be achieved by either the selective blockade of NK receptors implicated in the anti-OV response or the development of ways to stimulate the critical receptor-ligand interactions that are essential for downstream immunotherapy.

6. Future Perspectives

Nearly two decades after the first published report of oncolytic viral therapy [40], investigators using these viruses have made remarkable progress in their preclinical testing and evaluation in clinical trials. Moreover, the recent approval of the H101 oncolytic adenovirus in China [109], the numerous clinical trials in place within the United States and Europe, and the acquisition of BioVex by Amgen suggest that virotherapy will gradually enter the armamentarium of tomorrow's physicians. In order to achieve widespread clinical applicability, however, certain obstacles must be overcome. For instance, infected cells have various antiviral defense mechanisms that must be circumvented to achieve sustained viral replication; however, circumventing this response must be countered with concerns about uncontrolled viral replication and toxicity. Additionally, host immunity, particularly innate immunity, is a first

line of defense against foreign pathogens that has been demonstrated to impede virotherapy; however, immune suppression potentially impedes the antitumor immune response that has been shown to synergize with viral oncolysis. Lastly, coadministration of pharmacological agents that cooperate with viral mediated tumor clearance shows significant promise; however, the comparative effectiveness of treating various tumors with the appropriate virus/drug combination must be thoroughly evaluated in clinical trials.

In recent years, significant attention has been directed towards the host immune response to oncolytic viruses. In particular, the role of initial immune responder cells, including NK cells, macrophages, and neutrophils, has been questioned. With their antiviral and antitumor properties combined with their ability to mediate macrophage activation, the NK cell response to virotherapy has elicited significant attention.

Despite recent progress, there are certain challenges that should be addressed in order to expand our knowledge of the NK response to OV therapy. To date, limitations in mouse strain susceptibility to certain oncolytic viral vectors have impeded the ability to evaluate the host immune response in fully immunocompetent animal models [110, 111]. Thus, there is an increasing need to study the NK response to OV in syngeneic, orthotopic tumor models that include the presence of tumor initiating cells and recapitulate the immunosuppressive tumor microenvironment that is frequently found in human cancers.

In order to fully investigate the role of NK cells following OV infection, future studies should also attempt to test OV in a syngeneic mouse model with specific NK deficiencies. By testing for OV efficacy and downstream immune cell activation, including macrophage and T-cell polarization, in each of these groups, it would be possible to delineate the critical NK components in antiviral and antitumor immunity to OV. Additionally, NKp46 is the only NCR present on murine NK cells. As a result, we are limited in our ability to test the significance of NKp44 and NKp30 against OV *in vivo*. To circumvent this problem, it would be possible to evaluate OV in the context of a humanized mouse model [112] where NKp46, NKp44, and NKp30 are expressed.

While a variety of cotherapies have been shown cooperate with viral oncolysis through immune cell suppression, additional work can be done to fine-tune this approach. For instance, it is shortsighted to think that antitumor immunity has no part in viral oncolysis. Rather, future work must identify the delicate balance between an initial suppression of antiviral immunity that facilitates initial rounds of viral replication and a downstream stimulation of antitumor immunity against tumor or viral antigens. This could potentially take the form of targeting NK cell depletion in a temporal manner whereby NK cells are attenuated initially after viral infection and then allowed to repopulate the site of infection a few days after viral inoculation. This later response would take advantage of their antitumor properties, macrophage activating properties, and their ability to induce an antitumor T-cell response.

An additional approach could focus on M1/M2 macrophage polarization following OV infection. Clinical reports have confirmed that tumors, such as glioblastoma, are typically associated with generalized immunosuppression, TGF-β production, and an M2 macrophage phenotype [103]. oHSV inoculation elicits an M1 macrophage response that is detrimental to OV efficacy [91]. Taken together, future studies could attempt to discern whether a temporary maintenance of the M2 phenotype initially after infection followed by a switch towards an inflammatory M1 state is effective at initially enhancing viral titers while eliciting antitumor immunity at later timepoints.

The role of NK receptor-ligand interactions deserves further exploration. Future studies should investigate the identity of the NK receptor ligands in virally infected cells; whether the ligands are viral in origin or expressed following cellular stress; whether they are expressed following infection with other oncolytic viral vectors. The identifying of ligands expressed after OV infection will be useful on multiple fronts. First it could potentially be targeted to enhance viral efficacy. For instance, if NKp30 and NKp46 are key receptors mediating premature viral clearance, suppressing the expression of these ligands could be particularly advantageous. Targeted suppression could be achieved through either pharmacologic means or through the creation of a novel oHSV that expresses a decoy for NKp30/NKp46 or inhibits ligand presentation on the infected cell surface. Second, while NK killing of virally infected cells may be deleterious for viral replication, it represents a novel target for mediating antitumor immunity. As a result, in instances where NK-mediated antitumor immunity is deemed beneficial, such as later time points after infection once productive viral replication is established, eliciting NKp30/NKp46-mediated tumor killing could be pursued as a viable therapeutic option.

With a number of oncolytic viral clinical trials in the pipeline, it will be critical for investigators to include the evaluation of NK cells in the immunological response to viral administration. Attention should be directed towards NK cell numbers in the periphery, within the tumor microenvironment and their distribution within the virally infected tumor. Moreover, the activation/developmental state of these NK cells should be evaluated. For instance, NK cells should be tested for CD56 and CD94 expression, whether the recruited/circulating NK cells are NCR^{bright} or NCR^{dim}, and their functional capacity. By collecting this data from human samples, we can test the validity of our preclinical models and guide future experimental trials.

References

[1] C. Alvarez-Breckenridge, B. Kaur, and E. A. Chiocca, "Pharmacologic and chemical adjuvants in tumor virotherapy," *Chemical Reviews*, vol. 109, no. 7, pp. 3125–3140, 2009.

[2] R. J. Prestwich, F. Errington, R. M. Diaz et al., "The case of oncolytic viruses vs the immune system: waiting on the judgment of Solomon," *Human Gene Therapy*, vol. 20, no. 10, pp. 1119–1132, 2009.

[3] J. S. Orange, "Human natural killer cell deficiencies," *Current Opinion in Allergy and Clinical Immunology*, vol. 6, no. 6, pp. 399–409, 2006.

[4] J. S. Orange, "Human natural killer cell deficiencies and susceptibility to infection," *Microbes and Infection*, vol. 4, no. 15, pp. 1545–1558, 2002.

[5] C. A. Biron, K. S. Byron, and J. L. Sullivan, "Severe herpesvirus infections in an adolescent without natural killer cells," *The New England Journal of Medicine*, vol. 320, no. 26, pp. 1731–1735, 1989.

[6] S. Coca, J. Perez-Piqueras, D. Martinez et al., "The prognostic significance of intratumoral natural killer cells in patients with colerectal carcinoma," *Cancer*, vol. 79, no. 12, pp. 2320–2328, 1997.

[7] S. Ishigami, S. Natsugoe, K. Tokuda et al., "Prognostic value of intratumoral natural killer cells in gastric carcinoma," *Cancer*, vol. 88, no. 3, pp. 577–583, 2000.

[8] I. Takanami, K. Takeuchi, and M. Giga, "The prognostic value of natural killer cell infiltration in resected pulmonary adenocarcinoma," *Journal of Thoracic and Cardiovascular Surgery*, vol. 121, no. 6, pp. 1058–1063, 2001.

[9] F. R. Villegas, S. Coca, V. G. Villarrubia et al., "Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer," *Lung Cancer*, vol. 35, pp. 23–28, 2002.

[10] S. Kim, K. Iizuka, H. S. P. Kang et al., "In vivo developmental stages in murine natural killer cell maturation," *Nature Immunology*, vol. 3, no. 6, pp. 523–528, 2002.

[11] N. Anfossi, P. Andre, S. Guia et al., "Human NK cell education by inhibitory receptors for MHC class I," *Immunity*, vol. 25, no. 2, pp. 331–342, 2006.

[12] M. A. Cooper, T. A. Fehniger, and M. A. Caligiuri, "The biology of human natural killer-cell subsets," *Trends in Immunology*, vol. 22, no. 11, pp. 633–640, 2001.

[13] G. Ferlazzo and C. Munz, "NK cell compartments and their activation by dendritic cells," *Journal of Immunology*, vol. 172, no. 3, pp. 1333–1339, 2004.

[14] Y. Hayakawa and M. J. Smyth, "CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity," *Journal of Immunology*, vol. 176, no. 3, pp. 1517–1524, 2006.

[15] E. Vivier, E. Tomasello, M. Baratin, T. Walzer, and S. Ugolini, "Functions of natural killer cells," *Nature Immunology*, vol. 9, no. 5, pp. 503–510, 2008.

[16] T. Walzer, M. Dalod, S. H. Robbins, L. Zitvogel, and E. Vivier, "Natural-killer cells and dendritic cells: 'l'union fait la force'," *Blood*, vol. 106, no. 7, pp. 2252–2258, 2005.

[17] M. Lucas, W. Schachterle, K. Oberle, P. Aichele, and A. Diefenbach, "Dendritic cells prime natural killer cells by trans-presenting interleukin 15," *Immunity*, vol. 26, no. 4, pp. 503–517, 2007.

[18] K. Kurozumi, J. Hardcastle, R. Thakur et al., "Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy," *Journal of the National Cancer Institute*, vol. 99, no. 23, pp. 1768–1781, 2007.

[19] H. Wakimoto, G. Fulci, E. Tyminski, and E. A. Chiocca, "Altered expression of antiviral cytokine mRNAs associated with cyclophosphamide's enhancement of viral oncolysis," *Gene Therapy*, vol. 11, no. 2, pp. 214–223, 2004.

[20] T. Walzer, M. Blery, J. Chaix et al., "Identification, activation, and selective in vivo ablation of mouse NK cells via NKp46," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 9, pp. 3384–3389, 2007.

[21] M. Vitale, C. Bottino, S. Sivori et al., "NKp44, a novel triggering surface molecule specifically expressed by activated natural killer cells, is involved in non-major histocompatibility complex-restricted tumor cell lysis," *Journal of Experimental Medicine*, vol. 187, no. 12, pp. 2065–2072, 1998.

[22] A. Moretta, C. Bottino, M. Vitale et al., "Activating receptors and coreceptors involved in human natural killer cell-mediated cytolysis," *Annual Review of Immunology*, vol. 19, pp. 197–223, 2001.

[23] T. I. Arnon, G. Markel, and O. Mandelboim, "Tumor and viral recognition by natural killer cells receptors," *Seminars in Cancer Biology*, vol. 16, no. 5, pp. 348–358, 2006.

[24] N. Bloushtain, U. Qimron, A. Bar-Ilan et al., "Membrane-associated heparan sulfate proteoglycans are involved in the recognition of cellular targets by NKp30 and NKp46," *Journal of Immunology*, vol. 173, no. 4, pp. 2392–2401, 2004.

[25] G. Ferlazzo, M. L. Tsang, L. Moretta, G. Melioli, R. M. Steinman, and C. Munz, "Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells," *Journal of Experimental Medicine*, vol. 195, no. 3, pp. 343–351, 2002.

[26] R. B. Mailliard, Y. I. Son, R. Redlinger et al., "Dendritic cells mediate NK cell help for Th1 and CTL responses: two-signal requirement for the induction of NK cell helper function," *Journal of Immunology*, vol. 171, no. 5, pp. 2366–2373, 2003.

[27] T. I. Arnon, H. Achdout, O. Levi et al., "Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus," *Nature Immunology*, vol. 6, no. 5, pp. 515–523, 2005.

[28] M. A. Degli-Esposti and M. J. Smyth, "Close encounters of different kinds: dendritic cells and NK cells take centre stage," *Nature Reviews Immunology*, vol. 5, no. 2, pp. 112–124, 2005.

[29] C. S. Brandt, M. Baratin, E. C. Yi et al., "The B7 family member B7-H6 is a tumor cell ligand for the activating natural killer cell receptor NKp30 in humans," *Journal of Experimental Medicine*, vol. 206, no. 7, pp. 1495–1503, 2009.

[30] E. P. von Strandmann, V. R. Simhadri, B. von Tresckow et al., "Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells," *Immunity*, vol. 27, no. 6, pp. 965–974, 2007.

[31] K. Shinozaki, O. Ebert, C. Kournioti, Y. S. Tai, and S. L. C. Woo, "Oncolysis of multifocal hepatocellular carcinoma in the rat liver by hepatic artery infusion of vesicular stomatitis virus," *Molecular Therapy*, vol. 9, no. 3, pp. 368–376, 2004.

[32] G. Fulci, L. Breymann, D. Gianni et al., "Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 34, pp. 12873–12878, 2006.

[33] C. A. Biron, K. B. Nguyen, G. C. Pien, L. P. Cousens, and T. P. Salazar-Mather, "Natural killer cells in antiviral defense: function and regulation by innate cytokines," *Annual Review of Immunology*, vol. 17, pp. 189–220, 1999.

[34] B. D. Anderson, T. Nakamura, S. J. Russell, and K. W. Peng, "High CD46 receptor density determines preferential killing of tumor cells by oncolytic measles virus," *Cancer Research*, vol. 64, no. 14, pp. 4919–4926, 2004.

[35] L. Baraz, E. Khazanov, R. Condiotti, M. Kotler, and A. Nagler, "Natural killer (NK) cells prevent virus production in cell culture," *Bone Marrow Transplantation*, vol. 24, no. 2, pp. 179–189, 1999.

[36] M. A. Cooper, T. A. Fehniger, S. C. Turner et al., "Human natural killer cells: a unique innate immunoregulatory role for the CD56(bright) subset," *Blood*, vol. 97, no. 10, pp. 3146–3151, 2001.

[37] W. F. Hickey, "Leukocyte traffic in the central nervous system: the participants and their roles," *Seminars in Immunology*, vol. 11, no. 2, pp. 125–137, 1999.

[38] D. L. Thomas and N. W. Fraser, "HSV-1 therapy of primary tumors reduces the number of metastases in an immune-competent model of metastatic breast cancer," *Molecular Therapy*, vol. 8, no. 4, pp. 543–551, 2003.

[39] M. L. M. Lamfers, G. Fulci, D. Gianni et al., "Cyclophosphamide increases transgene expression mediated by an oncolytic adenovirus in glioma-bearing mice monitored by bioluminescence imaging," *Molecular Therapy*, vol. 14, no. 6, pp. 779–788, 2006.

[40] R. L. Martuza, A. Malick, J. M. Markert, K. L. Ruffner, and D. M. Coen, "Experimental therapy of human glioma by means of a genetically engineered virus mutant," *Science*, vol. 252, no. 5007, pp. 854–856, 1991.

[41] G. Fulci and E. A. Chiocca, "Oncolytic viruses for the therapy of brain tumors and other solid malignancies: a review," *Frontiers in Bioscience*, vol. 8, pp. e346–e360, 2003.

[42] J. M. Markert, P. G. Liechty, W. Wang et al., "Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM," *Molecular Therapy*, vol. 17, no. 1, pp. 199–207, 2008.

[43] R. M. Diaz, F. Galivo, T. Kottke et al., "Oncolytic immunovirotherapy for melanoma using vesicular stomatitis virus," *Cancer Research*, vol. 67, no. 6, pp. 2840–2848, 2007.

[44] E. de Vries, H. R. Koene, J. M. Vossen et al., "Identification of an unusual Fc gamma receptor IIIa (CD16) on natural killer cells in a patient with recurrent infections," *Blood*, vol. 88, no. 8, pp. 3022–3027, 1996.

[45] S. Jawahar, C. Moody, M. Chan, R. Finberg, R. Geha, and T. Chatila, "Natural killer (NK) cell deficiency associated with an epitope-deficient Fc receptor type IIIA (CD16-II)," *Clinical and Experimental Immunology*, vol. 103, no. 3, pp. 408–413, 1996.

[46] C. Lopez, D. Kirkpatrick, S. E. Read et al., "Correlation between low natural killing of fibroblasts infected with herpes simplex virus type 1 and susceptibility to herpesvirus infections," *The Journal of Infectious Diseases*, vol. 147, pp. 1030–1035, 1983.

[47] C. Klein, N. Philippe, F. Le Deist et al., "Partial albinism with immunodeficiency (Griscelli syndrome)," *Journal of Pediatrics*, vol. 125, no. 6 I, pp. 886–895, 1994.

[48] A. J. Mancini, L. S. Chan, and A. S. Paller, "Partial albinism with immunodeficiency: Griscelli syndrome: report of a case and review of the literature," *Journal of the American Academy of Dermatology*, vol. 38, no. 2, pp. 295–300, 1998.

[49] A. Plebani, B. Ciravegna, M. Ponte, M. C. Mingari, and L. Moretta, "Intefieukin-2 mediated restoration of natural killer cell function in a patient with griscelli syndrome," *European Journal of Pediatrics*, vol. 159, no. 9, pp. 713–714, 2000.

[50] D. C. Anderson, F. C. Schmalsteig, M. J. Finegold et al., "The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features," *The Journal of Infectious Diseases*, vol. 152, pp. 668–689, 1985.

[51] Y. L. Lau, L. C. K. Low, B. M. Jones, and J. W. M. Lawton, "Defective neutrophil and lymphocyte function in leucocyte adhesion deficiency," *Clinical and Experimental Immunology*, vol. 85, no. 2, pp. 202–208, 1991.

[52] T. K. Kishimoto, N. Hollander, T. M. Roberts, D. C. Anderson, and T. A. Springer, "Heterogeneous mutations in the beta subunit common to the LFA-1, Mac-1, and p150,95 glycoproteins cause leukocyte adhesion deficiency," *Cell*, vol. 50, no. 2, pp. 193–202, 1987.

[53] K. Shibuya, L. L. Lanier, J. H. Phillips et al., "Physical and functional association of LFA-1 with DNAM-1 adhesion molecule," *Immunity*, vol. 11, no. 5, pp. 615–623, 1999.

[54] F. Ghiringhelli, L. Apetoh, F. Housseau, G. Kroemer, and L. Zitvogel, "Links between innate and cognate tumor immunity," *Current Opinion in Immunology*, vol. 19, no. 2, pp. 224–231, 2007.

[55] I. Waldhauer and A. Steinle, "NK cells and cancer immunosurveillance," *Oncogene*, vol. 27, no. 45, pp. 5932–5943, 2008.

[56] P. A. Albertsson, P. H. Basse, M. Hokland et al., "NK cells and the tumour microenvironment: implications for NK-cell function and anti-tumour activity," *Trends in Immunology*, vol. 24, no. 11, pp. 603–609, 2003.

[57] G. Esendagli, K. Bruderek, T. Goldmann et al., "Malignant and non-malignant lung tissue areas are differentially populated by natural killer cells and regulatory T cells in non-small cell lung cancer," *Lung Cancer*, vol. 59, no. 1, pp. 32–40, 2008.

[58] E. J. Shillitoe and C. Pellenz, "Factors that limit the effectiveness of herpes simplex virus type 1 for treatment of oral cancer in mice," *Clinical Cancer Research*, vol. 11, no. 8, pp. 3109–3116, 2005.

[59] B. G. Derubertis, B. M. Stiles, A. Bhargava et al., "Cytokine-secreting herpes viral mutants effectively treat tumor in a murine metastatic colorectal liver model by oncolytic and T-cell-dependent mechanisms," *Cancer Gene Therapy*, vol. 14, no. 6, pp. 590–597, 2007.

[60] C. G. Drake, "Prostate cancer as a model for tumour immunotherapy," *Nature Reviews Immunology*, vol. 10, no. 8, pp. 580–593, 2010.

[61] J. W. Vieweg, "Prostate cancer: targeting complexity," *Current Opinion in Urology*, vol. 18, no. 3, pp. 261–262, 2008.

[62] S. A. Gujar, D. A. Pan, P. Marcato, K. A. Garant, and P. W. Lee, "Oncolytic virus-initiated protective immunity against prostate cancer," *Molecular Therapy*, vol. 19, pp. 797–804, 2008.

[63] C. G. Miller and N. W. Fraser, "Requirement of an integrated immune response for successful neuroattenuated HSV-1 therapy in an intracranial metastatic melanoma model," *Molecular Therapy*, vol. 7, no. 6, pp. 741–747, 2003.

[64] F. Benencia, M. C. Courrèges, J. R. Conejo-Garcia et al., "HSV oncolytic therapy upregulates interferon-inducible chemokines and recruits immune effector cells in ovarian cancer," *Molecular Therapy*, vol. 12, no. 5, pp. 789–802, 2005.

[65] C. L. White, K. R. Twigger, L. Vidal et al., "Characterization of the adaptive and innate immune response to intravenous oncolytic reovirus (dearing type 3) during a phase I clinical trial," *Gene Therapy*, vol. 15, no. 12, pp. 911–920, 2008.

[66] T. Kottke, F. Galivo, P. Wongthida et al., "Treg depletion-enhanced IL-2 treatment facilitates therapy of established tumors using systemically delivered oncolytic virus," *Molecular Therapy*, vol. 16, no. 7, pp. 1217–1226, 2008.

[67] R. Baluna and E. S. Vitetta, "Vascular leak syndrome: a side effect of immunotherapy," *Immunopharmacology*, vol. 37, no. 2-3, pp. 117–132, 1997.

[68] S. A. Rosenberg, J. J. Mule, P. J. Spiess, C. M. Reichert, and S. L. Schwarz, "Regression of established pulmonary metastases and subcutaneous tumor mediated by the systemic administration of high-dose recombinant interleukin 2," *Journal of Experimental Medicine*, vol. 161, no. 5, pp. 1169–1188, 1985.

[69] T. Kottke, J. Thompson, R. M. Diaz et al., "Improved systemic delivery of oncolytic reovirus to established tumors using preconditioning with cyclophosphamide-mediated

treg modulation and interleukin-2," *Clinical Cancer Research*, vol. 15, no. 2, pp. 561–569, 2009.

[70] N. Matsushita, S. A. Pilon-Thomas, L. M. Martin, and A. I. Riker, "Comparative methodologies of regulatory T cell depletion in a murine melanoma model," *Journal of Immunological Methods*, vol. 333, no. 1-2, pp. 167–179, 2008.

[71] M. E. C. Lutsiak, R. T. Semnani, R. De Pascalis, S. V. S. Kashmiri, J. Schlom, and H. Sabzevari, "Inhibition of CD4+25+ T regulatory cell function implicated in enhanced immune response by low-dose cyclophosphamide," *Blood*, vol. 105, no. 7, pp. 2862–2868, 2005.

[72] F. Galivo, R. M. Diaz, P. Wongthida et al., "Single-cycle viral gene expression, rather than progressive replication and oncolysis, is required for VSV therapy of B16 melanoma," *Gene Therapy*, vol. 17, no. 2, pp. 158–170, 2010.

[73] P. Wongthida, R. M. Diaz, F. Galivo et al., "Type III IFN interleukin-28 mediates the antitumor efficacy of oncolytic virus VSV in immune-competent mouse models of cancer," *Cancer Research*, vol. 70, no. 11, pp. 4539–4549, 2010.

[74] T. Granot, L. Venticinque, J. C. Tseng, and D. Meruelo, "Activation of cytotoxic and regulatory functions of NK cells by Sindbis viral vectors," *PLoS One*, vol. 6, no. 6, Article ID e20598, 2011.

[75] H. Ogbomo, M. Michaelis, J. Kreuter, H. W. Doerr, and J. Cinatl, "Histone deacetylase inhibitors suppress natural killer cell cytolytic activity," *FEBS Letters*, vol. 581, no. 7, pp. 1317–1322, 2007.

[76] S. Armeanu, M. Bitzer, U. M. Lauer et al., "Natural killer cell-mediated lysis of hepatoma cells via specific induction of NKG2D ligands by the histone deacetylase inhibitor sodium valproate," *Cancer Research*, vol. 65, no. 14, pp. 6321–6329, 2005.

[77] J. Altomonte, L. Wu, M. Meseck et al., "Enhanced oncolytic potency of vesicular stomatitis virus through vector-mediated inhibition of NK and NKT cells," *Cancer Gene Therapy*, vol. 16, no. 3, pp. 266–278, 2009.

[78] D. Schepis, M. D'Amato, M. Studahl, T. Bergstrom, K. Karre, and L. Berg, "Herpes simplex virus infection downmodulates NKG2D ligand expression," *Scandinavian Journal of Immunology*, vol. 69, pp. 429–436, 2009.

[79] S. E. Chisholm, K. Howard, M. V. Gómez, and H. T. Reyburn, "Expression of ICP0 is sufficient to trigger natural killer cell recognition of herpes simplex virus-infected cells by natural cytotoxicity receptors," *Journal of Infectious Diseases*, vol. 195, no. 8, pp. 1160–1168, 2007.

[80] R. Bhat, S. Dempe, C. Dinsart, and J. Rommelaere, "Enhancement of NK cell anti-tumour responses using an oncolytic parvovirus," *International Journal of Cancer*, vol. 128, no. 4, pp. 908–919, 2011.

[81] H. Ogbomo, M. Michaelis, J. Geiler et al., "Tumor cells infected with oncolytic influenza A virus prime natural killer cells for lysis of resistant tumor cells," *Medical Microbiology and Immunology*, vol. 199, pp. 93–101, 2010.

[82] K. Ikeda, H. Wakimoto, T. Ichikawa et al., "Complement depletion facilitates the infection of multiple brain tumors by an intravascular, replication-conditional herpes simplex virus mutant," *Journal of Virology*, vol. 74, no. 10, pp. 4765–4775, 2000.

[83] K. Ikeda, T. Ichikawa, H. Wakimoto et al., "Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses," *Nature Medicine*, vol. 5, no. 8, pp. 881–887, 1999.

[84] W. Arap, R. Pasqualini, and E. Ruoslahti, "Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model," *Science*, vol. 279, no. 5349, pp. 377–380, 1998.

[85] S. M. Wahl, J. B. Allen, K. L. Hines et al., "Synthetic fibronectin peptides suppress arthritis in rats by interrupting leukocyte adhesion and recruitment," *Journal of Clinical Investigation*, vol. 94, no. 2, pp. 655–662, 1994.

[86] S. Elitok, S. V. Brodsky, D. Patschan et al., "Cyclic arginine-glycine-aspartic acid peptide inhibits macrophage infiltration of the kidney and carotid artery lesions in apo-E-deficient mice," *American Journal of Physiology—Renal Physiology*, vol. 290, no. 1, pp. F159–F166, 2006.

[87] X. Zhang, A. Chen, D. De Leon et al., "Atomic force microscopy measurement of leukocyte-endothelial interaction," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 286, no. 1, pp. H359–H367, 2004.

[88] A. Otsuki, A. Patel, K. Kasai et al., "Histone deacetylase inhibitors augment antitumor efficacy of herpes-based oncolytic viruses," *Molecular Therapy*, vol. 16, no. 9, pp. 1546–1555, 2008.

[89] M. G. Katze, Y. He, and M. Gale Jr., "Viruses and interferon: a fight for supremacy," *Nature Reviews Immunology*, vol. 2, no. 9, pp. 675–687, 2002.

[90] A. Friedman, J. P. Tian, G. Fulci, E. A. Chiocca, and J. Wang, "Glioma virotherapy: effects of innate immune suppression and increased viral replication capacity," *Cancer Research*, vol. 66, no. 4, pp. 2314–2319, 2006.

[91] G. Fulci, N. Dmitrieva, D. Gianni et al., "Depletion of peripheral macrophages and brain microglia increases brain tumor titers of oncolytic viruses," *Cancer Research*, vol. 67, no. 19, pp. 9398–9406, 2007.

[92] H. Li, Z. Zeng, X. Fu, and X. Zhang, "Coadministration of a herpes simplex virus-2-based oncolytic virus and cyclophosphamide produces a synergistic antitumor effect and enhances tumor-specific immune responses," *Cancer Research*, vol. 67, no. 16, pp. 7850–7855, 2007.

[93] J. Qiao, H. Wang, T. Kottke et al., "Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus," *Clinical Cancer Research*, vol. 14, no. 1, pp. 259–269, 2008.

[94] R. M. Myers, S. M. Greiner, M. E. Harvey et al., "Preclinical pharmacology and toxicology of intravenous MV-NIS, an oncolytic measles virus administered with or without cyclophosphamide," *Clinical Pharmacology and Therapeutics*, vol. 82, no. 6, pp. 700–710, 2007.

[95] N. Van Rooijen and A. Sanders, "Liposome mediated depletion of macropbages: mechanism of action, preparation of liposomes and applications," *Journal of Immunological Methods*, vol. 174, no. 1-2, pp. 83–93, 1994.

[96] C. J. Breitbach, J. M. Paterson, C. G. Lemay et al., "Targeted inflammation during oncolytic virus therapy severely compromises tumor blood flow," *Molecular Therapy*, vol. 15, no. 9, pp. 1686–1693, 2007.

[97] J. Altomonte, L. Wu, L. Chen et al., "Exponential enhancement of oncolytic vesicular stomatitis virus potency by vector-mediated suppression of inflammatory responses in vivo," *Molecular Therapy*, vol. 16, no. 1, pp. 146–153, 2008.

[98] H. Kambara, Y. Saeki, and E. A. Chiocca, "Cyclophosphamide allows for in vivo dose reduction of a potent oncolytic virus," *Cancer Research*, vol. 65, no. 24, pp. 11255–11258, 2005.

[99] T. Todo, S. D. Rabkin, P. Sundaresan et al., "Systemic antitumor immunity in experimental brain tumor therapy using a multimutated, replication-competent herpes simplex

virus," *Human Gene Therapy*, vol. 10, no. 17, pp. 2741–2755, 1999.

[100] M. Toda, S. D. Rabkin, H. Kojima, and R. L. Martuza, "Herpes simplex virus as an in situ cancer vaccine for the induction of specific anti-tumor immunity," *Human Gene Therapy*, vol. 10, no. 3, pp. 385–393, 1999.

[101] K. Nakano, T. Todo, K. Chijiiwa, and M. Tanaka, "Therapeutic efficacy of G207, a conditionally replicating herpes simplex virus type 1 mutant, for gallbladder carcinoma in immunocompetent hamsters," *Molecular Therapy*, vol. 3, no. 4, pp. 431–437, 2001.

[102] R. Castriconi, A. Daga, A. Dondero et al., "NK cells recognize and kill human glioblastoma cells with stem cell-like properties," *Journal of Immunology*, vol. 182, no. 6, pp. 3530–3539, 2009.

[103] K. Gousias, M. Markou, V. Arzoglou et al., "Frequent abnormalities of the immune system in gliomas and correlation with the WHO grading system of malignancy," *Journal of Neuroimmunology*, vol. 226, no. 1-2, pp. 136–142, 2010.

[104] J. Yu, M. Wei, B. Becknell et al., "Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells," *Immunity*, vol. 24, no. 5, pp. 575–590, 2006.

[105] R. Trotta, J. Dal Col, J. Yu et al., "TGF-beta utilizes SMAD3 to inhibit CD16-mediated IFN-gamma production and antibody-dependent cellular cytotoxicity in human NK cells," *Journal of Immunology*, vol. 181, no. 6, pp. 3784–3792, 2008.

[106] D. M. Paulnock, K. P. Demick, and S. P. Coller, "Analysis of interferon-gamma-dependent and -independent pathways of macrophage activation," *Journal of Leukocyte Biology*, vol. 67, no. 5, pp. 677–682, 2000.

[107] A. Wu, S. Wiesner, J. Xiao et al., "Expression of MHC I and NK ligands on human CD133 + glioma cells: possible targets of immunotherapy," *Journal of Neuro-Oncology*, vol. 83, no. 2, pp. 121–131, 2007.

[108] M. Bergmann, I. Romirer, M. Sachet et al., "A genetically engineered influenza A virus with ras-dependent oncolytic properties," *Cancer Research*, vol. 61, no. 22, pp. 8188–8193, 2001.

[109] K. Garber, "China approves world's first oncolytic virus therapy for cancer treatment," *Journal of the National Cancer Institute.*, vol. 98, no. 5, pp. 298–300, 2006.

[110] L. F. Kastrukoff, A. S. Lau, F. Takei et al., "Redundancy in the immune system restricts the spread of HSV-1 in the central nervous system (CNS) of C57BL/6 mice," *Virology*, vol. 400, no. 2, pp. 248–258, 2010.

[111] P. Lundberg, C. Ramakrishna, J. Brown et al., "The immune response to herpes simplex virus type 1 infection in susceptible mice is a major cause of central nervous system pathology resulting in fatal encephalitis," *Journal of Virology*, vol. 82, no. 14, pp. 7078–7088, 2008.

[112] E. A. Pek, T. Chan, S. Reid, and A. A. Ashkar, "Characterization and IL-15 dependence of NK cells in humanized mice," *Immunobiology*, vol. 216, no. 1-2, pp. 218–224, 2011.

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 798526, 15 pages
doi:10.1155/2012/798526

Review Article

Retargeting of Viruses to Generate Oncolytic Agents

M. H. Verheije[1] and P. J. M. Rottier[2]

[1] *Pathology Division, Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands*
[2] *Virology Division, Department of Infectious Diseases & Immunology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 1, 3584 CL Utrecht, The Netherlands*

Correspondence should be addressed to M. H. Verheije, m.h.verheije@uu.nl

Received 30 June 2011; Revised 25 August 2011; Accepted 26 August 2011

Academic Editor: R. Mark L. Buller

Oncolytic virus therapy is based on the ability of viruses to effectively infect and kill tumor cells without destroying the normal tissues. While some viruses seem to have a natural preference for tumor cells, most viruses require the modification of their tropism to specifically enter and replicate in such cells. This review aims to describe the transductional targeting strategies currently employed to specifically redirect viruses towards surface receptors on tumor cells. Three major strategies can be distinguished; they involve (i) the incorporation of new targeting specificity into a viral surface protein, (ii) the incorporation of a scaffold into a viral surface protein to allow the attachment of targeting moieties, and (iii) the use of bispecific adapters to mediate targeting of a virus to a specified moiety on a tumor cell. Of each strategy key features, advantages and limitations are discussed and examples are given. Because of their potential to cause sustained, multiround infection—a desirable characteristic for eradicating tumors—particular attention is given to viruses engineered to become self-targeted by the genomic expression of a bispecific adapter protein.

1. Introduction

Cancer is one of the major health problems of our times. Though the prognosis for people diagnosed with, at least some forms of, cancer has increased considerably, it is more typical a disease of which treatment is initially effective, to be followed later by an irreversible and eventually fatal relapse. Already for decades, cancer treatment is based on three types of approaches: surgery, radio-, and chemotherapy. While the scientific and technological advancements have improved the efficacy of each of these classical approaches tremendously, and while also some new therapies have evolved including immunotherapy, the treatments apparently fail to eradicate all residual tumor cells or metastases completely. Therefore, additional means are urgently required to support or replace the conventional therapies. Hence, a variety of new approaches is currently being explored, one of which is based on the use of viruses.

Oncolytic viruses are defined by their ability to specifically kill tumor cells, but to leave the normal tissues unharmed. Their most characteristic features, thus, are their target specificity and their cytolytic capacity. Ideally, they exhibit additional features including, but not limited to, a high reproductive capacity *in vivo*, the ability to recruit uninfected neighboring cells (syncytia formation), the ability to infect both dividing and nondividing cells, a high stability *in vivo*, the inability of chromosomal integration, the lack of disease induction, and the general absence of preexisting antibodies to the virus in the host population.

Infection of cells by viruses primarily depends on their successful entry of these cells. As a first step, virus-binding to the cell relies on the specific interaction between the viral attachment protein(s) and the cellular receptor(s). Only very few viruses have a natural preference for replication in tumor cells. Some acquired such tropism by serial passage in cell culture cells; examples include measles virus, mumps virus and Newcastle disease virus (recently reviewed in [1]), vesicular stomatitis virus [2], and reovirus [3]. Many viruses, however, lack the means for selective binding to tumor cell epitopes. To adapt these viruses for oncolytic therapy, their

natural tropism needs to be altered to allow binding to tumor-specific receptors, an approach called transductional targeting.

This review outlines the recent developments in transductional targeting of viruses. It will focus on the three strategies for retargeting of viruses that seem most promising for the development of new oncolytic viruses. Section 2 will review the strategy through which viruses could be successfully provided new tropism by introducing targeting information into one of the viral surface proteins. As this strategy has been investigated most actively, we will limit the overview of the examples to those viruses in which the new targeting specificity could be genomically encoded. In Section 3, approaches are described by which scaffold-based modifications of viral surface proteins were applied to direct virions to new target cells, including the use of biotin or antibody-binding moieties. Section 4 will review the use of bispecific adapter proteins as mediators of binding virions to tumor cells. Most often such adapters were simply combined with the respective viruses thereby enabling single-round infection. In some cases, these targeting devices were incorporated genetically into the virus so as to generate self-targeted agents able to independently spread through a tumor. Finally, the review will be completed with general conclusions on the current status of the field of oncolytic virotherapy and with views on its future.

2. Modification of Viral Surface Proteins

The most popular approach to generate oncolytic viruses has been by adapting their surface-exposed components. Viral surface proteins can be modified to express ligands that bind to receptors preferentially or exclusively expressed on tumor cells. Viruses can be genetically adapted to express those modifications to redirect them towards tumor cells (Figures 1(a) and 1(b)). The main advantage of this strategy is that the targeting specificity is inherent to the viral genome and will, thus, be maintained upon replication. Progeny virus is then able to infect neighboring cells harboring the target receptor, thereby establishing a multiround infection that will be maintained until no further tumor target cells remain. For this strategy, the ability to genetically modify the viral genome is crucial. Furthermore, detailed structural information about the viral surface protein to be modified is indispensible to predict at which location targeting motifs might be tolerated and will be exposed. Such motifs should not only allow binding of the modified virion to the cells but should also not be detrimental to the entry mechanism of the particular virus, not interfering for instance with the fusion of viral and cellular membrane. In addition, the targeting ligand introduced into the viral protein will have to meet size limitations. Small peptides, thus, seem the first and most obvious choice. The development of targeting strategies of viruses is, however, severely limited by a shortage of naturally existing molecules available for use as targeting ligands. Therefore, other sources of binding ligands have been investigated and incorporated into viral proteins for this purpose. These include (parts of) antibodies, like scFvs (single-chain variable fragments, composed of a fusion of the variable regions of the heavy (V_H) and light chains (V_L) of an immunoglobulin) or Fabs (antigen-binding fragments, composed of one constant and one variable domain from each heavy and light chain of the antibody). The feasibility of modifying viral coat proteins has been demonstrated for a number of viruses as is summarized below.

2.1. Adenoviruses. Adenoviruses are among the most extensively studied viruses for oncolytic viral therapy. In a wild-type infection, adenovirus-binding to the cells is mediated by its major attachment factor, the fiber protein. Via its carboxy terminal knob domain, this protein binds to the primary cellular receptor coxsackie/adenovirus receptor (CAR). Following viral attachment, internalization is mediated through interaction of RGD motifs in the penton base with cellular α_v integrins. In order to achieve CAR-independent infection by adenoviruses, the viral tropism can be modified via genetic engineering of adenovirus capsid proteins.

The list of reviews describing the development of genetically redirected adenoviruses through incorporation of ligands into viral surface proteins is numerous. In summary, heterologous peptide ligands have been successfully engineered into many adenoviral proteins, including the HI loop of the fiber, the C terminus of the fiber, the L1 loop in the hexon, and the RGD loop in the penton base and in the minor capsid protein IX. Most commonly, targeting moieties are inserted in the HI loop of the fiber knob. For this protein, the importance of the insertion site of the ligand was demonstrated when introducing a model peptide CDCRGDCFC into the knob [4]. The insertion of the ligand into three of five analyzed loops of the knob still allowed trimerization of the knob protein, and the resulting adenoviruses showed superior infectivity to that of viruses with the same peptide fused to the fiber C terminus. That the precise ligand positioning is pivotal was further demonstrated by the lack of enhancement of infectivity when the ligand-flanking linkers were extended and when tandem copies of the ligand peptide were inserted [5]. Interestingly, also antibody-based targeting could be achieved for adenoviruses by generating fiber chimeras [6] or fusions of scFvs with the capsid protein IX [7]. For the most recent reviews on transductionally targeted adenoviruses, the reader is referred to [8–11]. For adenoviruses, the possibility to combine transductional with transcriptional targeting to increase adenoviral specificity makes this group of viruses particularly interesting for future therapy; however, their strong immunogenic nature might seriously hamper their efficacy *in vivo*.

2.2. Paramyxoviruses-Measles Virus. The measles virus is another virus well studied for oncolytic therapy, as the attenuated measles virus strain Edmonston has the ability to selectively destroy neoplastic tissue (reviewed in [12]). Measles virus has two envelope glycoproteins: the hemagglutinin (H) attachment protein and the fusion (F) protein. Virus attachment, entry, and subsequent cell-cell fusion are mediated via the two measles receptors: CD46 and the signaling lymphocyte activation molecule (SLAM).

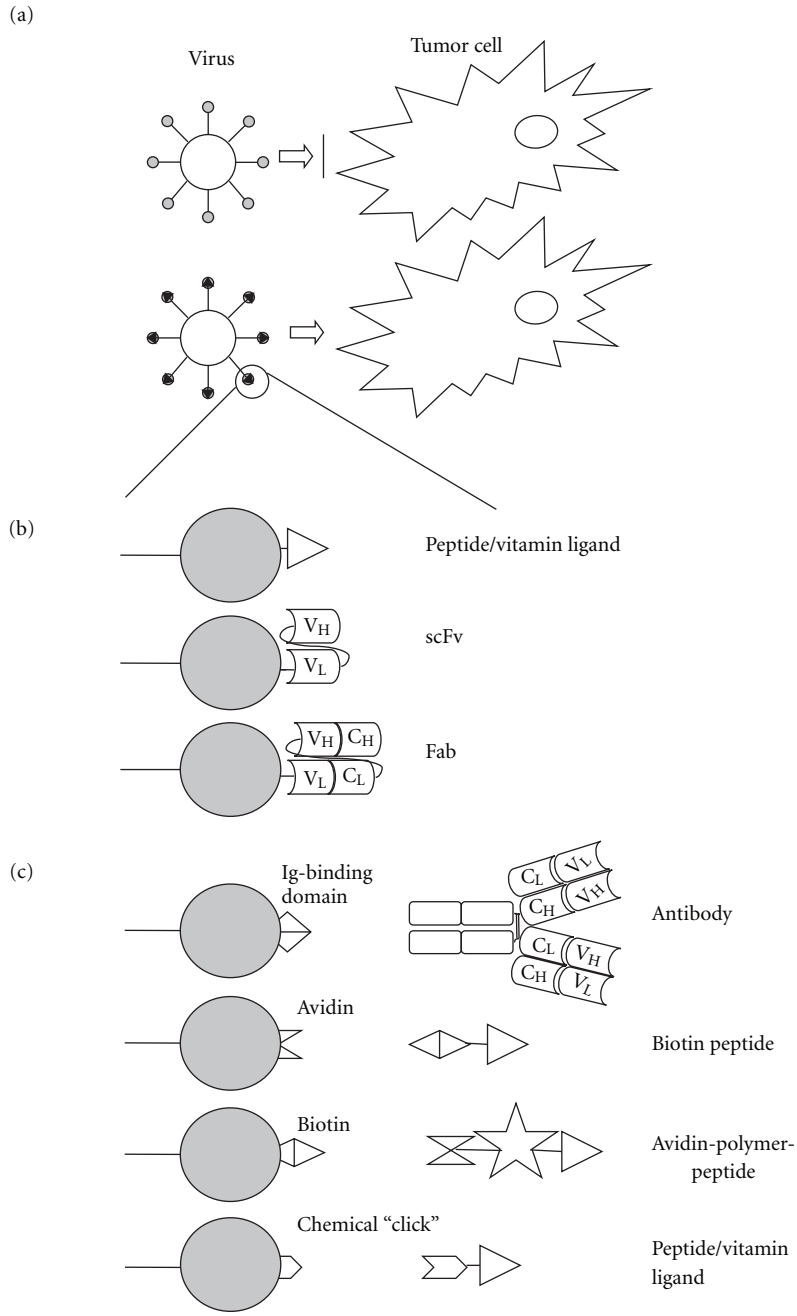


Figure 1: Transductional targeting through the modification of viral surface proteins. (a) Principle of redirecting viruses by the insertion of tumor-specific ligands into the viral coat, without modification of viral surface protein (upper part) no infection; (b) Schematic representation of a viral surface protein (represented by the grey-filled circle) on which tumor-binding peptides or antibodies are exposed. Ligands can be introduced at the N- or C-terminus of the protein or internally, provided that the correct folding of the viral protein and its accessibility for binding to the cell surface receptor are maintained; (c) Schematic representation of a viral surface protein on which a scaffold is exposed. The targeting ligand, examples of which are shown schematically, is then provided as a separate entity, binding on the one hand to the virion and on the other hand to the cell surface receptor of choice.

To improve the specificity of the infection, tumor-specific ligands have been introduced as C-terminal extensions of the H protein. A range of ligands, including both peptides and scFvs, were tolerated and, in addition, allowed the redirection of the virus to cells expressing the appropriate virus receptor. Again, the nature of the ligands was pivotal. Thus, though the length of the linkers separating the V_H and V_L domains was not of importance for scFvs to be incorporated into virions, it certainly affected the membrane fusion ability of the virus [13]. For reviews on redirected measles virus, please see [12, 14–16]. As the Edmonston strain has been used for vaccination for over 50 years now, its safety profile is impressive and might provide a good basis for future application in oncolytic therapy.

2.3. Herpesviruses-Herpes Simplex Virus (HSV). Another field of active study involves the use of herpes simplex virus for tumor therapy (reviewed in [17, 18]). Herpesvirus infects cells by attachment to heparan sulfate proteoglycans, mediated by the viral glycoproteins gC and gB, followed by the interaction of the glycoprotein gD with one of two alternative protein receptors. One, designated herpesvirus entry mediator, is a member of the family of tumor necrosis factors receptors. The second involves nectin1 and nectin2, both intercellular adhesion molecules belonging to the immunoglobulin (Ig) superfamily.

Retargeting of HSV could be achieved by the insertion of ligands and scFvs into the gC and/or the gD protein, with subsequent increased infectivity of target cells expressing the appropriate virus receptor. The current strategies to redirect HSV towards tumor cells have recently been reviewed [18, 19]. For another herpesvirus, the gamma herpes virus saimiri the native binding region of the viral glycoprotein ORF51 to heparan sulphate was replaced with that of a peptide sequence interacting with somatostatin receptors, known to be overexpressed on hepatocellular carcinoma cells. The subsequent recombinant virus appeared to infect the carcinoma cells as well as the wild-type virus, while showing reduced infectivity for other cell lines. The reason for these observations is unclear [20]. In conclusion, herpesviruses remain promising as candidates for oncolytic therapy as they can be redirected to tumor cells and are considered reasonably safe due to the induction of a self-limited disease in humans. On the other hand, their wide natural tropism and the presence of viral antibodies in the human population might hamper their effectiveness *in vivo*.

2.4. Parvoviruses-Adenoassociated Virus (AAV). A less frequently studied candidate for development as oncolytic agent is AAV (reviewed in [21]). AAV has a broad host cell range due to the widespread distribution of its primary cellular receptor heparan sulfate proteoglycan. The viral capsid protein is responsible for the interaction with this host cell receptor.

Transductional targeting independent of the native tropism could be demonstrated by genetically incorporating the 14-amino-acid targeting peptide L14 [22] into six different putative loops of the AAV2 capsid protein. The results showed that all mutant capsids were efficiently incorporated, that three mutants expressed L14 on the capsid surface, but that only one of these efficiently infected wild-type AAV2-resistant cell lines that expressed the integrin receptor recognized by L14. The importance of the incorporation site and of the peptide sequence was further elucidated in other studies, showing that the assembly, the generation of infectious particles, and the ability to transduce target cells depends both on the position in the capsid and on the ligand introduced [23, 24]. Successful targeting was demonstrated towards RGD [25] and towards the human luteinizing hormone receptor [24]. Due to the broad cell tropism of wild-type AAV, retargeting in combination with ablation of its natural tropism will remain crucial to develop this virus into a safe oncolytic vector.

2.5. Retroviruses-Murine Leukemia Virus (MuLV). Replication-competent retroviruses have gained interest as oncolytic agents, in particular because of their high transduction efficiency (reviewed in [26, 27]). Of MuLV different classes can be distinguished, of which the host range is based on the interaction between the envelope glycoprotein and a particular cell surface receptor. While the ecotropic MuLVs are particularly capable of infecting mouse and rat cells, amphotropic MuLV infects a range of mammalian, including human cells via the widely expressed Pit-2 receptor.

Initial studies to redirect ecotropic MuLV towards human tumor cells pointed towards the importance of the interaction between the envelope glycoprotein and its original virus receptor. Despite the correct folding of chimeric envelope glycoproteins displaying scFvs, their incorporation into viral particles, and the binding of pseudotyped virus particles carrying chimeric ecotropic Env to human cells, the resulting viruses were not infectious for the targeted cells (reviewed in [28]). When expanding these studies using amphotropic MuLV, targeted infection could be achieved only when incorporating the high molecular weight melanoma-associated antigen (HMWMAA), while targeting towards the EGF [29], IGF [30], and folate [31] receptors was unsuccessful, despite the observed binding to cells expressing those receptors. It was proposed that trafficking of the virus particles to lysosomes and subsequent degradation caused the lack of infectivity, but attempts to overcome this problem by inserting a translocation domain of exotoxin A of *Pseudomonas aeruginosa* into the envelope protein, in order to translocate the virion from endosomes to the cytoplasm, were unsuccessful [29]. Clearly, the choice of receptor will be of ultimate importance for the successful targeting of retrovirus vectors towards tumor cells.

2.6. Poxviruses-Vaccinia Virus. Vaccinia virus has been studied for its antitumor properties already for a long time. Despite its entry into a wide range of cells, for several vaccinia virus strains, a natural preference for replication in cancer tissue has been reported. While the identity of the natural receptor is still under debate, it likely involves a widely expressed surface component, like heparan sulfate or chondroitin sulfate proteoglycans. Tumor targeting can be improved by deleting vaccinia virus genes that are necessary for replication in normal cells but not in cancer cells (recently reviewed in [32]).

To increase the specificity of their tropism, tumor-specific scFvs have been displayed on the surface of vaccinia virus particles. Targeting moieties were introduced by fusing an scFv directed against the tumor-associated antigen ErbB2 to the N-terminus of the nonessential hemagglutinin HA protein in vaccinia virus strain IHD-J [33]. Similarly, the nonessential p14 membrane-associated protein of vaccinia strain MVA could be replaced with a p14 fusion molecule carrying an inserted scFv directed against the tumor-associated antigen MUC-1 [34]. The resulting fusion proteins could be expressed, were exposed on the envelope of the recombinant virus, and were able to bind the target cells. No preferential infection of the target cells was, however, observed, likely

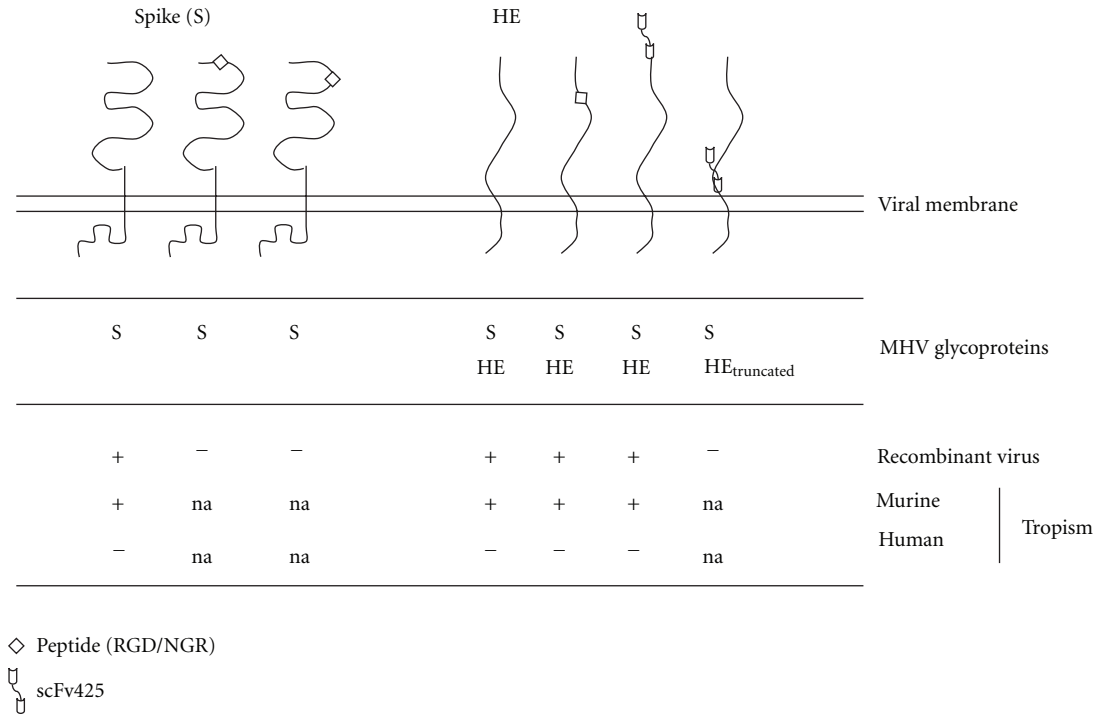


Figure 2: Modification of viral surface proteins for redirecting coronavirus to tumor cells. Schematic representation of MHV surface glycoproteins spike (S) and hemagglutinin-esterase (HE) and of modifications applied to redirect the virus to novel target cell antigens. Modifications tested include: insertion of small peptide ligands, including RGD and NGR, and extension with the anti-EGFR scFv425. Recombinant MHV viruses encoding such mutated S proteins or modified HE proteins (in the presence of wild type spike proteins) were generated by targeted recombination [35]. Indicated is whether the intended recombinant viruses could actually be isolated (confirmed by RT-PCR and sequencing). Also indicated is the tropism of each successfully generated recombinant virus for murine and for human cells (Verheije and Rottier, unpublished data).

because the recombinant viruses still contained wild-type host cell attachment proteins, providing the infection with a broad cell range. Therefore, the future challenge for the transductional targeting of vaccinia virus towards tumor cells will lie in the elimination of its natural tropism.

2.7. Coronaviruses-Mouse Hepatitis Virus (MHV). The favorable characteristics of—particularly the nonhuman—coronaviruses as potential oncolytic agents have been recognized only recently. In these viruses, the spike (S) protein is responsible for receptor binding and subsequent cell entry through virus-cell membrane fusion. The aminoterminal S1 domain is required for virus-binding to the cells, and, while undergoing ordered structural changes, the S2 domain mediates fusion with the cell membrane. Infection of cells by coronaviruses depends on the expression of specific cellular receptors, which makes these viruses highly species-specific. For example, entry by MHV is mediated by the murine carcinoembryonic antigen (CEACAM1a) receptor.

Attempts to redirect coronaviruses, in particular MHV, by mutation of the viral surface proteins were unsuccessful. Incorporation of ligands, such as RGD and NGR, into various nonconserved domains in the S1 domain of the spike protein appeared to be not tolerated, as the selection of retargeted recombinant viruses based on the new binding properties of the modified spike was not successful (Figure 2; Verheije and Rottier, unpublished data). Obviously, without much knowledge of the tertiary structure of the coronavirus spike protein and of its conformational changes during cell entry, chances are high that the introduction of even small ligands affects its proper functioning. Some MHV strains carry an accessory hemagglutinin-esterase (HE) surface glycoprotein. Attempts to also use this glycoprotein for retargeting were equally unsuccessful. Though some HE gene modifications, such as insertions of small peptide ligands and terminal extensions with the anti-EGFR scFv425, could be incorporated into the viral genome, the resulting recombinant viruses were unable to redirect MHV to human tumor cells (Figure 2; Verheije and Rottier, unpublished data).

3. Introduction of Scaffolds into Viral Surface Proteins

Rather than incorporating specific tumor targeting information into a viral surface protein, an alternative approach involves the incorporation of a scaffold moiety into such a protein to which subsequently various types of targeting modules can be linked (schematically depicted in Figure 1(c)). The main strategic difference relative to the previous method is that the moiety incorporated is not a tumor ligand itself but represents an attachment site for exogenously provided targeting moieties that, besides to

the scaffold, also bind to the receptor of interest (compare Figures 1(b) and 1(c)). An essential operational limitation is that this targeting strategy provides viruses that can only establish single-round infection, remaining dependent on the external supply of the targeting module. Yet, it has the advantage of flexibility as the targeting device, binding always to the same, previously modified viral protein, can be changed relatively easy. Some of these strategies are based on antibody targeting, giving the opportunity to redirect the oncolytic virus to virtually every tumor surface epitope. A particular application based on this principle relies on the biotin-(strept)avidin-coupling method (reviewed in [36]).

3.1. Adenoviruses. For adenoviruses, single-round targeted virus particles could be generated by using the biotin-streptavidin coupling system. After incorporating a biotinacceptor peptide into the fiber, metabolically biotinylated adenovirus was coupled to an EGF-streptavidin complex and found to successfully infect EGFR expressing target cells [37]. Similarly, a biotin-polyethylene glycol (PEG)-EGF conjugate coupled to an avidin-modified adenovirus could redirect the virus to a nonnative receptor [38]. In another study, small protein ligands capable of selective binding to human IgG and IgA were incorporated as model ligands for tropism-modified adenoviruses. Viable viruses that had genetically incorporated scaffolds into their fiber gene could be rescued and were, after incubation with antibodies, able to enter cells displaying the Fc receptor on their surface [39]. Recently, it has been demonstrated that specific chemoselective modification of the adenoviral particle could also function as a scaffold for targeting devices. By metabolic incorporation of noncanonical monosaccharides and amino acids in adenoviral particles, conjugation with a folate targeting motif in combination with a taxoid was achieved. Initial results demonstrated increased toxicity in vitro [40].

3.2. Adenoassociated Virus. The Ig-binding fragment of protein A was tested as a possible scaffold to redirect AAV. The fragment was successfully introduced into the capsid protein providing a versatile platform for antibody-mediated AAV targeting [41]. In a more recent study, a biotinacceptor peptide was incorporated into AAV particles. Subsequent biotin labeling of the viruses with the biotin ligase BirA and attachment of an RGD peptide to target integrins resulted in a significant increase in the transduction of endothelial cells, demonstrating again the feasibility of this approach [42].

3.3. Togaviruses-Sindbis Virus. Sindbis virus has inherent oncolytic properties and has been studied quite extensively as an oncolytic virus (reviewed in [43]). One of the surface proteins on mammalian cells to mediate the Sindbis virus infection is the laminin receptor, which is overexpressed on various human tumors. The envelope protein E2 of Sindbis virus is responsible for receptor binding.

To increase the specificity of the infection, researchers combined the introduction of ligands into the viral envelope with the use of targeting molecules. To this end, virus particles were generated which contained the IgG-binding domain of protein A inserted into their envelope protein E2. When combined with antibodies that bound to specific surface antigens on nonsusceptible cells, the chimeric virus was able to infect these otherwise refractory cells [44]. A comparable combination approach was taken by introducing Ig-binding domains as N-terminal extensions of the E2 glycoprotein. After adding species-matched antibodies, Fc receptor-positive cell lines could be successfully infected [45].

3.4. Murine Leukemia Virus. Also for MLV, studies were performed to introduce the IgG-binding domain of protein A to enable modular use of antibodies of various specificities for vector targeting. By inserting this binding domain into the hinge region of the viral envelope protein virions were generated that were capable of capturing anti-HER2 antibodies. Subsequent efficient binding of the virus-antibody complex to HER2-positive target cells and enhancement of transduction of these cells was observed [46].

4. Transductional Targeting of Viruses Using Bispecific Adapters

4.1. Bispecific Adapters. An elegant strategy currently employed to target viruses towards tumor cells makes use of bispecific adapters. Such proteins consist of two domains ("arms"), one binding to the virion, the other to a cell surface epitope of interest, thereby enabling indirect interaction of virus and tumor cell. The composition of the adapter proteins can vary greatly, depending on the design of the arms. The virus-binding domains that have been used include soluble receptor fragments (so-called pseudoreceptors), polymers like PEG, (parts of) antibodies, including scFvs or Fabs. Moieties that have been applied for cell-binding are natural peptide or vitamin ligands for receptors, and again scFvs or Fabs directed against a cell epitope of interest. The specificity for two different antigens is achieved either by joining the arms together chemically or by combining the two moieties in one fusion protein, often with a flexible linker, the targeting arm typically being at the C-terminus. The principle of redirecting viruses towards nonnative cells using bispecific proteins is shown in Figure 3(a), with typical examples of the two domains being depicted in Figure 3(b). In Table 1, an overview is provided of combinations of arms in bispecific adapter proteins actually generated to target viruses to tumor cells.

4.2. Advantages and Disadvantages of Bispecific Adapters for Targeting. The use of adapters to redirect viruses towards tumor cells has several advantages over the introduction of targeting or scaffold moieties into viral attachment proteins. First, no detailed structural information is required about the viral surface proteins, as the manipulation of these proteins is not required. Second, as the size of the targeting part of the adapter protein seems less crucial than when introducing this moiety into a viral protein, the choice of targets can easily be expanded by using (parts of) antibodies. In this way, the selection of targeting receptors becomes virtually

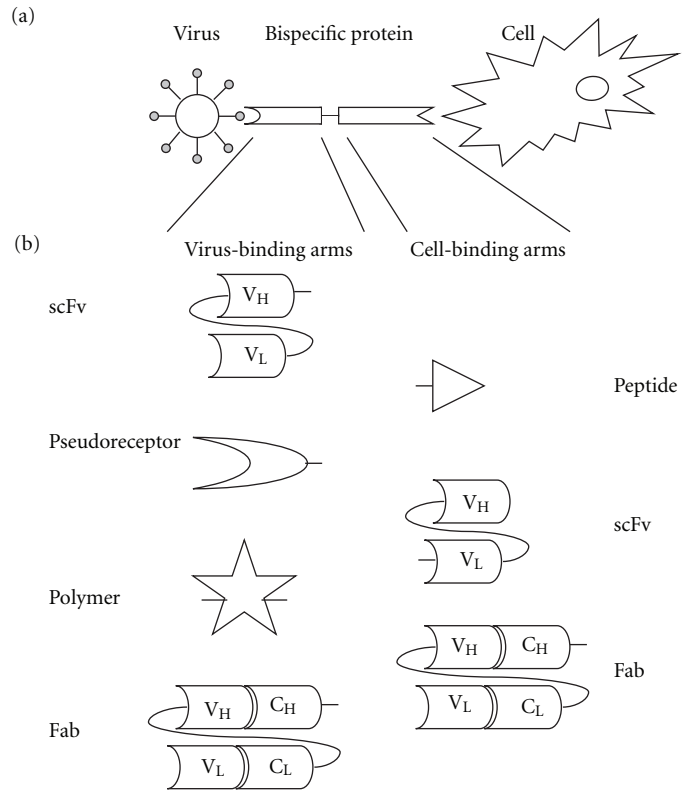


Figure 3: Bispecific adapter targeting principle and composition. (a) Principle of targeting viruses towards tumor cells using bispecific adapters. (b) Typical examples of virus-binding moieties (left) and cell-binding moieties (right) of bispecific adapters. In theory, all combinations of virus-binding and cell-binding arms are possible. An overview of the composition of the bispecific adapters used to target particular viruses to tumor cells is provided in Table 1.

Table 1: Overview of the composition of bispecific adapters used to reroute viruses for oncolytic purposes.

Adaptor protein moiety binding to		Targeting demonstrated for
Virion	Cell	
Antibody (scFv or Fab)	Antibody (scFv or Fab)	Adenovirus Adenoassociated virus Coronavirus
Antibody (scFv or Fab)	Ligand peptide	Adenovirus Paramyxovirus
Soluble/pseudo receptor	Antibody (scFv or Fab)	Adenovirus Herpesvirus Coronavirus
Soluble/pseudo receptor	Ligand peptide	Adenovirus Coronavirus
Polymer	Antibody (scFv or Fab)	Adenovirus
Polymer	Ligand peptide	Adenovirus

unlimited, as antibodies can be generated relatively easy, once the receptor of interest has been identified. Third, as adapter proteins are straightforward to construct, expanding the repertoire of target receptors becomes relatively easy. Finally, the binding of adapter proteins to the virion has, at least in some cases, been reported to ablate the virus' natural tropism, which is especially useful when the oncolytic virus of choice has a preference for normal cells in the host.

There are also disadvantages of using bispecific proteins in targeting oncolytic viruses. As bispecific proteins are artificial polypeptides composed of parts that do not occur linked together naturally, their proper biogenesis with

independent folding of both moieties and efficient secretion may be impaired. Moreover, unless expressed by the oncolytic virus itself, production and purification of the adaptors is a challenge.

4.3. Application of Bispecific Adapters. To redirect oncolytic viruses towards tumor cells, bispecific proteins can be applied in two ways. First, after recombinant production or chemical synthesis, the proteins can be precomplexed with virions before applying them *in vitro* or *in vivo*. A major drawback of this approach is, however, that it allows single-round infection only; progeny virus will not be able to infect neighboring cells as the amount of adapter protein will be limiting. Consequently, the use of such adapter-precomplexed viruses *in vivo* is likely to be restricted to local, rather than systemic application. It will probably also require repeated administration of high doses of the adapter proteins. Little is known about the potential risks of such approach.

As an alternative, the genetic information for the adapter protein can be incorporated into the viral genome. When properly expressed, this ensures the local production of the targeting device together with the progeny virus in the infected cell. This approach will enable multiround infection and lateral spread of the oncolytic virus. The time span and, hence, the efficacy of this kind of therapy will be limited by the emergence of immunity against the bispecific protein and/or the virus. The feasibility of this strategy depends on the availability of a genetic modification system to introduce the adapter-encoding gene into the viral genome as an additional expression cassette. While such modification systems are currently available for most viruses, the capacity of the genome to accept such insertions can be limited; hence, the size of the targeting moiety might be restricted. Finally, the genetic stability of such recombinant viruses might be an issue, in particular when the adapter protein is used to ablate the natural tropism of the virus.

The feasibility of using adapter proteins for oncolytic viral therapy has been explored for a number of viruses. Below we first present an overview of the studies in which targeting to tumor cells was performed by coadministration of viruses and adaptor proteins (Section 4.3.1). Thereafter, we discuss the studies describing genetic targeting of oncolytic viruses generated by the incorporation of genes coding for bispecific proteins into the viral genome (Section 4.3.2).

4.3.1. Single-Round Transductional Targeting Using Bispecific Adapters

Adenoviruses. To redirect adenoviruses to tumor cells, neutralizing antiknob fiber antibodies have been used extensively. The first demonstration of their potential application for retargeting of these viruses to a nonadenovirus cellular receptor was in 1996, when such antibodies were chemically conjugated to folate and shown to mediate infection of folate-receptor expressing cells [47]. Many nonnative receptors have since been targeted by conjugating an antiknob antibody fragment to either ligand peptides or antibody domains directed against a cellular receptor. This resulted in successful targeting towards the EGF receptor (EGFR) [48, 49], FGF receptor [50–52], integrins [53, 54], EGP-2 (also known as EpCAM) [55, 56], the melanoma-associated antigen HMWMAA receptor [57], carbonic anhydrase IX protein G250 [58], CD40 [59, 60], various organ- and tumor homing peptide receptors [61], mesothelin MSLN [62], prostate-specific membrane antigen PSMA [54, 63], VEGFR2 [54], Ly-6D [64], and Tie2 receptors [54].

Similar approaches have been explored using antibodies directed against other adenoviral proteins. Thus, Fabs directed against the penton base of the fiber in combination with targeting ligands, such as EGF, IGF, and TNFα could mediate the infection of target cells expressing the appropriate receptors [65]. Also Fabs directed against the hexon protein chemically linked to Fabs specifically binding to an antigen highly overexpressed on human hepatocellular carcinoma were successfully applied to redirect adenoviruses to a nonnative receptor [66].

Another strategy successfully employed for the same purpose made use of pseudoreceptors. In this approach, bispecific proteins were generated by fusion of a soluble form of CAR (sCAR) to EGF [67, 68], to the Fc region of human IgG1 [69], and to scFvs against ErbB2 [70] and CEA [71].

In yet another approach, polymers were exploited as targeting ligands in a single round fashion. Here, adenovirus particles were coated to inhibit their natural tropism after which ligands, including peptides and scFvs, were attached. Several types of polymers have been used [72–77], including polyethylene and metacrylamide derivatives, to successfully target adenoviruses to FGF2 [78], RGD [79], TNFα [80], and the HER2 receptor [81]. It has been proposed that adenoviral coating with polymers might have enhanced potential for systemic delivery, as it prolongs the viral plasma half-live and reduces the hepatotoxicity *in vivo* [80].

Finally, another type of bispecific molecule based on the binding ability of the Gla domain of coagulation factor X to the hexon was exploited for targeting. Upon fusion of Gla to scFv proteins, increased infection of tumor cells by adenovirus could be observed. However, the anticipated reduction in liver transduction was not observed [82].

Adenoassociated Viruses. AAV has a broad host cell range due to the widespread distribution of its primary cellular receptor heparan sulfate proteoglycan. To achieve a more specific infection, a bispecific Fab was tested of which one arm recognized the cell-surface integrins αIIbβ3 while the other bound to the AAV capsid [83]. Targeting this way did not inhibit downstream steps required for productive infection. Moreover, a decrease of infection of normally permissive cells was observed, indicating that the bispecific protein was able to ablate the normal tropism.

Herpesviruses-Herpes Simplex Virus. HSV binding to the cell is mediated by several widely expressed cell surface receptors, including nectin1. HSV was successfully redirected to the EGFR by means of a soluble adapter protein comprising the N-terminal domain of nectin1 fused to an scFv directed

against EGFR [84]. Adapter-mediated entry was, however, promoted by the presence of heparan sulfate proteoglycans on cells, which are also required for wild-type HSV infection.

Paramyxoviruses-Newcastle Disease Virus (NDV). In the avian paramyxovirus NDV, the hemagglutinin-neuraminidase (HN) protein is responsible for sialic acid receptor attachment, while the F protein mediates the fusion of viral envelope and cellular membrane. NDV has oncolytic properties by nature; however, it has a broad cell tropism due to the widespread occurrence of sialic acids on many cells. To narrow its specificity, the use of bispecific adapter proteins has been investigated. Preincubation of NDV with a recombinant bispecific protein composed of an scFv against HN that blocks the native receptor binding site and the interleukin-2 peptide clearly enhanced the specificity of the virus [85] and reduced its side-effects when applied systemically *in vivo* [86].

Coronaviruses-Mouse Hepatitis Coronavirus (MHV) and Feline Infectious Peritonitis Virus (FIPV). The first demonstration of retargeting of coronaviruses was achieved by exchanging the viral spike ectodomains. Thus, felinized MHV (fMHV) [87] and murinized FIPV (mFIPV) [35] were generated in which the murine viruses carried the feline S ectodomain and *vice versa*. These otherwise highly species-specific recombinant viruses were able to cross species barriers; fMHV had acquired feline cell tropism but completely lost its murine cell tropism while the opposite was true for mFIPV. To extend the species tropism of nonhuman coronaviruses towards human tumor cells, bispecific adapter proteins were generated. Proteins composed of a bispecific scFv directed against both the feline spike protein and the EGFR could mediate FIPV and fMHV infection of EGFR-expressing human cancer cells, with subsequent syncytia formation typical of a productive coronavirus infection [88].

Subsequent studies to redirect murine coronavirus MHV to human tumor cells were based on an adapter protein that consisted of a pseudoreceptor, composed of the N-terminal domain of murine CEACAM1a (soluble receptor; soR), fused to an scFv directed against the EGFR [89] or to the EGF ligand [90]. Again, such adapter proteins could mediate EGFR-specific entry of MHV into human cancer cells. However, in contrast to many of the previous examples, no ablation of the natural tropism of the virus was observed.

4.3.2. Multiple-Round Transductional Targeting Using Bispecific Adapters. To overcome the major drawback inherent to single-round targeting, a number of investigations focused on the expression of the bispecific adapters from the viral genome in order to allow the recombinant viruses to produce their own targeting device and sustain the infection. The feasibility of this approach has so far only been demonstrated for some adenoviruses and coronaviruses.

Adenoviruses. To redirect adenoviruses to nonnative surface receptors, conditionally replicating adenoviruses (CRAds) seem to be the viruses of choice, due to their selective replication in tumor cells. The first experiments demonstrating the ability to redirect CRAds towards tumor cells were performed using dual-virus mixtures consisting of a CRAd and an adenovirus secreting a bispecific adapter protein consisting of a fusion between the soluble CAR receptor and the EGF ligand [91]. Dual virus infections resulted in increased oncolytic activity *in vitro* and improved therapeutic efficacy *in vivo*.

Subsequently, CRAds were engineered to express the bispecific adapter proteins by themselves. Van Beusechem et al. [92] developed such a CRAd encoding a bispecific protein composed of the anti-EGFR scFv 425 and antifiber knob scFv s11. The resulting virus AdΔ24-425S11 produced the bispecific protein 425-s11 during replication in cancer cells, yielding progeny virus with enhanced infectivity and oncolytic properties on EGFR-positive, CAR-deficient tumor cells. However, in addition to infection mediated by EGFR, the virus retained its capacity to infect cells through binding to the native receptors CAR and integrins. To abolish the native tropism, mutations were introduced that eliminated CAR and integrin binding [93], resulting in a recombinant virus with a strictly EGFR-dependent targeting profile and reduced replication in EGFR-negative cells. Both viruses displayed similar oncolytic potency in cell lines and tissue specimens [93]. Also when applied in a mouse model by intrajugular or intramuscular injection, the native tropism of adenoviruses appeared to be reduced after the removal of both the CAR and integrin-binding sites [94]. Strikingly, however, when expressing the soluble CAR-EGF targeting moiety from a CRAd rather than from a dual virus system, its oncolytic potential was severely impaired [95], suggesting that the expression of biologically active proteins can be counterproductive to virus replication.

To overcome the biosynthetic differences between the bispecific proteins translated and secreted via the ER-Golgi route and the adenovirus with translation in the cytoplasm but assembly in the nucleus, an elegant strategy was developed by tagging of the adenovirus fiber and the scFv each with a synthetic leucine zipper-like dimerization domain [96]. Tagging of the proteins with the zipper peptide sequences preserved both the trimerization capability of the adenovirus fiber and the recognition of the EGFR by the zipper-scFv protein, but, most importantly, it gave rise to receptor-specific infection of the target cells.

Several studies have shown the feasibility of using bispecific proteins for redirecting adenoviruses towards target cells *ex vivo* or *in vivo* in laboratory animal models, including [71, 92, 97–102]. Although quite effective, these studies were all based on a two-component strategy, requiring the mixing of virions with bispecific proteins before administration. To our knowledge, no *in vivo* studies have yet been performed using recombinant adenoviruses expressing a bispecific adapter from their viral genome to establish whether they have superior targeting and cell-killing abilities.

Coronaviruses. To generate self-targeted coronaviruses, initially the coding sequence for a bispecific adapter protein composed of the soluble mCEACAM1a receptor linked to

a His-tag was incorporated into the MHV viral genome by targeted recombination, creating the virus-designated MHVsoR-His [103]. The presence of this additional expression cassette was tolerated and the resulting recombinant viruses indeed expressed the adapter protein. Inoculation of target cells expressing the artificial His-receptor on their surface showed the recombinant viruses to be able to establish a multiround, receptor-dependent infection. Furthermore, extensive cell-cell fusion and rapid cell killing of infected target cells was observed, demonstrating the possibility of generating genetically redirected coronaviruses [103].

The expression cassette was subsequently extended by inserting the sequence encoding the EGF peptide between that of the soluble receptor and the His-tag [90]. Again, the generated recombinant MHVsoR-EGF-His thereby acquired the ability to cause multiround infection of other-wise non-susceptible, EGFR-expressing cell cultures *in vitro*, with subsequent efficient cytolytic activity [90]. More importantly, the redirected virus demonstrated oncolytic capacity also *in vivo* in an orthotopic U87dEGFR xenograft mouse model. Survival rates of the mice were significantly longer when the tumor-bearing animals were treated with MHVsoR-EGF-His than after treatment with control virus MHVsoR-His or with PBS (Figure 4(a)). In none of the MHVsoR-EGF-His treated mice-recurrent tumor load could be detected, demonstrating the strong oncolytic capacity of such viruses *in vivo* [90] (Figure 4(b)). Despite the impressive oncolytic effect *in vivo* of the redirected MHV, replication of MHV in non-tumor tissue of the natural host was observed (Figure 4(c)), presumably because the natural tropism of MHV was not ablated.

Further experiments demonstrated that the composition of the bispecific protein is of critical importance for the success of generating recombinant oncolytic coronaviruses. In particular, viable recombinant coronaviruses expressing a bispecific scFv from an additional expression cassette in the viral genome could not be rescued (Figure 5; Verheije and Rottier, unpublished data). Subsequent introduction of a bispecific gene encoding the soR fused to a scFv against the EGFR did generate viable viruses; however, such viruses were genetically highly unstable, loosing the foreign gene usually already within one passage (Figure 5; Verheije and Rottier, unpublished data). As successful incorporation of other, even larger, foreign genes at the same position in the MHV genome has been reported (including for example the gene-encoding luciferase [104]), the instability of the scFvs is likely due to their particular sequence composition rather than to their size.

In conclusion, oncolytic coronaviruses expressing a soluble receptor that is C-terminally extended with a peptide ligand have great potential for oncolytic therapy. By expanding the targeting repertoire through exchange of peptide ligands, coronaviruses can probably be redirected towards various tumor epitopes, provided that the binding and fusion ability of the viral proteins are maintained. As murine coronaviruses display great species specificity in their infection, ablation of the natural tropism will probably not be required, making MHV a safe candidate oncolytic agent for use in other mammals, including humans.

5. Conclusions and Perspectives

Appealing as the idea of oncolytic virotherapy may be, its realization is still disconcertingly remote. What this overview emphatically reveals is that the field of retargeting of viruses for therapeutic use is in its early infancy. In fact, for the majority of the viruses studied, scientists are still struggling with the most fundamental aspects of changing their cell tropism. Robust platforms for retargeting have actually not been established yet for any of the viruses. On the positive side, the feasibility of retargeting was, at least *in vitro,* demonstrated for an increasing number of viruses in the past years. Clearly the most attractive goal will be to generate oncolytic viruses that carry the retargeting information in their genome. Only then will the viruses be able to sustain their replication in the tumor tissue, irrespective of the retargeting principle used, that is, whether through the modification of the viral attachment protein or through expression of an adapter protein. However, (pre)clinical data on the efficacy—let alone safety—of such transductionally targeted viruses *in vivo* are very limited. With one exception (a transductionally and transcriptionally targeted adenovirus [105]), none of such genetically modified, tropism modified oncolytic viruses have entered phase I clinical trial. This makes it virtually impossible to compare the viruses reviewed here. Thus, many hurdles have yet to be overcome before new oncolytic viruses will reach the clinic. Below, some of the important future tasks and challenges for the field are summarized.

One major challenge at the base of the idea of oncolytic virotherapy is the availability of suitable target receptors on tumor cells. Ideally, such receptors are unique or highly overexpressed in order to provide sufficient specificity for the infection. Recent developments in the proteomics field have already recognized various proteins that are overexpressed in tumor cells as compared to normal tissue and many more will hopefully be identified. It remains, however, questionable whether truly unique tumor surface proteins exist. This stresses the need to increase specificity of oncolytic viruses in other ways. This can be achieved, for example, by combining transductional targeting with either transcriptional targeting or attenuation of the viral genome, both increasing tumor-specific replication. In transcriptional targeting, viral genes essential for replication are placed under control of a tumor-specific promoter—which is particularly feasible for DNA viruses—or under the control of an IRES element in the case of RNA viruses. Attenuation of the viral genome might be achieved by the deletion of viral genes that eliminate functions dispensable in tumor cells, but not in normal tissue. The feasibility of combining both transductional and translational targeting has already been demonstrated for DNA viruses, including adenovirus, while for RNA viruses investigations rather focus on the transductional targeting of attenuated viruses.

The natural tropism of the therapeutic virus is another aspect which needs to be taken into account with regard to safety. Ablation of the native tropism might be required for those viruses naturally infecting humans, to prevent the infection of normal tissue, but also when the virus has a preference for binding, for instance, to blood substances or

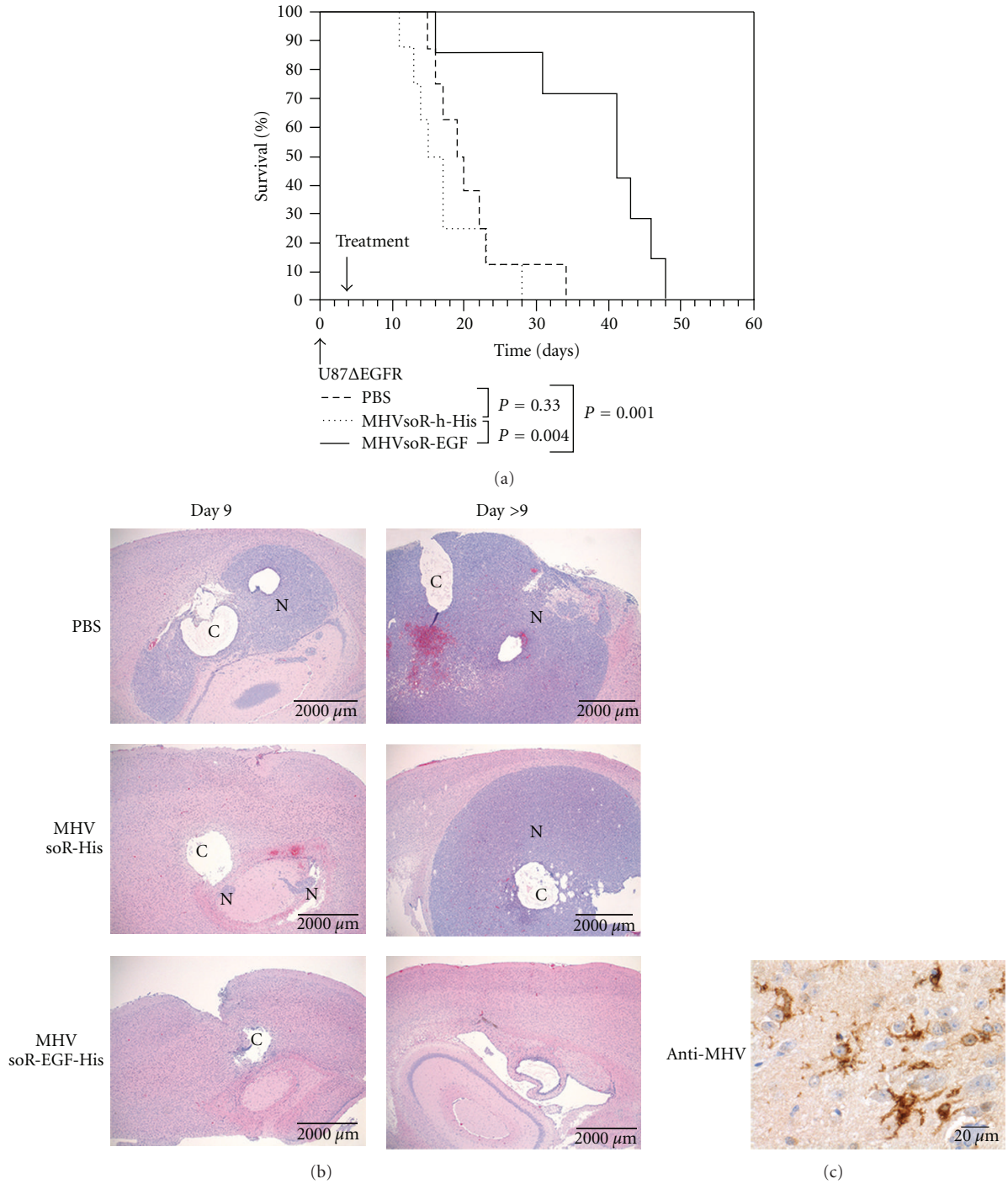


Figure 4: *In vivo* oncolytic activity of murine coronavirus MHV. Mice with established intracranial U87ΔEGFR tumors were treated with MHV genetically redirected to the EGFR (MHVsoR-EGF-His), the His-receptor (MHVsoR-his), or with PBS. (a) Survival curves. (b) Histopathological analysis of brains at day 9 posttreatment and at the day of euthanasia (day > 9). Large neoplasms and cystic structures are indicated by "N" and "C," respectively. (c) Immunostaining of brains after treatment with MHVsoR-EGF-His using polyclonal anti-MHV antibodies (Copyright © American Society for Microbiology, Journal of Virology, Vol. 83, No. 15, P. 7507-16, 2009, DOI 10.1128/JVI.00495-09).

when it exhibits hepatic tropism, both being a major cause of loss of infectious virus *in vivo*. The use of nonhuman viruses for oncolytic therapy gains interest, as such viruses are usually nonpathogenic for humans and, in addition, no preexisting antibodies circulate which might limit their efficacy. However, when adapting to the new host, these viruses might also pose a risk, as was reviewed in [106].

In order to achieve effective eradication of all tumor cells, a desirable characteristic of oncolytic viruses is their ability to cause sustained, multiround infection. This can be achieved

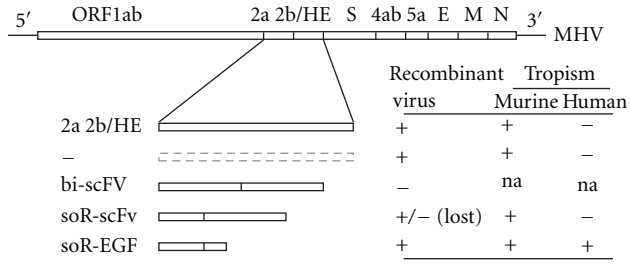


Figure 5: Introduction of bispecific adapter cassettes into the coronaviral genome. Schematic representation of the MHV genome and of the bispecific expression cassettes introduced herein. The plus-strand RNA genome contains, from 5′ to 3′, the polymerase precursor gene (ORF1ab), the accessory genes 2a and 2b/HE, the S gene, the nonessential genes 4ab and 5a, and the genes encoding the virion proteins E, M, and N. In the recombinant viruses, the gene cluster 2a + 2b/HE was replaced with an expression cassette downstream of the translation regulation sequence for protein 2a. Recombinant MHV viruses were generated by targeted recombination [35]. Indicated is whether the particular recombinant virus could be isolated, as confirmed by RT-PCR on virus RNA. In addition, the ability of such viruses to infect murine and human cells is depicted (Verheije and Rottier, unpublished data). Abbreviations of adapters as specified in the text: "na": not applicable.

by viruses genetically redirected through the incorporation of tumor-binding ligands and those having incorporated a bispecific adapter into their viral genome The stability of such recombinant viruses might, however, be a matter of concern, in particular when the targeting protein is required to ablate the natural human tropism. In general, DNA viruses are considered to be more stable than RNA viruses in which, in addition, the mutation rate is relatively high.

Irrespective of the origin of the virus, immunity induced upon (repeated) viral treatment against viral antigens but also against foreign proteins like the bispecific adapters expressed from the viral genome might limit the effectiveness of the therapy. To shield viruses from antibodies, polymers might be used to coat the virion [107]. Yet, this might compromise the binding of the virus to tumor cells and can technically only be performed upon application and not during lateral spread of the virus. Other ways to increase the delivery of oncolytic virus to tumor cells, especially when applied systemically, might be the use of carrier cells, which have the ability to home to the tumor [108].

In conclusion, transductionally targeted viruses may provide a much needed tumor-specific therapy, but researchers will have to face, besides the technological challenges, a delicate balance between safety and effectiveness during development of such new viruses for clinical use. Yet, despite all problems and concerns, the importance of the ultimate goal of winning the fight against cancer warrants the sacrifice of all the energy and creativity needed for its realization.

References

[1] P. J. Lech and S. J. Russell, "Use of attenuated paramyxoviruses for cancer therapy," *Expert Review of Vaccines*, vol. 9, no. 11, pp. 1275–1302, 2010.

[2] G. N. Barber, "Vesicular stomatitis virus as an oncolytic vector," *Viral Immunology*, vol. 17, no. 4, pp. 516–527, 2004.

[3] C. Thirukkumaran and D. G. Morris, "Oncolytic viral therapy using reovirus," *Methods in Molecular Biology*, vol. 542, pp. 607–634, 2009.

[4] A. Hesse, D. Kosmides, R. E. Kontermann, and D. M. Nettelbeck, "Tropism modification of adenovirus vectors by peptide ligand insertion into various positions of the adenovirus serotype 41 short-fiber knob domain," *Journal of Virology*, vol. 81, no. 6, pp. 2688–2699, 2007.

[5] D. T. Rein, M. Breidenbach, H. Wu et al., "Gene transfer to cervical cancer with fiber-modified adenoviruses," *International Journal of Cancer*, vol. 111, no. 5, pp. 698–704, 2004.

[6] N. Belousova, G. Mikheeva, J. Gelovani, and V. Krasnykh, "Modification of adenovirus capsid with a designed protein ligand yields a gene vector targeted to a major molecular marker of cancer," *Journal of Virology*, vol. 82, no. 2, pp. 630–637, 2008.

[7] K. L. Poulin, R. M. Lanthier, A. C. Smith et al., "Retargeting of adenovirus vectors through genetic fusion of a single-chain or single-domain antibody to capsid protein IX," *Journal of Virology*, vol. 84, no. 19, pp. 10074–10086, 2010.

[8] R. Waehler, S. J. Russell, and D. T. Curiel, "Engineering targeted viral vectors for gene therapy," *Nature Reviews Genetics*, vol. 8, no. 8, pp. 573–587, 2007.

[9] J. M. Mathis, M. A. Stoff-Khalili, and D. T. Curiel, "Oncolytic adenoviruses—selective retargeting to tumor cells," *Oncogene*, vol. 24, no. 52, pp. 7775–7791, 2005.

[10] J. N. Glasgow, M. Everts, and D. T. Curiel, "Transductional targeting of adenovirus vectors for gene therapy," *Cancer Gene Therapy*, vol. 13, no. 9, pp. 830–844, 2006.

[11] K. Hall, M. E. Blair Zajdel, and G. E. Blair, "Unity and diversity in the human adenoviruses: exploiting alternative entry pathways for gene therapy," *Biochemical Journal*, vol. 431, no. 3, pp. 321–336, 2010.

[12] T. Nakamura and S. J. Russell, "Oncolytic measles viruses for cancer therapy," *Expert Opinion on Biological Therapy*, vol. 4, no. 10, pp. 1685–1692, 2004.

[13] A. L. Hammond, R. K. Plemper, J. Zhang, U. Schneider, S. J. Russell, and R. Cattaneo, "Single-chain antibody displayed on a recombinant measles virus confers entry through the tumor-associated carcinoembryonic antigen," *Journal of Virology*, vol. 75, no. 5, pp. 2087–2096, 2001.

[14] E. Galanis, "Therapeutic potential of oncolytic measles virus: promises and challenges," *Clinical Pharmacology and Therapeutics*, vol. 88, no. 5, pp. 620–625, 2010.

[15] B. Blechacz and S. J. Russell, "Measles virus as an oncolytic vector platform," *Current Gene Therapy*, vol. 8, no. 3, pp. 162–175, 2008.

[16] S. J. Russell and K. W. Peng, "Measles virus for cancer therapy," *Current Topics in Microbiology and Immunology*, vol. 330, pp. 213–241, 2009.

[17] K. Shah and X. O. Breakefield, "HSV amplicon vectors for cancer therapy," *Current Gene Therapy*, vol. 6, no. 3, pp. 361–370, 2006.

[18] G. Campadelli-Fiume, C. De Giovanni, V. Gatta, P. Nanni, P. L. Lollini, and L. Menotti, "Rethinking herpes simplex virus: the way to oncolytic agents," *Reviews in Medical Virology*, vol. 21, no. 4, pp. 213–226, 2011.

[19] R. Manservigi, R. Argnani, and P. Marconi, "HSV recombinant vectors for gene therapy," *The Open Virology Journal*, vol. 4, pp. 123–156, 2010.

[20] S. J. Turrell and A. Whitehouse, "Mutation of herpesvirus saimiri ORF51 glycoprotein specifically targets infectivity to hepatocellular carcinoma cell lines," *Journal of Biomedicine and Biotechnology*, vol. 2011, Article ID 785158, 14 pages, 2011.

[21] K. Park, W. J. Kim, Y. H. Cho et al., "Cancer gene therapy using adeno-associated virus vectors," *Frontiers in Bioscience*, vol. 13, no. 7, pp. 2653–2659, 2008.

[22] A. Girod, M. Ried, C. Wobus et al., "Genetic capsid modifications allow efficient re-targeting of adeno-associated virus type 2," *Nature Medicine*, vol. 5, no. 9, pp. 1052–1056, 1999.

[23] M. Grifman, M. Trepel, P. Speece et al., "Incorporation of tumor-targeting peptides into recombinant adeno-associated virus capsids," *Molecular Therapy*, vol. 3, no. 6, pp. 964–975, 2001.

[24] W. Shi, G. S. Arnold, and J. S. Bartlett, "Insertional mutagenesis of the adeno-associated virus type 2 (AAV2) capsid gene and generation of AAV2 vectors targeted to alternative cell-surface receptors," *Human Gene Therapy*, vol. 12, no. 14, pp. 1697–1711, 2001.

[25] W. Shi and J. S. Bartlett, "RGD inclusion in VP3 provides adeno-associated virus type 2 (AAV2)-based vectors with a heparan sulfate-independent cell entry mechanism," *Molecular Therapy*, vol. 7, no. 4, pp. 515–525, 2003.

[26] C. Dalba, D. Klatzmann, C. R. Logg, and N. Kasahara, "Beyond oncolytic virotherapy: replication-competent retrovirus vectors for selective and stable transduction of tumors," *Current Gene Therapy*, vol. 5, no. 6, pp. 655–667, 2005.

[27] C. K. Tai and N. Kasahara, "Replication-competent retrovirus vectors for cancer gene therapy," *Frontiers in Bioscience*, vol. 13, no. 8, pp. 3083–3095, 2008.

[28] S. J. Russell and F. L. Cosset, "Modifying the host range properties of retroviral vectors," *Journal of Gene Medicine*, vol. 1, no. 5, pp. 300–311, 1999.

[29] O. Erlwein, W. Wels, and B. S. Schnierle, "Chimeric ecotropic MLV envelope proteins that carry EGF receptor-specific ligands and the Pseudomonas exotoxin a translocation domain to target gene transfer to human cancer cells," *Virology*, vol. 302, no. 2, pp. 333–341, 2002.

[30] M. P. Chadwick, F. J. Morling, F. L. Cosset, and S. J. Russell, "Modification of retroviral tropism by display of IGF-I," *Journal of Molecular Biology*, vol. 285, no. 2, pp. 485–494, 1999.

[31] M. Pizzato, E. D. Blair, M. Fling et al., "Evidence for nonspecific adsorption of targeted retrovirus vector particles to cells," *Gene Therapy*, vol. 8, no. 14, pp. 1088–1096, 2001.

[32] K. Guse, V. Cerullo, and A. Hemminki, "Oncolytic vaccinia virus for the treatment of cancer," *Expert Opinion on Biological Therapy*, vol. 11, no. 5, pp. 595–608, 2011.

[33] M. C. Galmiche, L. Rindisbacher, W. Wels, R. Wittek, and F. Buchegger, "Expression of a functional single chain antibody on the surface of extracellular enveloped vaccinia virus as a step towards selective tumour cell targeting," *Journal of General Virology*, vol. 78, part 11, pp. 3019–3027, 1997.

[34] S. Paul, M. Geist, K. Dott et al., "Specific tumor cell targeting by a recombinant MVA expressing a functional single chain antibody on the surface of intracellular mature virus (IMV) particles," *Viral Immunology*, vol. 20, no. 4, pp. 664–671, 2007.

[35] L. Kuo, G. J. Godeke, M. J. Raamsman, P. S. Masters, and P. J. Rottier, "Retargeting of coronavirus by substitution of the spike glycoprotein ectodomain: crossing the host cell species barrier," *Journal of Virology*, vol. 74, no. 3, pp. 1393–1406, 2000.

[36] H. P. Lesch, M. U. Kaikkonen, J. T. Pikkarainen, and S. Yla-Herttuala, "Avidin-biotin technology in targeted therapy," *Expert Opinion on Drug Delivery*, vol. 7, no. 5, pp. 551–564, 2010.

[37] L. Pereboeva, S. Komarova, J. Roth, S. Ponnazhagan, and D. T. Curiel, "Targeting EGFR with metabolically biotinylated fiber-mosaic adenovirus," *Gene Therapy*, vol. 14, no. 8, pp. 627–637, 2007.

[38] J. W. Park, H. Mok, and T. G. Park, "Epidermal growth factor (EGF) receptor targeted delivery of PEGylated adenovirus," *Biochemical and Biophysical Research Communications*, vol. 366, no. 3, pp. 769–774, 2008.

[39] P. Henning, M. K. Magnusson, E. Gunneriusson et al., "Genetic modification of adenovirus 5 tropism by a novel class of ligands based on a three-helix bundle scaffold derived from staphylococcal protein A," *Human Gene Therapy*, vol. 13, no. 12, pp. 1427–1439, 2002.

[40] P. S. Banerjee, E. S. Zuniga, I. Ojima, and I. S. Carrico, "Targeted and armed oncolytic adenovirus via chemoselective modification," *Bioorganic and Medicinal Chemistry Letters*, vol. 21, no. 17, pp. 4985–4988, 2011.

[41] L. Gigout, P. Rebollo, N. Clement et al., "Altering AAV tropism with mosaic viral capsids," *Molecular Therapy*, vol. 11, no. 6, pp. 856–865, 2005.

[42] M. D. Stachler, I. Chen, A. Y. Ting, and J. S. Bartlett, "Site-specific modification of AAV vector particles with biophysical probes and targeting ligands using biotin ligase," *Molecular Therapy*, vol. 16, no. 8, pp. 1467–1473, 2008.

[43] J. I. Quetglas, M. Ruiz-Guillen, A. Aranda, E. Casales, J. Bezunartea, and C. Smerdou, "Alphavirus vectors for cancer therapy," *Virus Research*, vol. 153, no. 2, pp. 179–196, 2010.

[44] K. Ohno, K. Sawai, Y. Lijima, B. Levin, and D. Meruelo, "Cell-specific targeting of sindbis virus vectors displaying IgG-binding domains of protein A," *Nature Biotechnology*, vol. 15, no. 8, pp. 763–767, 1997.

[45] W. B. Klimstra, J. C. Williams, K. D. Ryman, and H. W. Heidner, "Targeting Sindbis virus-based vectors to Fc receptor-positive cell types," *Virology*, vol. 338, no. 1, pp. 9–21, 2005.

[46] C. K. Tai, C. R. Logg, J. M. Park, W. F. Anderson, M. F. Press, and N. Kasahara, "Antibody-mediated targeting of replication-competent retroviral vectors," *Human Gene Therapy*, vol. 14, no. 8, pp. 789–802, 2003.

[47] J. T. Douglas, B. E. Rogers, M. E. Rosenfeld, S. I. Michael, M. Feng, and D. T. Curiel, "Targeted gene delivery by tropism-modified adenoviral vectors," *Nature Biotechnology*, vol. 14, no. 11, pp. 1574–1578, 1996.

[48] S. J. Watkins, V. V. Mesyanzhinov, L. P. Kurochkina, and R. E. Hawkins, "The "adenobody" approach to viral targeting: specific and enhanced adenoviral gene delivery," *Gene Therapy*, vol. 4, no. 10, pp. 1004–1012, 1997.

[49] C. R. Miller, D. J. Buchsbaum, P. N. Reynolds et al., "Differential susceptibility of primary and established human glioma cells to adenovirus infection: targeting via the epidermal growth factor receptor achieves fiber receptor-independent gene transfer," *Cancer Research*, vol. 58, no. 24, pp. 5738–5748, 1998.

[50] B. E. Rogers, J. T. Douglas, C. Ahlem, D. J. Buchsbaum, J. Frincke, and D. T. Curiel, "Use of a novel cross-linking method to modify adenovirus tropism," *Gene Therapy*, vol. 4, no. 12, pp. 1387–1392, 1997.

[51] C. K. Goldman, B. E. Rogers, J. T. Douglas et al., "Targeted gene delivery to Kaposi's sarcoma cells via the fibroblast growth factor receptor," *Cancer Research*, vol. 57, no. 8, pp. 1447–1451, 1997.

[52] D. L. Gu, A. M. Gonzalez, M. A. Printz et al., "Fibroblast growth factor 2 retargeted adenovirus has redirected cellular tropism: evidence for reduced toxicity and enhanced antitumor activity in mice," *Cancer Research*, vol. 59, no. 11, pp. 2608–2614, 1999.

[53] T. J. Wickham, D. M. Segal, P. W. Roelvink et al., "Targeted adenovirus gene transfer to endothelial and smooth muscle cells by using bispecific antibodies," *Journal of Virology*, vol. 70, no. 10, pp. 6831–6838, 1996.

[54] H. J. Haisma, G. K. Kamps, A. Bouma et al., "Selective targeting of adenovirus to $\alpha v\beta 3$ integrins, VEGFR2 and Tie2 endothelial receptors by angio-adenobodies," *International Journal of Pharmaceutics*, vol. 391, no. 1-2, pp. 155–161, 2010.

[55] H. J. Haisma, H. M. Pinedo, A. Rijswijk et al., "Tumor-specific gene transfer via an adenoviral vector targeted to the pan-carcinoma antigen EpCAM," *Gene Therapy*, vol. 6, no. 8, pp. 1469–1474, 1999.

[56] D. A. Heideman, P. J. Snijders, M. E. Craanen et al., "Selective gene delivery toward gastric and esophageal adenocarcinoma cells via EpCAM-targeted adenoviral vectors," *Cancer Gene Therapy*, vol. 8, no. 5, pp. 342–351, 2001.

[57] D. M. Nettelbeck, A. A. Rivera, J. Kupsch et al., "Retargeting of adenoviral infection to melanoma: combining genetic ablation of native tropism with a recombinant bispecific single-chain diabody (scDb) adapter that binds to fiber knob and HMWMAA," *International Journal of Cancer*, vol. 108, no. 1, pp. 136–145, 2004.

[58] W. Jongmans, K. van den Oudenalder, D. M. Tiemessen et al., "Targeting of adenovirus to human renal cell carcinoma cells," *Urology*, vol. 62, no. 3, pp. 559–565, 2003.

[59] B. W. Tillman, T. D. de Gruijl, S. A. Luykx-de Bakker et al., "Maturation of dendritic cells accompanies high-efficiency gene transfer by a CD40-targeted adenoviral vector," *Journal of Immunology*, vol. 162, no. 11, pp. 6378–6383, 1999.

[60] B. W. Tillman, T. L. Hayes, T. D. DeGruijl, J. T. Douglas, and D. T. Curiel, "Adenoviral vectors targeted to CD40 enhance the efficacy of dendritic cell-based vaccination against human papillomavirus 16-induced tumor cells in a murine model," *Cancer Research*, vol. 60, no. 19, pp. 5456–5463, 2000.

[61] M. Trepel, M. Grifman, M. D. Weitzman, and R. Pasqualini, "Molecular adaptors for vascular-targeted adenoviral gene delivery," *Human Gene Therapy*, vol. 11, no. 14, pp. 1971–1981, 2000.

[62] M. Breidenbach, D. T. Rein, M. Everts et al., "Mesothelin-mediated targeting of adenoviral vectors for ovarian cancer gene therapy," *Gene Therapy*, vol. 12, no. 2, pp. 187–193, 2005.

[63] R. Kraaij, A. L. van Rijswijk, M. H. Oomen, H. J. Haisma, and C. H. Bangma, "Prostate specific membrane antigen (PSMA) is a tissue-specific target for adenoviral transduction of prostate cancer in vitro," *Prostate*, vol. 62, no. 3, pp. 253–259, 2005.

[64] H. J. van Zeeburg, V. W. van Beusechem, A. Huizenga et al., "Adenovirus retargeting to surface expressed antigens on oral mucosa," *Journal of Gene Medicine*, vol. 12, no. 4, pp. 365–376, 2010.

[65] E. Li, S. L. Brown, D. J. von Seggern, G. B. Brown, and G. R. Nemerow, "Signaling antibodies complexed with adenovirus circumvent CAR and integrin interactions and improve gene delivery," *Gene Therapy*, vol. 7, no. 18, pp. 1593–1599, 2000.

[66] S. K. Yoon, L. Mohr, C. R. O'Riordan, A. Lachapelle, D. Armentano, and J. R. Wands, "Targeting a recombinant adenovirus vector to HCC cells using a bifunctional Fab-antibody conjugate," *Biochemical and Biophysical Research Communications*, vol. 272, no. 2, pp. 497–504, 2000.

[67] I. Dmitriev, E. Kashentseva, B. E. Rogers, V. Krasnykh, and D. T. Curiel, "Ectodomain of coxsackievirus and adenovirus receptor genetically fused to epidermal growth factor mediates adenovirus targeting to epidermal growth factor receptor-positive cells," *Journal of Virology*, vol. 74, no. 15, pp. 6875–6884, 2000.

[68] J. G. Wesseling, P. J. Bosma, V. Krasnykh et al., "Improved gene transfer efficiency to primary and established human pancreatic carcinoma target cells via epidermal growth factor receptor and integrin-targeted adenoviral vectors," *Gene Therapy*, vol. 8, no. 13, pp. 969–976, 2001.

[69] C. Ebbinghaus, A. Al-Jaibaji, E. Operschall et al., "Functional and selective targeting of adenovirus to high-affinity Fcγ receptor I-positive cells by using a bispecific hybrid adapter," *Journal of Virology*, vol. 75, no. 1, pp. 480–489, 2001.

[70] E. A. Kashentseva, T. Seki, D. T. Curiel, and I. P. Dmitriev, "Adenovirus targeting to c-erbB-2 oncoprotein by single-chain antibody fused to trimeric form of adenovirus receptor ectodomain," *Cancer Research*, vol. 62, no. 2, pp. 609–616, 2002.

[71] H. J. Li, M. Everts, L. Pereboeva et al., "Adenovirus tumor targeting and hepatic untargeting by a coxsackie/adenovirus receptor ectodomain anticarcinoembryonic antigen bispecific adapter," *Cancer Research*, vol. 67, no. 11, pp. 5354–5361, 2007.

[72] N. K. Green, J. Morrison, S. Hale et al., "Retargeting polymer-coated adenovirus to the FGF receptor allows productive infection and mediates efficacy in a peritoneal model of human ovarian cancer," *Journal of Gene Medicine*, vol. 10, no. 3, pp. 280–289, 2008.

[73] J. Moselhy, S. Sarkar, M. C. Chia et al., "Evaluation of copolymers of N-isopropylacrylamide and 2-dimethyl(aminoethyl)methacrylate in nonviral and adenoviral vectors for gene delivery to nasopharyngeal carcinoma," *International Journal of Nanomedicine*, vol. 2, no. 3, pp. 461–478, 2007.

[74] J. Han, D. Zhao, Z. Zhong, Z. Zhang, T. Gong, and X. Sun, "Combination of adenovirus and cross-linked low molecular weight PEI improves efficiency of gene transduction," *Nanotechnology*, vol. 21, no. 10, Article ID 105106, 2010.

[75] H. Bachtarzi, M. Stevenson, V. Subr, K. Ulbrich, L. W. Seymour, and K. D. Fisher, "Targeting adenovirus gene delivery to activated tumour-associated vasculature via endothelial selectins," *Journal of Controlled Release*, vol. 150, no. 2, pp. 196–203, 2011.

[76] K. D. Fisher, N. K. Green, A. Hale, V. Subr, K. Ulbrich, and L. W. Seymour, "Passive tumour targeting of polymer-coated adenovirus for cancer gene therapy," *Journal of Drug Targeting*, vol. 15, no. 7-8, pp. 546–551, 2007.

[77] M. Stevenson, A. B. Hale, S. J. Hale et al., "Incorporation of a laminin-derived peptide (SIKVAV) on polymer-modified adenovirus permits tumor-specific targeting via $\alpha 6$-integrins," *Cancer Gene Therapy*, vol. 14, no. 4, pp. 335–345, 2007.

[78] J. Lanciotti, A. Song, J. Doukas et al., "Targeting adenoviral vectors using heterofunctional polyethylene glycol FGF2 conjugates," *Molecular Therapy*, vol. 8, no. 1, pp. 99–107, 2003.

[79] Y. Eto, J. Q. Gao, F. Sekiguchi et al., "PEGylated adenovirus vectors containing RGD peptides on the tip of PEG show high transduction efficiency and antibody evasion ability," *Journal of Gene Medicine*, vol. 7, no. 5, pp. 604–612, 2005.

[80] J. Q. Gao, Y. Eto, Y. Yoshioka et al., "Effective tumor targeted gene transfer using PEGylated adenovirus vector via systemic administration," *Journal of Controlled Release*, vol. 122, no. 1, pp. 102–110, 2007.

[81] Y. Jung, H. J. Park, P. H. Kim et al., "Retargeting of adenoviral gene delivery via Herceptin-PEG-adenovirus conjugates to breast cancer cells," *Journal of Controlled Release*, vol. 123, no. 2, pp. 164–171, 2007.

[82] C. Y. Chen, S. M. May, and M. A. Barry, "Targeting adenoviruses with factor X-single-chain antibody fusion proteins," *Human Gene Therapy*, vol. 21, no. 6, pp. 739–749, 2010.

[83] J. S. Bartlett, J. Kleinschmidt, R. C. Boucher, and R. J. Samulski, "Targeted adeno-associated virus vector transduction of nonpermissive cells mediated cells by a bispecific F(ab'γ)2 antibody," *Nature Biotechnology*, vol. 17, no. 2, pp. 181–186, 1999.

[84] K. Nakano, R. Asano, K. Tsumoto et al., "Herpes simplex virus targeting to the EGF receptor by a gD-Specific soluble bridging molecule," *Molecular Therapy*, vol. 11, no. 4, pp. 617–626, 2005.

[85] H. Bian, P. Fournier, R. Moormann, B. Peeters, and V. Schirrmacher, "Selective gene transfer in vitro to tumor cells via recombinant newcastle disease virus," *Cancer Gene Therapy*, vol. 12, no. 3, pp. 295–303, 2005.

[86] H. Bian, H. Wilden, P. Fournier, B. Peeters, and V. Schirrmacher, "In vivo efficacy of systemic tumor targeting of a viral RNA vector with oncolytic properties using a bispecific adapter protein," *International Journal of Oncology*, vol. 29, no. 6, pp. 1359–1369, 2006.

[87] B. J. Haijema, H. Volders, and P. J. Rottier, "Switching species tropism: an effective way to manipulate the feline coronavirus genome," *Journal of Virology*, vol. 77, no. 8, pp. 4528–4538, 2003.

[88] T. Wurdinger, M. H. Verheije, M. Raaben et al., "Targeting non-human coronaviruses to human cancer cells using a bispecific single-chain antibody," *Gene Therapy*, vol. 12, no. 18, pp. 1394–1404, 2005.

[89] T. Wurdinger, M. H. Verheije, K. Broen et al., "Soluble receptor-mediated targeting of mouse hepatitis coronavirus to the human epidermal growth factor receptor," *Journal of Virology*, vol. 79, no. 24, pp. 15314–15322, 2005.

[90] M. H. Verheije, M. L. Lamfers, T. Wurdinger et al., "Coronavirus genetically redirected to the epidermal growth factor receptor exhibits effective antitumor activity against a malignant glioblastoma," *Journal of Virology*, vol. 83, no. 15, pp. 7507–7516, 2009.

[91] A. Hemminki, I. Dmitriev, B. Liu, R. A. Desmond, R. Alemany, and D. T. Curiel, "Targeting oncolytic adenoviral agents to the epidermal growth factor pathway with a secretory fusion molecule," *Cancer Research*, vol. 61, no. 17, pp. 6377–6381, 2001.

[92] V. W. van Beusechem, D. C. Mastenbroek, P. B. van den Doel et al., "Conditionally replicative adenovirus expressing a targeting adapter molecule exhibits enhanced oncolytic potency on CAR-deficient tumors," *Gene Therapy*, vol. 10, no. 23, pp. 1982–1991, 2003.

[93] J. E. Carette, H. C. Graat, F. H. Schagen et al., "A conditionally replicating adenovirus with strict selectivity in killing cells expressing epidermal growth factor receptor," *Virology*, vol. 361, no. 1, pp. 56–67, 2007.

[94] D. A. Einfeld, R. Schroeder, P. W. Roelvink et al., "Reducing the native tropism of adenovirus vectors requires removal of both CAR and integrin interactions," *Journal of Virology*, vol. 75, no. 23, pp. 11284–11291, 2001.

[95] A. Hemminki, M. Wang, T. Hakkarainen, R. A. Desmond, J. Wahlfors, and D. T. Curiel, "Production of an EGFR targeting molecule from a conditionally replicating adenovirus impairs its oncolytic potential," *Cancer Gene Therapy*, vol. 10, no. 8, pp. 583–588, 2003.

[96] J. N. Glasgow, G. Mikheeva, V. Krasnykh, and D. T. Curiel, "A strategy for adenovirus vector targeting with a secreted single chain antibody," *Plos One*, vol. 4, no. 12, Article ID e8355, 2009.

[97] V. W. Van Beusechem, J. Grill, D. C. Jeroen Mastenbroek et al., "Efficient and selective gene transfer into primary human brain tumors by using single-chain antibody-targeted adenoviral vectors with native tropism abolished," *Journal of Virology*, vol. 76, no. 6, pp. 2753–2762, 2002.

[98] S. D. Barker, I. P. Dmitriev, D. M. Nettelbeck et al., "Combined transcriptional and transductional targeting improves the specificity and efficacy of adenoviral gene delivery to ovarian carcinoma," *Gene Therapy*, vol. 10, no. 14, pp. 1198–1204, 2003.

[99] M. Everts, S. A. Kim-Park, M. A. Preuss et al., "Selective induction of tumor-associated antigens in murine pulmonary vasculature using double-targeted adenoviral vectors," *Gene Therapy*, vol. 12, no. 13, pp. 1042–1048, 2005.

[100] H. J. Li, M. Everts, M. Yamamoto, D. T. Curiel, and H. R. Herschman, "Combined transductional untargeting/retargeting and transcriptional restriction enhances adenovirus gene targeting and therapy for hepatic colorectal cancer tumors," *Cancer Research*, vol. 69, no. 2, pp. 554–564, 2009.

[101] P. N. Reynolds, S. A. Nicklin, L. Kaliberova et al., "Combined transductional and transcriptional targeting improves the specificity of transgene expression in vivo," *Nature Biotechnology*, vol. 19, no. 9, pp. 838–842, 2001.

[102] P. N. Reynolds, K. R. Zinn, V. D. Gavrilyuk et al., "A targetable, injectable adenoviral vector for selective gene delivery to pulmonary endothelium in vivo," *Molecular Therapy*, vol. 2, no. 6, pp. 562–578, 2000.

[103] M. H. Verheije, T. Wurdinger, V. W. van Beusechem, C. A. de Haan, W. R. Gerritsen, and P. J. Rottier, "Redirecting coronavirus to a nonnative receptor through a virus-encoded targeting adapter," *Journal of Virology*, vol. 80, no. 3, pp. 1250–1260, 2006.

[104] C. A. de Haan, L. van Genne, J. N. Stoop, H. Volders, and P. J. Rottier, "Coronaviruses as vectors: position dependence of foreign gene expression," *Journal of Virology*, vol. 77, no. 21, pp. 11312–11323, 2003.

[105] K. J. Kimball, M. A. Preuss, M. N. Barnes et al., "A phase I study of a tropism-modified conditionally replicative adenovirus for recurrent malignant gynecologic diseases," *Clinical Cancer Research*, vol. 16, no. 21, pp. 5277–5287, 2010.

[106] D. Koppers-Lalic and R. C. Hoeben, "Non-human viruses developed as therapeutic agent for use in humans," *Reviews in Medical Virology*, vol. 21, no. 4, pp. 227–239, 2011.

[107] N. K. Green, C. W. Herbert, S. J. Hale et al., "Extended plasma circulation time and decreased toxicity of polymer-coated adenovirus," *Gene Therapy*, vol. 11, no. 16, pp. 1256–1263, 2004.

[108] H. Nakashima, B. Kaur, and E. A. Chiocca, "Directing systemic oncolytic viral delivery to tumors via carrier cells," *Cytokine and Growth Factor Reviews*, vol. 21, no. 2-3, pp. 119–126, 2010.

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 186512, 8 pages
doi:10.1155/2012/186512

Review Article

Oncolytic Virotherapy for Hematological Malignancies

Swarna Bais,[1] Eric Bartee,[2] Masmudur M. Rahman,[2] Grant McFadden,[2] and Christopher R. Cogle[1]

[1] *Division of Hematology and Oncology, Department of Medicine, College of Medicine, University of Florida, ARB R4-202, P.O. Box 100278, Gainesville, FL 32610-0278, USA*
[2] *Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, ARB R4-295, P.O. Box 100266, Gainesville, FL 32610, USA*

Correspondence should be addressed to Grant McFadden, grantmcf@ufl.edu and Christopher R. Cogle, c@ufl.edu

Received 6 July 2011; Accepted 31 August 2011

Academic Editor: Nanhai G. Chen

Hematological malignancies such as leukemias, lymphomas, multiple myeloma (MM), and the myelodysplastic syndromes (MDSs) primarily affect adults and are difficult to treat. For high-risk disease, hematopoietic stem cell transplant (HCT) can be used. However, in the setting of autologous HCT, relapse due to contamination of the autograft with cancer cells remains a major challenge. *Ex vivo* manipulations of the autograft to purge cancer cells using chemotherapies and toxins have been attempted. Because these past strategies lack specificity for malignant cells and often impair the normal hematopoietic stem and progenitor cells, prior efforts to *ex vivo* purge autografts have resulted in prolonged cytopenias and graft failure. The ideal *ex vivo* purging agent would selectively target the contaminating cancer cells while spare normal stem and progenitor cells and would be applied quickly without toxicities to the recipient. One agent which meets these criteria is oncolytic viruses. This paper details experimental progress with reovirus, myxoma virus, measles virus, vesicular stomatitis virus, coxsackievirus, and vaccinia virus as well as requirements for translation of these results to the clinic.

1. Hematological Malignancies

Hematological malignancies include leukemias, lymphomas, multiple myeloma (MM), and the myelodysplastic syndromes (MDSs) that most often affect individuals older than 60 years of age. These blood cancers affect approximately 10% of Americans diagnosed with cancer each year, and an estimated 140,000 were newly diagnosed in 2010 (National Cancer Institute, Surveillance Epidemiology, and End Results). Unfortunately, despite best available therapies, an estimated 50,000 individuals died from these diseases in 2010.

The causes of hematological cancers vary depending on the specific malignancy. Exposure to environmental toxins such as benzenes, prior cytotoxic treatment such as radiotherapy or chemotherapy for an antecedent cancer, as well as infections have all been implicated as causative factors in initiating hematological malignancies. In contrast, recurrent cytogenetic abnormalities have also been observed in hematological malignancies. These abnormalities often form the basis for assigning prognosis. For example, in acute myeloid leukemia (AML), recurrent mutations that portend for a high risk of relapse after conventional treatment include those with chromosome 7 abnormalities, chromosome 5 abnormalities, complex karyotypic abnormalities, and mutations in the *FLT3* gene. Genetic information can also indicate the most appropriate therapy. For instance, in patients with acute promyelocytic leukemia with the abnormal *PML-RARA* gene fusion, treatment with all transretinoic acid (ATRA) and cytotoxic chemotherapy can cure approximately 90% of patients [1]. In patients with MDS and deletion of chromosome 5q, treatment with lenalidomide can improve blood counts in 75% of patients [2].

Based on the utility of genetic information in determining prognosis and type of treatment in hematological malignancies, increased attention has been given to fully assessing the blood cancer genome. Recently, whole genome sequencing of an AML patient's DNA revealed several novel mutations never before associated with oncogenesis [3]. This technology also recently led to the discovery of *TET2*

mutations as common gene mutations in MDS and emphasized the importance of epigenetic dysregulation in this disease [4, 5]. Because of the abnormal DNA methylation that occurs after *TET2* mutations, finding this mutation in an MDS patient's genome may indicate treatment with a hypomethylating agent such as azacitidine or decitabine [6]. Recently, whole genome sequencing was reported useful in determining the best treatment for a patient with AML [7]. Thus, genome analysis has the strong potential for personalized medicine in hematological malignancies.

In some hematological malignancies, such as MDS, abnormalities in bone marrow stromal cells are believed to affect hematopoietic stem and progenitor cells, leading to neoplastic transformation [8]. Evidence that the bone marrow microenvironment is an important factor in the oncogenesis of hematological malignancies has spurred great interest in regulating microenvironmental interactions as a means for improved therapies. We have targeted blood vessels in the leukemia niche with the novel vascular disrupting combretastatin, OXi4503, and have successfully regressed disease [9]. This work has been translated into a phase I clinical study (http://www.ClinicalTrials.gov Identifier NCT01085656).

Cancer stem cells have been identified for some hematological malignancies [10]. In the specific case of acute myeloid leukemia (AML), a small subpopulation of cancer stem cells have been identified in the CD34+CD38−CD123+ fraction [11, 12]. In MM, myeloma stem cells have been found in the CD138− B cell fraction, which replicate and differentiate into CD138+ malignant plasma cells [13]. In chronic myeloid leukemia (CML), hematopoietic progenitor cells are believed to be the cancer-initiating cells which are endowed with cancer stem cell properties after acquiring the abnormal *BCR/ABL* gene fusion [14]

2. Treatment of Hematological Malignancies

The cornerstone of conventional therapy for hematological malignancies includes agents that block cell division such as antimetabolites (e.g., cytarabine), DNA alkylating agents (e.g., cyclophosphamide), and anthracyclines (e.g., daunorubicin). Treatment with these agents induces initial remission in a high percentage of patients; however, relapsed disease remains a major challenge in treating patients with hematological malignancies.

For example, in cases of AML, remission rates with standard induction chemotherapy such as seven days of continuously infused cytarabine and three days of anthracycline bring about initial complete remissions in approximately 30–70% of patients. However, in older individuals, who are more commonly diagnosed with AML, long-term prognosis can be grim with only 10–20% of patients surviving without disease [15]. For patients with high risk AML, allogeneic HCT is used and can be curative in approximately 40% of patients. With this procedure, the donor immune system recognizes any residual leukemia in the recipient as foreign because of minor human leukocyte antigen mismatches and/or unique AML antigens, resulting in elimination and persistent surveillance for the malignant cells. By similar mechanisms, the donor immune system can recognize the recipient's normal organs (skin, gastrointestinal tract, liver, lungs, joints) as foreign and elicit graft versus host disease (GVHD). Although potentially curative, most AML patients due to their age-related comorbidities are not fit for the high risks associated with allogeneic HCT (e.g., GVHD, life-threatening infections, organ toxicity) and/or do not have a suitable allogeneic stem cell donor. Experimental therapies for AML have recently included specific mutation-targeting agents such as FLT3 inhibitors for patients with internal tandem duplications in the *FLT3* gene of the AML clone. However, results from these clinical trials have been disappointing.

For patients with MM, treatment decisions are often based on risk for refractory and relapsed disease. Certain chromosome abnormalities, such as deletion of chromosome 13, portend for poor prognosis. In addition, gene expression profiling can be used to risk-stratify MM disease [16]. For patients with standard risk MM, initial treatment is dependent on the patient's eligibility for high-dose chemotherapy followed by autologous HCT, which can prolong disease-free and overall survival but carries treatment-related risks of organ toxicity, need for transfusions, and life-threatening infection. Patients eligible for autologous HCT are treated with nonalkylating agent induction therapies such as thalidomide and dexamethasone or lenalidomide and dexamethasone [17, 18]. After this initial therapy, patients have the option of early versus delayed autologous HCT. If early HCT is used, then a second HCT can be performed in patients who do not achieve a very good partial remission or better [19]. If the patient elects for delayed high-dose chemotherapy followed by autologous HCT, then transplant is not performed until initial induction therapy brings about a plateau in response or progressed disease develops. Even using these treatments, however, autologous HCT rarely brings about cures for MM, as the disease nearly always relapses.

3. Autograft Contamination and Disease Relapse after Transplant

Despite the significant increase in use of autologous HCT for hematologic malignancies, disease relapse is a primary cause of death after transplant. Graft contamination is thought to be the chief reason for posttransplant relapse. This premise is supported by multiple lines of evidence. First, transplant of HSPC from syngeneic (identical twin) donors leads to lower incidences of disease relapse in patients with multiple myeloma, low-grade non-Hodgkin's lymphoma, AML, and ALL [20–22]. Second, numerous reports show that transplanted autografts contain minimal residual disease (MRD) in a variety of patients with cancer [23–39]. The level of MRD, detected by flow cytometry, immunohistochemistry, and molecular methods, directly correlated with risk of disease relapse and death. Whereas these lines of evidence show a strong correlation, direct proof of contaminated autografts through tracing studies are most compelling. Thus, the third line of evidence comes from gene

marking studies [40–42]. In these clinical studies, autologous HSPCs were genetically tagged and then transplanted. Relapsed disease was evaluated for the tag. In a variety of leukemias and cancers, the posttransplant relapsed disease contained the pretransplant tag. Together, these lines of evidence support the premise that contaminating cells within the autologous transplant graft can be the origin of relapsed disease after transplant.

4. Purging Strategies

Considering the high rates of refractory and relapsed in patients with hematological malignancies and evidence of contaminating cancer cells in autologous HCT grafts, it is possible that graft purging of contaminating cells may improve posttransplant disease-free and overall survival. Ideally, a safe and effective purging strategy should specifically target the contaminating malignant clone and spare normal HSPC needed for reconstitution of immunity, erythropoieis, and platelets.

Several purging strategies have been attempted to selectively target malignant cells from autologous HCT grafts. One strategy is to treat the autologous graft after collection but prior to transplant back into the patient. A number of these *ex vivo* purging techniques have been tested such as:

(i) chemotherapy with antiproliferative drugs such mafosfamide and 4-hydroperoxycyclophosphamide (4-HC, active metabolite of cyclophosphamide) [43, 44];

(ii) CD34+ stem/progenitor cell enrichment using immunomagnetic selection [45];

(iii) immunotoxins or hybrid cytotoxic proteins designed to selectively kill cancer cells such as heregulin (HRG) *Pseudomonas* exotoxin (PE) 40 [46];

(iv) immunomagnetic removal of tumor cells when the tumor cells express a unique antigen [47];

(v) monoclonal antibodies such as alemtuzumab (anti-CD52) and rituximab (anti-CD20) [48];

(vi) photodynamic purging by rhodamine [49].

Unfortunately most of these *ex vivo* purging techniques also impaired normal hematopoietic stem and progenitor (HSPC) function and therefore have not translated to routine clinical practice for autologous HCT [50]. Purging strategies utilizing cytotoxic chemotherapy can be nonselective to cancer cells, and HSPC can be susceptible to the cytotoxic drugs [43, 44]. Immunomagnetic selection based on one cell surface marker (i.e., CD34) can enrich for normal HSPC; however, this selection process is never 100%, and the positive fraction may contain contaminating cancer cells [45, 47]. Moreover, discriminating normal from malignant HSPC can be difficult when using just cell surface markers because the two populations are sometimes indistinguishable by immunophenotyping. Given similarities in immunophenotype, immunotoxins may target both malignant and normal HSPC, leading to impaired normal hematopoiesis and posttransplant hematopoietic reconstitution [46, 48].

Ideally, *ex vivo* purging is selective for the cancer cells yet spares normal HSPC. Moreover, the ideal purging technique should be applied quickly (within minutes-to-hours) so that the transplant process is not delayed and any modifications to standard transplantation protocols are minimized. Cell viability is a time-dependent variable, and the quicker the manipulation the higher cell viability for transplant. In the postthaw setting, cell viability can diminish within hours, thus purging techniques applied to thawed products should be especially time sensitive in order to provide patients with the highest cell viability for transplant.

5. Oncolytic Virotherapy for Hematological Malignancies

Oncolytic viruses may meet criteria as ideal purging agents for hematological malignancies. Specifically, certain oncolytic viruses selectively target malignant hematopoietic cells such as multiple myeloma and leukemia cells while sparing normal HSPCs [51]. This capacity to purge autologous HCT grafts makes oncolytic viruses particular attractive for potential use in the clinical transplant setting (Figure 1). A few oncolytic viruses have already been translated into the clinic (Table 1).

One potential purging agent is coxsackievirus A21 (CVA21) based on its ability to selectively target hematological malignant cells [52]. CVA21, a common enterovirus, exhibited a potent cytostatic and cytocidal effect against three MM cell lines with reduced cytotoxicity against normal human peripheral blood mononuclear cells (PBMCs) [53]. CVA21 specificity is believed to be related to expression of intercellular adhesion molecule-1 (ICAM-1) and decay accelerating factor (DAF) on the surface of target cells. While the immunocompromised status of MM patients receiving chemotherapy poses a concern for the use of virotherapy, it may be in these patients that CVA21 virotherapy will have the most successful outcome due to the lack of antiviral immunity. Disseminated CVA21 infection can be controlled by antiviral compounds, such as pleconaril [54] or immunoglobulin [55]. CVA21 has already been administered to end-stage melanoma patients without adverse effects [56], and further human trials are currently underway to evaluate safety.

Another potential oncolytic virus for the treatment of hematological malignancies is reovirus [57]. Reovirus is a double-stranded RNA virus that is replication competent and preferentially infects cells with hyperactivated signaling, for example, in the Ras pathway. When reovirus was used to *ex vivo* purge MM cells from admixtures of apheresis products, purging was incomplete: only 50% of the MM cells were effectively purged. Also, reovirus was unable to purge follicular lymphoma and Burkitt's lymphoma cells [58]. A major advantage with reovirus is that it does not affect normal HSPCs. Therefore, reovirus may have potential in certain hematological malignancies, but it remains to be defined how clinically effective the virus is at eliminating each type of cancer.

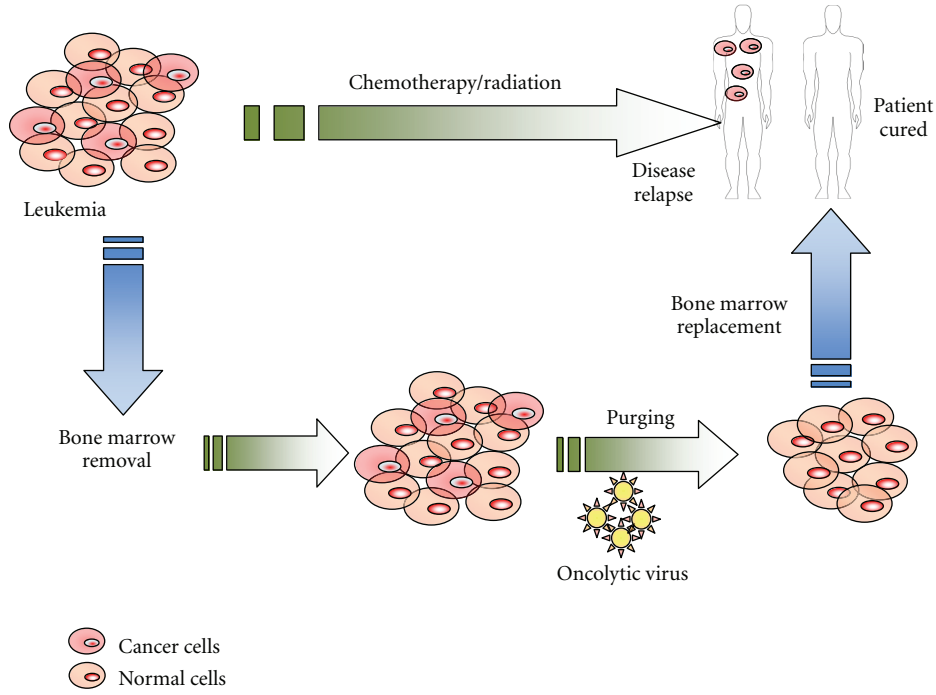


Figure 1: Proposed treatment schema of oncolytic virotherapy for patients with hematological malignancies undergoing high-dose chemotherapy and autologous HCT.

Vesicular stomatitis virus (VSV) is another virus with oncolytic potential [59]. This negative strand RNA virus lacks toxicity for HSPCs in culture and has oncolytic activity against AML cell lines. Moreover, VSV can purge MM from mobilized PBSC CD34+ cells [60].

The Edmonston-B vaccine strain of measles virus (MV-Edm) also has reported oncolytic activity against MM. Using six clinical MM samples and a transplant model into immunodeficient mice, this measles virus successfully purged myeloma cells [61]. The intrinsic tumor selective cytotoxicity is an attractive feature of this agent. They also noted that administration of MV-Edm into MV-susceptible transgenic mice expressing the human CD46 receptor resulted in infection of macrophages in spleen, lymph nodes, and peritoneal cavity [62]. To enhance virus specificity, they generated an anti-CD38 scFv and demonstrated that display of scFv redirected virus binding and entry into CD38 receptor positive cells that were devoid of natural measles receptors [63]. The MV-Edm virus is currently in a phase I clinical study for recurrent or refractory MM where it is administered systemically via intravenous route along with cyclophosphamide chemotherapy (http://www.ClinicalTrials.gov ID NCT00450814) [64]. In this trial, the investigators are using the MV-NIS Edmonston lineage which was genetically engineered to express the human sodium iodide symporter (NIS). Insertion of the NIS protein into MV enables pharmacokinetic monitoring of the virus by means of radioactive iodine (^{123}I) administration. Cells infected with MV-NIS will show increased uptake of the radioactive iodine, and this uptake can be serially tracked in real time. The patient's normal thyroid function is protected by coadministration of a normal thyroid hormone, triiodothyronine (T3).

Table 1: Oncolytic viruses for the treatment of hematological malignancies.

Virus	Disease targets	Clinical studies	References
Reovirus	MM, NHL, CLL	In development	[57]
Myxoma virus	AML, MM	In development	[51]
Measles virus	MM	Ongoing (NCT00450814)	[64]
Vaccinia virus	MM	Case report	[76]

MM: multiple myeloma; NHL: non-Hodgkin's lymphoma; CLL: chronic lymphocytic leukemia; AML: acute myeloid leukemia.

Live attenuated measles virus (MV) has potent oncolytic activity against MM tumor xenografts. The virus is tumor selective and preferentially targets cells that express high levels of CD46 receptors [65]. A vaccine strain of MV causes regression of large established human lymphoma xenografts in immunodeficient mice. MV is a negative-strand RNA virus, and, interestingly, the presence of anti-MV antibodies does not compromise the oncolytic effect of MV [66].

Adachi et al. reported a midkine promoter based conditionally replicative adenovirus (Ad) for the treatment of pediatric solid tumors and bone marrow tumor purging. A conditionally replicative Ad in which the expression of E1 is controlled by the MK promoter achieved high levels of viral replication in neuroblastoma or Ewing's sarcoma cells and induced tumor cell killing. No damage to CD34+ cells was

seen, even after three hours of infection at 1000 MOI [67]. Adenovirus serotype 5 (Ad5) and other low-seroprevalence adenoviruses may have utility as oncolytic agents against MM and other hematological malignancies [68].

Tumor-specific double-deleted Vaccinia virus has also been tested in multiple myeloma [69]. Esfandyari et al. were the first to document permissiveness of lymphoma cells to oncolytic herpes viruses and introduced ELK as a suitable factor for predicting tumor susceptibility to novel anticancer agents [70].

Oncolytic rat parvovirus, H-1PV, may be a potential candidate for the treatment of some non-Hodgkin's B-cell lymphomas, including those resistant to apoptosis induction by rituximab. H-1PV efficiently killed through necrosis while sparing normal B lymphocytes [71].

Recently, we showed that myxoma virus (MYXV) has the capacity to selectively target primary human leukemia cells while spare normal HSPCs [51]. Poxviruses such as MYXV can bind and initiate entry into most mammalian cells but then discriminates permissive versus nonpermissive cells by virtue of the cell signaling circuitry of the infected cell. We have shown that upregulated AKT signaling, either as constitutive phosphorylation or induced by virus infection [72], regulates MYXV permissiveness in a wide variety of human solid tumor cell lines [73]. Considering the complexity and heterogeneity of cancer cells, this pathway is likely not the only mechanism for cancer cell specificity and there may be other mechanisms to explain the virus' discrimination between leukemia cells and normal HSPC. For example, when normal macrophages are infected with MYXV, the cells rapidly coinduce two antiviral cytokines (tumor necrosis factor and type I interferon) by a RIG-I-dependent signaling mechanism, which then aborts MYXV infection in normal somatic cells in a paracrine-like manner [74]. Thus, it could be that normal HSPCs are competent for this synergy, whereas malignant HSPCs, such as AML cells, are defective in some aspect of the tumor necrosis factor/interferon pathway. The mechanism for selective killing of cancer is still being studied, but two important factors include (1) most human cancer cells lack type I IFN and TNF synergy responses [75] and (2) most cancer cells have excessive levels of activated Akt, which facilitates MYXV replication [73].

6. Clinical Translation of Oncolytic Viruses as Purging Agents for HCT

For successful clinical translation, there are some unique requirements for oncolytic virotherapy in the setting of purging cancer cells prior to HCT. First, the OV must spare normal HSPCs. Second, the purging strategy should be simple and quick, especially when using cryopreserved stem cell products. After thawing autologous cryopreserved HSPC, cell viability decreases quickly (within minutes to an hour); thus any postthaw intervention must be quickly performed to ensure transplant of an adequate number of viable HSPC. Finally, the oncolytic virus must show limited to no infection of recipient somatic cells or tissues considering that all transplant recipients are highly immunocompromised after high-dose chemotherapy and autologous HCT.

In addition to showing preclinical safety and efficacy, the translation of oncolytic virotherapy for hematological malignancies will also require the ability to massively scale up manufacture of clinical grade virus under good manufacturing process (GMP) conditions. This process will necessitate facilities with expertise in virus production.

Currently, there are no clinical studies of oncolytic viruses as purging agents prior to autologous HCT. However, if a virus system can be optimized to meet minimum clinical criteria, then oncolytic virotherapy would have major impact in how we treat patients with blood cancers. Already, promising experimental progress indicates that early phase clinical studies of oncolytic viruses as purging agents for HCT are imminently approaching.

Acknowledgments

This work is supported by grants from the Bankhead Coley Foundation (1BT02) and NCI (CA138541-01).

References

[1] B. U. Mueller, T. Pabst, J. Fos et al., "ATRA resolves the differentiation block in t(15;17) acute myeloid leukemia by restoring PU.1 expression," *Blood*, vol. 107, no. 8, pp. 3330–3338, 2006.

[2] R. S. Komrokji and A. F. List, "Lenalidomide for treatment of myelodysplastic syndromes: current status and future directions," *Hematology/Oncology Clinics of North America*, vol. 24, no. 2, pp. 377–388, 2010.

[3] D. C. Link, L. G. Schuettpelz, D. Shen et al., "Identification of a novel TP53 cancer susceptibility mutation through whole-genome sequencing of a patient with therapy-related AML," *Journal of the American Medical Association*, vol. 305, no. 15, pp. 1568–1576, 2011.

[4] M. Ko, Y. Huang, A. M. Jankowska et al., "Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2," *Nature*, vol. 468, no. 7325, pp. 839–843, 2010.

[5] R. Itzykson, O. Kosmider, T. Cluzeau et al., "Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias," *Leukemia*, vol. 25, no. 7, pp. 1147–1152, 2011.

[6] R. Itzykson, S. Thépot, V. Eclache et al., "Prognostic significance of monosomal karyotype in higher risk myelodysplastic syndrome treated with azacitidine," *Leukemia*, vol. 25, no. 7, pp. 1207–1209, 2011.

[7] J. S. Welch, P. Westervelt, L. Ding et al., "Use of whole-genome sequencing to diagnose a cryptic fusion oncogene," *Journal of the American Medical Association*, vol. 305, no. 15, pp. 1577–1584, 2011.

[8] M. H. G. P. Raaijmakers, S. Mukherjee, S. Guo et al., "Bone progenitor dysfunction induces myelodysplasia and secondary leukaemia," *Nature*, vol. 464, no. 7290, pp. 852–857, 2010.

[9] G. J. Madlambayan, A. M. Meacham, K. Hosaka et al., "Leukemia regression by vascular disruption and antiangiogenic therapy," *Blood*, vol. 116, no. 9, pp. 1539–1547, 2010.

[10] R. C. Sanders Jr., W. B. Slayton, C. R. Cogle, R. C. Fisher, and E. W. Scott, "Stem cell research," *Paediatric Respiratory Reviews*, vol. 7, no. 2, pp. 135–140, 2006.

[11] J. L. McKenzie, K. Takenaka, O. I. Gan, M. Doedens, and J. E. Dick, "Low rhodamine 123 retention identifies long-term human hematopoietic stem cells within the Lin-CD34+CD38- population," *Blood*, vol. 109, no. 2, pp. 543–545, 2007.

[12] F. Goodrum, C. T. Jordan, S. S. Terhune, K. High, and T. Shenk, "Differential outcomes of human cytomegalovirus infection in primitive hematopoietic cell subpopulations," *Blood*, vol. 104, no. 3, pp. 687–695, 2004.

[13] W. Matsui, C. A. Huff, Q. Wang et al., "Characterization of clonogenic multiple myeloma cells," *Blood*, vol. 103, no. 6, pp. 2332–2336, 2004.

[14] D. Perrotti, C. Jamieson, J. Goldman, and T. Skorski, "Chronic myeloid leukemia: mechanisms of blastic transformation," *Journal of Clinical Investigation*, vol. 120, no. 7, pp. 2254–2264, 2010.

[15] American Cancer Society, *Cancer Facts & Figures*, The Society, Atlanta, Ga, USA, 2011.

[16] A. K. Stewart and R. Fonseca, "Prognostic and therapeutic significance of myeloma genetics and gene expression profiling," *Journal of Clinical Oncology*, vol. 23, no. 26, pp. 6339–6344, 2005.

[17] S. V. Rajkumar, E. Blood, D. Vesole, R. Fonseca, and P. R. Greipp, "Phase III clinical trial of thalidomide plus dexamethasone compared with dexamethasone alone in newly diagnosed multiple myeloma: a clinical trial coordinated by the eastern cooperative oncology group," *Journal of Clinical Oncology*, vol. 24, no. 3, pp. 431–436, 2006.

[18] S. V. Rajkumar, S. Jacobus, N. S. Callander et al., "Lenalidomide plus high-dose dexamethasone versus lenalidomide plus low-dose dexamethasone as initial therapy for newly diagnosed multiple myeloma: an open-label randomised controlled trial," *The Lancet Oncology*, vol. 11, no. 1, pp. 29–37, 2010.

[19] B. Barlogie, M. Attal, J. Crowley et al., "Long-term follow-up of autotransplantation trials for multiple myeloma: update of protocols conducted by the intergroupe francophone du myelome, southwest oncology group, and university of arkansas for medical sciences," *Journal of Clinical Oncology*, vol. 28, no. 7, pp. 1209–1214, 2010.

[20] G. Gahrton, H. Svensson, B. Björkstrand et al., "Syngeneic transplantation in multiple myeloma—a case-matched comparison with autologous and allogeneic transplantation," *Bone Marrow Transplantation*, vol. 24, no. 7, pp. 741–745, 1999.

[21] V. Lj Lazarevic, H. Högglund, M. Remberger et al., "Long-term survival following allogeneic or syngeneic stem cell transplant for follicular lymphoma in Sweden," *Leukemia and Lymphoma*, vol. 52, no. 1, pp. 69–71, 2011.

[22] L. Fouillard, M. Labopin, A. Gratwohl et al., "Results of syngeneic hematopoietic stem cell transplantation for acute leukemia: risk factors for outcomes of adults transplanted in first complete remission," *Haematologica*, vol. 93, no. 6, pp. 834–841, 2008.

[23] W. Vogel, H. G. Kopp, L. Kanz, and H. Einsele, "Myeloma cell contamination of peripheral blood stem-cell grafts can predict the outcome in multiple myeloma patients after high-dose chemotherapy and autologous stem-cell transplantation," *Journal of Cancer Research and Clinical Oncology*, vol. 131, no. 4, pp. 214–218, 2005.

[24] S. Galimberti, F. Morabito, F. Guerrini et al., "Peripheral blood stem cell contamination evaluated by a highly sensitive molecular method fails to predict outcome of autotransplanted multiple myeloma patients," *British Journal of Haematology*, vol. 120, no. 3, pp. 405–412, 2003.

[25] A. M. Barbui, M. Galli, G. Dotti et al., "Negative selection of peripheral blood stem cells to support a tandem autologous transplantation programme in multiple myeloma," *British Journal of Haematology*, vol. 116, no. 1, pp. 202–210, 2002.

[26] J. W. Friedberg, H. Kim, S. Li et al., "Ex vivo B cell depletion using the Eligix B cell SC system and autologous peripheral blood stem cell transplantation in patients with follicular non-Hodgkin's lymphoma," *Bone Marrow Transplantation*, vol. 32, no. 7, pp. 681–686, 2003.

[27] L. De Rosa, M. Lalle, A. Pandolfi, C. Ruscio, and R. Amodeo, "Autologous bone marrow transplantation with negative immunomagnetic purging for aggressive B-cell non-Hodgkin's lymphoma in first complete remission," *Annals of Hematology*, vol. 81, no. 10, pp. 575–581, 2002.

[28] S. Galimberti, R. Marasca, F. Caracciolo et al., "The role of molecular monitoring in autotransplantation for non-Hodgkin's lymphoma," *Bone Marrow Transplantation*, vol. 29, no. 7, pp. 581–587, 2002.

[29] J. C. McCann, R. Kanteti, B. Shilepsky, K. B. Miller, M. Sweet, and D. P. Schenkein, "High degree of occult tumor contamination in bone marrow and peripheral blood stem cells of patients undergoing autologous transplantation for non-Hodgkin's lymphoma," *Biology of Blood and Marrow Transplantation*, vol. 2, no. 1, pp. 37–43, 1996.

[30] N. Feller, M. A. Van Der Pol, T. Waaijman et al., "Immunologic purging of autologous peripheral blood stem cell products based on CD34 and CD133 expression can be effectively and safely applied in half of the acute myeloid leukemia patients," *Clinical Cancer Research*, vol. 11, no. 13, pp. 4793–4801, 2005.

[31] T. Miyamoto, K. Nagafuji, M. Harada et al., "Quantitative analysis of AML1/ETO transcripts in peripheral blood stem cell harvests from patients with t(8;21) acute myelogenous leukaemia," *British Journal of Haematology*, vol. 91, no. 1, pp. 132–138, 1995.

[32] E. S. Sandler, A. Homans, L. Mandell et al., "Hematopoietic stem cell transplantation after first marrow relapse of non-T, non-B acute lymphoblastic leukemia: a Pediatric Oncology Group pilot feasibility study," *Journal of Pediatric Hematology/Oncology*, vol. 28, no. 4, pp. 210–215, 2006.

[33] J. Atta, F. Fauth, M. Keyser et al., "Purging in BCR-ABL-positive acute lymphoblastic leukemia using immunomagnetic beads: comparison of residual leukemia and purging efficiency in bone marrow vs peripheral blood stem cells by semiquantitative polymerase chain reaction," *Bone Marrow Transplantation*, vol. 25, no. 1, pp. 97–104, 2000.

[34] W. Leung, A. R. Chen, R. C. Klann et al., "Frequent detection of tumor cells in hematopoietic grafts in neuroblastoma and Ewing's sarcoma," *Bone Marrow Transplantation*, vol. 22, no. 10, pp. 971–979, 1998.

[35] S. A. Burchill et al., "Minimal residual disease at the time of peripheral blood stem cell harvest in patients with advanced neuroblastoma," *Medical and Pediatric Oncology*, vol. 36, no. 1, pp. 213–219, 2001.

[36] R. Schulze, M. Schulze, A. Wischnik et al., "Tumor cell contamination of peripheral blood stem cell transplants and bone marrow in high-risk breast cancer patients," *Bone Marrow Transplantation*, vol. 19, no. 12, pp. 1223–1228, 1997.

[37] A. M. Vannucchi, A. Bosi, S. Glinz et al., "Evaluation of breast tumour cell contamination in the bone marrow and leukapheresis collections by RT-PCR for cytokeratin-19 mRNA," *British Journal of Haematology*, vol. 103, no. 3, pp. 610–617, 1998.

[38] C. Bokemeyer, A. J.M. Gillis, K. Pompe et al., "Clinical impact of germ cell tumor cells in apheresis products of patients

receiving high-dose chemotherapy," *Journal of Clinical Oncology*, vol. 19, no. 12, pp. 3029–3036, 2001.

[39] T. Kasahara, N. Hara, V. Bilim et al., "Sequential detection of alphafetoprotein-bearing cells in blood stem cell fraction of germ cell tumour patients," *British Journal of Cancer*, vol. 85, no. 8, pp. 1119–1123, 2001.

[40] M. K. Brenner, D. R. Rill, R. C. Moen et al., "Gene-marking to trace origin of relapse after autologous bone-marrow transplantation," *The Lancet*, vol. 341, no. 8837, pp. 85–86, 1993.

[41] A. B. Deisseroth, Z. Zu, D. Claxton et al., "Genetic marking shows that Ph+ cells present in autologous transplants of chronic myelogenous leukemia (CML) contribute to relapse after autologous bone marrow in CML," *Blood*, vol. 83, no. 10, pp. 3068–3076, 1994.

[42] D. R. Rill, V. M. Santana, W. M. Roberts et al., "Direct demonstration that autologous bone marrow transplantation for solid tumors can return a multiplicity of tumorigenic cells," *Blood*, vol. 84, no. 2, pp. 380–383, 1994.

[43] M. R. Motta, S. Mangianti, S. Rizzi et al., "Pharmacological purging of minimal residual disease from peripheral blood stem cell collections of acute myeloblastic leukemia patients: preclinical studies," *Experimental Hematology*, vol. 25, no. 12, pp. 1261–1269, 1997.

[44] E. J. Shpall, R. B. Jones, R. C. Bast et al., "4-Hydroperoxycyclophosphamide purging of breast cancer from the mononuclear cell fraction of bone marrow in patients receiving high-dose chemotherapy and autologous marrow support: a phase I trial," *Journal of Clinical Oncology*, vol. 9, no. 1, pp. 85–93, 1991.

[45] R. Abonour, K. M. Scott, L. A. Kunkel et al., "Autologous transplantation of mobilized peripheral blood CD34+ cells selected by immunomagnetic procedures in patients with multiple myeloma," *Bone Marrow Transplantation*, vol. 22, no. 10, pp. 957–963, 1998.

[46] A. Spyridonidis, M. Schmidt, W. Bernhardt et al., "Purging of mammary carcinoma cells during ex vivo culture of CD34+ hematopoietic progenitor cells with recombinant immunotoxins," *Blood*, vol. 91, no. 5, pp. 1820–1827, 1998.

[47] A. Gee, T. Moss, V. Mansour et al., "Large-scale immunomagnetic separation system for the removal of tumor cells from bone marrow," *Progress in Clinical and Biological Research*, vol. 377, pp. 181–187, 1992.

[48] B. D. Cheson, "Monoclonal antibody therapy for B-cell malignancies," *Seminars in Oncology*, vol. 33, no. 2, supplement 5, pp. 2–14, 2006.

[49] T. F. Goggins and N. Chao, "Depletion of host reactive T cells by photodynamic cell purging and prevention of graft versus host disease," *Leukemia and Lymphoma*, vol. 44, no. 11, pp. 1871–1879, 2003.

[50] S. Y. Wang, K. L. Makl, L. Y. Chen, C. H. Tzeng, C. P. Hu, and C. Chang, "Elimination of malignant tumor cells from human bone marrow using monoclonal antibodies and immunomagnetic beads," *Anticancer Research*, vol. 13, no. 6 A, pp. 2281–2285, 1993.

[51] M. Kim, G. J. Madlambayan, M. M. Rahman et al., "Myxoma virus targets primary human leukemic stem and progenitor cells while sparing normal hematopoietic stem and progenitor cells," *Leukemia*, vol. 23, no. 12, pp. 2313–2317, 2009.

[52] G. G. Au, L. F. Lincz, A. Enno, and D. R. Shafren, "Oncolytic Coxsackievirus A21 as a novel therapy for multiple myeloma," *British Journal of Haematology*, vol. 137, no. 2, pp. 133–141, 2007.

[53] D. R. Shafren, D. J. Dorahy, S. J. Greive, G. F. Burns, and R. D. Barry, "Mouse cells expressing human intercellular adhesion molecule-1 are susceptible to infection by coxsackievirus A21," *Journal of Virology*, vol. 71, no. 1, pp. 785–789, 1997.

[54] J. M. Rogers, G. D. Diana, and M. A. McKinlay, "Pleconaril: a broad spectrum antipicornaviral agent," *Advances in Experimental Medicine and Biology*, vol. 458, pp. 69–76, 1999.

[55] H. A. Rotbart, "Antiviral therapy for enteroviral infections," *Pediatric Infectious Disease Journal*, vol. 18, no. 7, pp. 632–633, 1999.

[56] K. A. Parato, D. Senger, P. A. J. Forsyth, and J. C. Bell, "Recent progress in the battle between oncolytic viruses and tumours," *Nature Reviews Cancer*, vol. 5, no. 12, pp. 965–976, 2005.

[57] C. M. Thirukkumaran, J. M. Luider, D. A. Stewart et al., "Reovirus oncolysis as a novel purging strategy for autologous stem cell transplantation," *Blood*, vol. 102, no. 1, pp. 377–387, 2003.

[58] T. Alain, K. Hirasawa, K. J. Pon et al., "Reovirus therapy of lymphoid malignancies," *Blood*, vol. 100, no. 12, pp. 4146–4153, 2002.

[59] D. F. Stojdl, B. Lichty, S. Knowles et al., "Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus," *Nature Medicine*, vol. 6, no. 7, pp. 821–825, 2000.

[60] S. Deola, S. Scaramuzza, R. S. Birolo et al., "Molecular purging of multiple myeloma cells by ex-vivo culture and retroviral transduction of mobilized-blood CD34+ cells," *Journal of Translational Medicine*, vol. 5, article no. 35, 2007.

[61] K. W. Peng, G. J. Ahmann, L. Pham, P. R. Greipp, R. Cattaneo, and S. J. Russell, "Systemic therapy of myeloma xenografts by an attenuated measles virus," *Blood*, vol. 98, no. 7, pp. 2002–2007, 2001.

[62] B. Roscic-Mrkic, R. A. Schwendener, B. Odermatt et al., "Roles of macrophages in measles virus infection of genetically modified mice," *Journal of Virology*, vol. 75, no. 7, pp. 3343–3351, 2001.

[63] K. W. Peng, K. A. Donovan, U. Schneider, R. Cattaneo, J. A. Lust, and S. J. Russell, "Oncolytic measles viruses displaying a single-chain antibody against CD38, a myeloma cell marker," *Blood*, vol. 101, no. 7, pp. 2557–2562, 2003.

[64] R. M. Myers, S. M. Greiner, M. E. Harvey et al., "Preclinical pharmacology and toxicology of intravenous MV-NIS, an oncolytic measles virus administered with or without cyclophosphamide," *Clinical Pharmacology and Therapeutics*, vol. 82, no. 6, pp. 700–710, 2007.

[65] H. T. Ong, M. M. Timm, P. R. Greipp et al., "Oncolytic measles virus targets high CD46 expression on multiple myeloma cells," *Experimental Hematology*, vol. 34, no. 6, pp. 713–720, 2006.

[66] D. Grote, S. J. Russell, T. I. Cornu et al., "Live attenuated measles virus induces regression of human lymphoma xenografts in immunodeficient mice," *Blood*, vol. 97, no. 12, pp. 3746–3754, 2001.

[67] Y. Adachi, P. N. Reynolds, M. Yamamoto et al., "A midkine promoter-based conditionally replicative adenovirus for treatment of pediatric solid tumors and bone marrow tumor purging," *Cancer Research*, vol. 61, no. 21, pp. 7882–7888, 2001.

[68] J. S. Senac, K. Doronin, S. J. Russell, D. F. Jelinek, P. R. Greipp, and M. A. Barry, "Infection and killing of multiple myeloma by adenoviruses," *Human Gene Therapy*, vol. 21, no. 2, pp. 179–190, 2010.

[69] H. Deng, N. Tang, A. E. Stief et al., "Oncolytic virotherapy for multiple myeloma using a tumour-specific double-deleted vaccinia virus," *Leukemia*, vol. 22, no. 12, pp. 2261–2264, 2008.

[70] T. Esfandyari, A. Tefferi, A. Szmidt et al., "Transcription factors down-stream of Ras as molecular indicators for targeting malignancies with oncolytic herpes virus," *Molecular Oncology*, vol. 3, no. 5-6, pp. 464–468, 2009.

[71] A. L. Angelova, M. Aprahamian, G. Balboni et al., "Oncolytic rat parvovirus H-1PV, a candidate for the treatment of human lymphoma: in vitro and in vivo studies," *Molecular Therapy*, vol. 17, no. 7, pp. 1164–1172, 2009.

[72] G. McFadden, "Poxvirus tropism," *Nature Reviews Microbiology*, vol. 3, no. 3, pp. 201–213, 2005.

[73] G. Wang, J. W. Barrett, M. Stanford et al., "Infection of human cancer cells with myxoma virus requires Akt activation via interaction with a viral ankyrin-repeat host range factor," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 12, pp. 4640–4645, 2006.

[74] F. Wang, X. Gao, J. W. Barrett et al., "RIG-I mediates the co-induction of tumor necrosis factor and type I interferon elicited by myxoma virus in primary human macrophages," *PLoS Pathogens*, vol. 4, no. 7, Article ID e1000099, 2008.

[75] E. Bartee and G. McFadden, "Human cancer cells have specifically lost the ability to induce the synergistic state caused by tumor necrosis factor plus interferon-β," *Cytokine*, vol. 47, no. 3, pp. 199–205, 2009.

[76] A. Kawa and S. Arakawa, "The effect of attenuated vaccinia virus AS strain on multiple myeloma: a case report," *Japanese Journal of Experimental Medicine*, vol. 57, no. 1, pp. 79–81, 1987.

Hindawi Publishing Corporation
Advances in Virology
Volume 2012, Article ID 586389, 5 pages
doi:10.1155/2012/586389

Review Article

Oncolytic Viruses: The Power of Directed Evolution

Maxine Bauzon and Terry W. Hermiston

Bayer Healthcare, Biologics Research, 455 Mission Bay Boulevard South, San Francisco, CA 94158, USA

Correspondence should be addressed to Terry W. Hermiston, terry.hermiston@bayer.com

Received 26 April 2011; Accepted 26 May 2011

Academic Editor: Nanhai G. Chen

Attempts at developing oncolytic viruses have been primarily based on rational design. However, this approach has been met with limited success. An alternative approach employs directed evolution as a means of producing highly selective and potent anticancer viruses. In this method, diverse viruses are grown under conditions that maximize diversity and then passaged under conditions meant to mimic those encountered in the human cancer microenvironment. Viruses which evolve to thrive under this selective pressure are isolated and tested to identify those with increased potency (i.e., ability to replicate and spread) and/or an increased therapeutic window (i.e., differentiated replication and spread on tumor versus normal cells), both of which have potential value but the latter of which defines an oncolytic virus. Using ColoAd1, an oncolytic virus derived by this approach as a prototype, we highlight the benefits of directed evolution, discuss methods to "arm" these novel viruses, and introduce techniques for their genetic modulation and control.

1. Introduction

As our understanding of cancer increases, the complex nature of this disease which often involves multiple mutations, overlapping signaling pathways, and the ability to adapt and develop resistance to various therapeutics becomes more evident [1–3]. Such a complex disease necessitates equally complex therapies—such as oncolytic viruses [4]. By definition, these viruses infect and selectively replicate in tumor cells resulting in eventual cell lysis. This replication and lysis serves to eradicate the target tumor cells while amplifying the therapeutic in a tumor-dependent fashion, all the while sparing neighboring normal cells. Unfortunately, the promise of oncolytic viruses as agents that selectively find and kill tumor cells has not been fully realized [5–8]. This fact may be due in part to some prejudices taken by researchers in their pursuit of oncolytic viruses. For example, the majority of oncolytic viruses currently studied are Ad5 based primarily, because Ad5 has been widely characterized and methods for its genetic manipulation are well established, making it the practical starting point for most studies. However, there is no clear rationale why Ad5 would make a superior oncolytic virus as opposed to other Ad serotypes or other viral classes. Additionally, the genetic manipulation of today's viruses in an attempt to increase selectivity and or potency may be misguided due to our limited knowledge of the underlying causes and nature of cancer. Therefore, the plasticity and complexity of tumors may hinder the rational design of oncolytic viruses [9].

In an attempt to circumvent these issues, researchers are beginning to explore the use of directed evolution as a way to harness the power of natural selection and to derive desirable properties without concern for the mechanism(s) responsible for these properties [10–12]. Directed evolution is not a foreign concept in the field of virology and has been utilized as a way to modulate viral vectors and enhance gene delivery. Such experiments focus primarily on enhancing infectivity or modulating tropism by changes to the viral coat [13, 14]. For oncolytic viruses, the goal is obviously different, namely to drive the viruses to evolve for optimal proliferation in the tumor environment. Normally, viruses infect normal cells. For Adenoviruses, this is via an oral or nasal route of entry and involves the epithelial lining of the nose, throat, and/or gut resulting in a respiratory and/or gastrointestinal infection (Figure 1(a)). As a cancer therapy, the objective is to develop viruses that selectively infect a vastly different set of cells,

namely, transformed epithelial cells and tumor associated endothelium located in distinct locations throughout the body, most of which are not normally seen during the typical Adenovirus infection. Researchers are therefore asking a oncolytic virus to efficiently and selectively kill cells to which they would never normally be exposed (Figure 1(b)). The necessary biological alterations needed to reach this goal are complex, but unlike evolution in nature which takes an extended period of time for adaptations resulting in new and desirable traits to accumulate, directed evolution can quickly lead to the rise of novel "species". Importantly, directed evolution is dependent on 2 factors both of which are completely within the investigator's control, namely, the need for (1)) a diverse starting pool and (2)) selective pressure designed to favor a specific outcome. To maximize starting diversity, the researcher has a myriad of options ranging from a single mutated serotype to entire viral classes. Additionally, the ability of viruses to undergo recombination under certain conditions can increase this starting diversity. Similarly, the directed outcome (increased tumor proliferation) is determined by the selective pressure set by the experimental setup and can be modulated in a number of ways including the source of the tumor cells and growth conditions.

2. ColoAd1

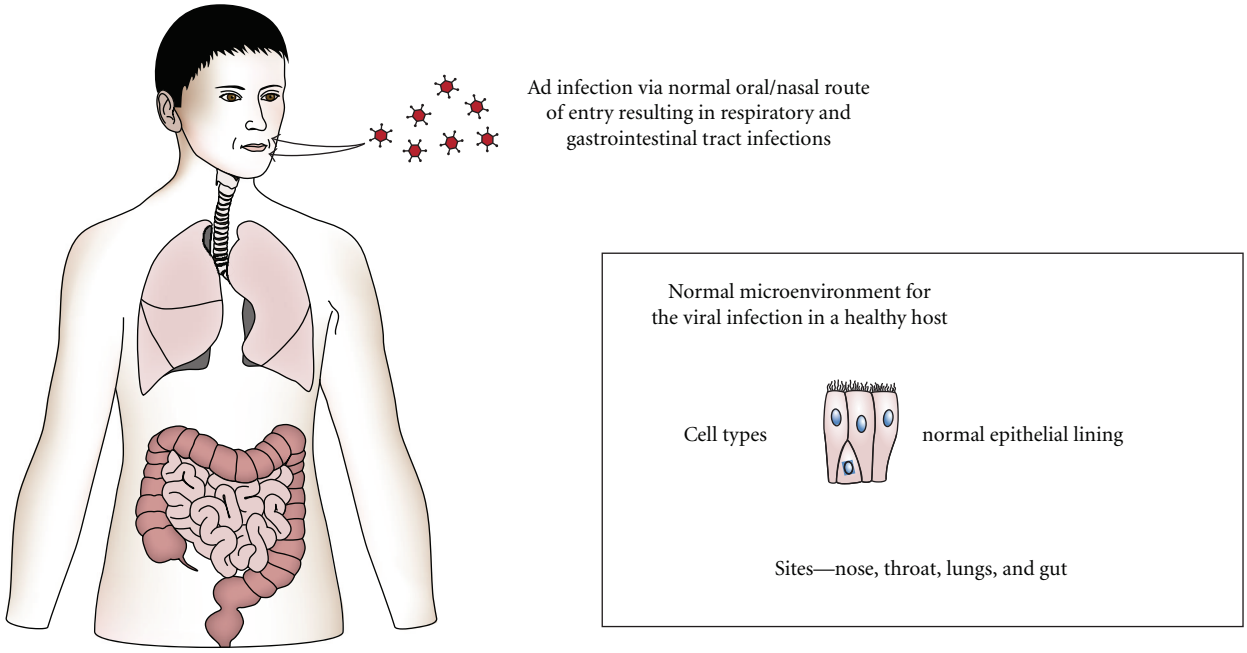
Human Adenovirus is comprised of 56 serotypes which are subdivided into groups A–G. These serotypes differ in a number of ways (e.g., pathology, hemagglutination properties, and cellular receptors used for entry) which help to distinguish them from one another and determine their group affiliation. Despite the diversity of human adenovirus, the majority of all oncolytic viruses are Ad5-based leaving the other serotypes and their oncolytic potential untapped. One way to explore these alternative serotypes is via "directed evolution". In this approach, Ad serotypes, representing the different Ad subgroups, are pooled and passaged at a high multiplicity of infection on human tumor cell lines representative of the target indication. This process invites recombination and creates selective pressure that gives rise to highly potent viral variants.

When this approach was applied to the colon cancer cell line, HT29, the result was ColoAd1, the first non-Ad5-based and first directed-evolution-derived oncolytic virus [10]. Chromatographic and sequence analysis revealed that ColoAd1 was Ad11p, a group B virus, with a nearly complete E3 region deletion, a smaller deletion in the E4 region, and a chimeric Ad3/Ad11p E2B region [10, 15]. In vitro studies showed ColoAd1 to be 2-3 logs more potent than either of its parent serotypes (Ad11p and Ad3), the standard Ad5, or the most clinically advanced oncolytic Ad, ONYX-015 [10, 16] Importantly, this increase in potency on cancer cells did not translate to increased potency on normal cells resulting in a therapeutic window that is 3-4 logs greater than Ad5 or Onyx-015. These *in vitro* results were supported by *in vivo* studies in a colon cancer liver seeding xenograft model and *ex vivo* on tumor tissue isolates from colon cancer patients. Parallel studies identified CD46 as a cellular attachment receptor for Ad11p, the parent virus of ColoAd1 [17, 18]. Interestingly, immunohistochemical studies staining for CD46 expression on excised colon cancer material, normal liver tissue and normal colon tissue revealed that strong CD46 staining was consistently seen in colon cancer tissue but was absent or weak in normal colon and liver tissue [10]. This suggests that CD46 expression may be a contributing factor to the observed tumor selectivity of ColoAd1. Importantly, CD46 overexpression has been described for a number of different cancer indications [19, 20], supporting the therapeutic value of this oncolytic virus. Theoretically, CD46 screening of tumor tissue could help guide physicians to identify which patients should be treated with this agent.

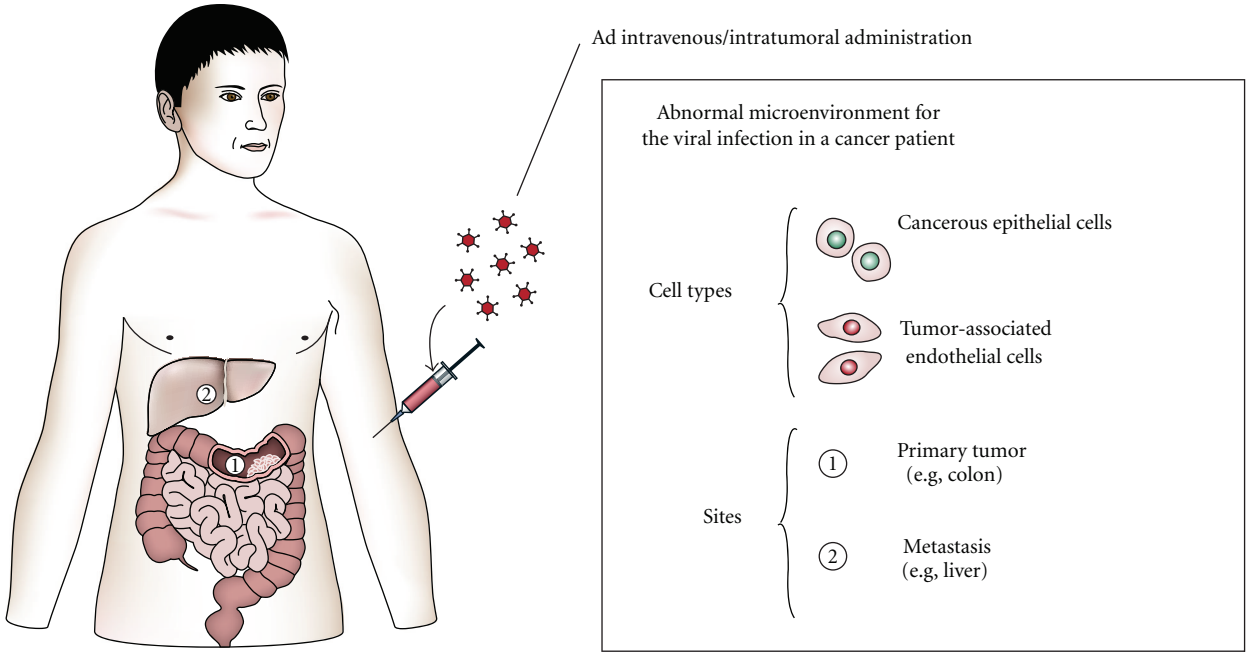
3. Manipulation and Control of Novel Viruses

Oncolytic viruses can and have been used to deliver exogenous genetic material, giving them much needed flexibility and complexity as a cancer treatment and augmenting their potential as viable cancer therapies [21]. The ability to engineer the genome of a replicating virus to carry foreign material is referred to as "arming". This strategy has been used in a variety of ways to alter viral selectivity, potency, safety, and utility [22, 23].

Like nonreplicating viruses, early armed oncolytic viruses controlled expression of foreign genes by using an exogenous constitutive or conditional promoter. This approach allowed for high levels of expression and, in the case of conditional promoters, some level of specificity. As the field advanced, researchers demonstrated that expression of exogenous genes could be achieved by using the endogenous expression machinery of the virus itself [24–26]. This latter method proved to have some advantages, the first being that it decreased the size of the foreign DNA that could be inserted into the viral genome. This outcome was beneficial given that viruses have genomic size constraints that greatly effect packaging efficiency. The second advantage of utilizing endogenous expression machinery is that depending on the endogenous promoter used, the timing and level of expression of the foreign gene could be controlled [24–26]. This not only allowed for efficient expression of the exogenous gene but could be used as an added measure of safety. Because the level of expression could be modulated depending on the endogenous promoter used, researchers and clinicians could have greater control over the levels of the armed therapeutic/exogenous gene. Furthermore, by utilizing an endogenous late promoter, it is possible to limit the expression of the gene to late in the viral cycle which, presumably, for an oncolytic virus would never be reached in a nonpermissive cell (normal cell) and, therefore, confine expression only to target cancer cells. Importantly, an armed therapeutic virus can express multiple genes [27] that potentially increase the therapeutic benefit of the virus and/or enable the treating physician with the opportunity to track the virus location (e.g., via imaging [28]) and viability (e.g., through the expression of a protein that can be detected in the blood or is secreted from the treated patient), as a means to better understand when additional treatments are warranted.



(a) What we have—natural viruses that infect normal cells in the context of their natural infection pathway



How do we re-evolve viruses to attack cancer cells and not normal cells?

(b) What we want—oncolytic viruses that attack tumor cells at their primary and associated sites of metastasis

Figure 1: Why we need directed evolution. (a) The starting point of all oncolytic viruses are naturally occurring viruses which infect normal cells they encounter along their standard route of entry. (b) The goal is to develop oncolytic viruses which selectively infect and replicate in cells they would never naturally encounter.

Arming is traditionally carried out by manipulating the viral genome and usually calls for a well characterized system. The novel viruses resulting from directed evolution will make rational insertion of therapeutic moieties extremely challenging, especially when one considers that they would not want to negatively impact the replication capabilities of these viruses which are critical to their clinical benefit. Clearly, innovative approaches will need to be taken in order

to manipulate viruses derived in this manner to ensure that this does not occur. One such approach taken uses a transposon-based method to "scan" a viral genome for insertion sites which are compatible with the viral lifecycle [29]. This approach makes it possible to arm these novel viruses with exogenous genes expressed from a foreign promoter or by the inclusion of a splice acceptor from an endogenous promoter [30]. Although this method, like many advances in the oncolytic field, was originally developed on Adenoviruses, it can be extended to any number of viral species whose genome can be cloned into a plasmid.

Given the potential to derive potent novel viruses from the directed evolution approach, it is very important to keep safety in mind. If these novel viruses are ever to advance as therapeutics, having the ability to control their replication in the treated patient is paramount. To this end, two approaches were taken with ColoAd1. The first was to study the effect on ColoAd1 of two clinically approved antivirals, ribovirin (RBV), and cidofovir (CDV), and the second was to genetically introduce drug sensitivity to the virus by inserting the HSV TK gene into the ColoAd1 genome using the transposon method [31].

Although there are no approved anti-Ad treatments, both RBV and CDV have been used to experimentally treat Adenoviral infections [32–34]. From these studies, RBV was found to be effective on Group C Ads but less so on Group B Ads [35, 36]. Not surprisingly, ColoAd1, being derived from 2 group B viruses was refractory to RBV treatment. CDV has been shown to have better Anti-Ad activity than RBV and when used to treat ColoAd1 was effective at inhibiting viral replication and spread on both tumor and normal cell lines. Interestingly, ColoAd1 was more sensitive to CDV treatment than either of its 2 parental strains, Ad11p or Ad3 suggesting that this increased CDV sensitivity was an outcome of the directed evolution process and not simply an inherited trait [31]. An alternative approach to the use of approved antivirals is the insertion of the HSV TK gene into the viral genome to create sensitivity to the approved drug ganciclovir (GCV) [37]. This approach, made possible by the transposon method of arming was successful in inhibiting ColoAd1 infection of both tumor and normal cells [29, 31]. The potential for using TK expression to track the virus also makes this approach appealing [38]. From these studies, it was demonstrated that ColoAd1 could be controlled through outside intervention.

The value gleaned from the ColoAd1 experience goes beyond a promising therapeutic, as it validates a new approach for the oncolytic virus field. The directed evolution method that resulted in ColoAd1 could be applied to other cancer types and other viral families. By utilizing this method, researchers are not longer limited to well characterized systems. Moreover, the ease by which viruses are molecularly manipulated need not be the deciding factor for proceeding. Although Adenoviruses have been covered in this paper, this approach is amendable to all viral families and opens up the possibility of harnessing inherent and novel oncolytic properties from a multitude of human viruses. Unlike more deliberately designed approaches, directed evolution capitalizes on the complexity of the tumor and can be directed towards an outcome that depends predominantly on the selective pressure applied by the researcher. Similar to Ad5-based oncolytic viruses, it is possible to arm these novel viruses without interfering with their lifecycle, thus unlocking the potential to modulate their characteristics or utility. Because these viruses are potentially highly potent, developing ways to control their replication should be a priority.

References

[1] R. P. Araujo, L. A. Liotta, and E. F. Petricoin, "Proteins, drug targets and the mechanisms they control: the simple truth about complex networks," *Nature Reviews Drug Discovery*, vol. 6, no. 11, pp. 871–880, 2007.

[2] C. L. Hann and J. R. Brahmer, "Who should receive epidermal growth factor receptor inhibitors for non-small cell lung cancer and when?" *Current Treatment Options in Oncology*, vol. 8, no. 1, pp. 28–37, 2007.

[3] D. D. Rosa, G. Ismael, L. D. Lago, and A. Awada, "Molecular-targeted therapies: lessons from years of clinical development," *Cancer Treatment Reviews*, vol. 34, no. 1, pp. 61–80, 2008.

[4] C. J. Breitbach, T. Reid, J. Burke, J. C. Bell, and D. H. Kirn, "Navigating the clinical development landscape for oncolytic viruses and other cancer therapeutics: no shortcuts on the road to approval," *Cytokine and Growth Factor Reviews*, vol. 21, no. 2-3, pp. 85–89, 2010.

[5] K. A. Parato, D. Senger, P. A. J. Forsyth, and J. C. Bell, "Recent progress in the battle between oncolytic viruses and tumours," *Nature Reviews Cancer*, vol. 5, no. 12, pp. 965–976, 2005.

[6] T. Reid, R. Warren, and D. Kirn, "Intravascular adenoviral agents in cancer patients: lessons from clinical trials," *Cancer Gene Therapy*, vol. 9, no. 12, pp. 979–986, 2002.

[7] D. C. Yu, P. Working, and D. Ando, "Selectively replicating oncolytic adenoviruses as cancer therapeutics," *Current Opinion in Molecular Therapeutics*, vol. 4, no. 5, pp. 435–443, 2002.

[8] J. A. Zwiebel, "Cancer gene and oncolytic virus therapy," *Seminars in Oncology*, vol. 28, no. 4, pp. 336–343, 2001.

[9] A. M. Crompton and D. H. Kirn, "From ONYX-015 to armed vaccinia viruses: the education and evolution of oncolytic virus development," *Current Cancer Drug Targets*, vol. 7, no. 2, pp. 133–139, 2007.

[10] I. Kuhn, P. Harden, M. Bauzon, C. Chartier, J. Nye, S. Thorne et al., "Directed evolution generates a novel oncolytic virus for the treatment of colon cancer," *PLoS ONE*, vol. 3, no. 6, Article ID e2409, 2008.

[11] W. Yan, G. Kitzes, F. Dormishian et al., "Developing novel oncolytic adenoviruses through bioselection," *Journal of Virology*, vol. 77, no. 4, pp. 2640–2650, 2003.

[12] A. Gros, J. Martínez-Quintanilla, C. Puig et al., "Bioselection of a gain of function mutation that enhances adenovirus 5 release and improves its antitumoral potency," *Cancer Research*, vol. 68, no. 21, pp. 8928–8937, 2008.

[13] I. Kwon and D. V. Schaffer, "Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer," *Pharmaceutical Research*, vol. 25, no. 3, pp. 489–499, 2008.

[14] L. Yang, J. Li, and X. Xiao, "Directed evolution of adeno-associated virus (AAV) as vector for muscle gene therapy," *Methods in Molecular Biology*, vol. 709, pp. 127–139, 2011.

[15] I. Kuhn, B. Larsen, C. Gross, and T. Hermiston, "High-performance liquid chromatography method for rapid assessment of viral particle number in crude adenoviral lysates of mixed serotype," *Gene Therapy*, vol. 14, no. 2, pp. 180–184, 2007.

[16] K. Garber, "China approves world's first oncolytic virus therapy for cancer treatment," *Journal of the National Cancer Institute*, vol. 98, no. 5, pp. 298–300, 2006.

[17] A. Gaggar, D. M. Shayakhmetov, and A. Lieber, "CD46 is a cellular receptor for group B adenoviruses," *Nature Medicine*, vol. 9, no. 11, pp. 1408–1412, 2003.

[18] M. Marttila, D. Persson, D. Gustafsson et al., "CD46 is a cellular receptor for all species B adenoviruses except types 3 and 7," *Journal of Virology*, vol. 79, no. 22, pp. 14429–14436, 2005.

[19] B. D. Anderson, T. Nakamura, S. J. Russell, and K. W. Peng, "High CD46 receptor density determines preferential killing of tumor cells by oncolytic measles virus," *Cancer Research*, vol. 64, no. 14, pp. 4919–4926, 2004.

[20] K. P. Murray, S. Mathure, R. Kaul et al., "Expression of complement regulatory proteins-CD 35, CD 46, CD 55, and CD 59-in benign and malignant endometrial tissue," *Gynecologic Oncology*, vol. 76, no. 2, pp. 176–182, 2000.

[21] T. Hermiston, "Fighting fire with fire: attacking the complexity of human tumors with armed therapeutic viruses," *Current Opinion in Molecular Therapeutics*, vol. 4, no. 4, pp. 334–342, 2002.

[22] T. W. Hermiston and I. Kuhn, "Armed therapeutic viruses: strategies and challenges to arming oncolytic viruses with therapeutic genes," *Cancer Gene Therapy*, vol. 9, no. 12, pp. 1022–1035, 2002.

[23] S. H. Thorne, T. Hermiston, and D. Kirn, "Oncolytic virotherapy: approaches to tumor targeting and enhancing antitumor effects," *Seminars in Oncology*, vol. 32, no. 6, pp. 537–548, 2005.

[24] L. K. Hawkins and T. Hermiston, "Gene delivery from the E3 region of replicating human adenovirus: evaluation of the E3B region," *Gene Therapy*, vol. 8, no. 15, pp. 1142–1148, 2001.

[25] L. K. Hawkins and T. W. Hermiston, "Gene delivery from the E3 region of replicating human adenovirus: evaluation of the ADP region," *Gene Therapy*, vol. 8, no. 15, pp. 1132–1141, 2001.

[26] L. K. Hawkins, L. Johnson, M. Bauzon et al., "Gene delivery from the E3 region of replicating human adenovirus: evaluation of the 6.7–K/gp19 K region," *Gene Therapy*, vol. 8, no. 15, pp. 1123–1131, 2001.

[27] M. Bauzon, D. Castro, M. Karr, L. K. Hawkins, and T. W. Hermiston, "Multigene expression from a replicating adenovirus using native viral promoters," *Molecular Therapy*, vol. 7, no. 4, pp. 526–534, 2003.

[28] D. Ding, S. J. Russell, and J. C. Morris III, "In vivo imaging and tumor therapy with the sodium iodide symporter," *Journal of Cellular Biochemistry*, vol. 90, no. 6, pp. 1079–1086, 2003.

[29] P. J. Kretschmer, F. Jin, C. Chartier, and T. W. Hermiston, "Development of a transposon-based approach for identifying novel transgene insertion sites within the replicating adenovirus," *Molecular Therapy*, vol. 12, no. 1, pp. 118–127, 2005.

[30] F. Jin, P. J. Kretschmer, and T. W. Hermiston, "Identification of novel insertion sites in the Ad5 genome that utilize the Ad splicing machinery for therapeutic gene expression," *Molecular Therapy*, vol. 12, no. 6, pp. 1052–1063, 2005.

[31] M. Bauzon, F. Jin, P. Kretschmer, and T. Hermiston, "In vitro analysis of cidofovir and genetically engineered TK expression as potential approaches for the intervention of ColoAd1-based treatment of cancer," *Gene Therapy*, vol. 16, no. 9, pp. 1169–1174, 2009.

[32] L. Lenaerts and L. Naesens, "Antiviral therapy for adenovirus infections," *Antiviral Research*, vol. 71, no. 2-3, pp. 172–180, 2006.

[33] P. Ljungman, "Viral infections: current diagnosis and treatment," *Hematology Journal*, vol. 5, 3, pp. S63–S68, 2004.

[34] D. Neofytos, A. Ojha, B. Mookerjee et al., "Treatment of adenovirus disease in stem cell transplant recipients with cidofovir," *Biology of Blood and Marrow Transplantation*, vol. 13, no. 1, pp. 74–81, 2007.

[35] M. Baba, S. Mori, S. Shigeta, and E. De Clercq, "Selective inhibitory effect of (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine and 2'-nor-cyclic GMP on adenovirus replication in vitro," *Antimicrobial Agents and Chemotherapy*, vol. 31, no. 2, pp. 337–339, 1987.

[36] F. Morfin, S. Dupuis-Girod, S. Mundweiler et al., "In vitro susceptibility of adenovirus to antiviral drugs is species-dependent," *Antiviral Therapy*, vol. 10, no. 2, pp. 225–229, 2005.

[37] O. Wildner, D. Hoffmann, C. Jogler, and K. Überla, "Comparison of HSV-1 thymidine kinase-dependent and -independent inhibition of replication-competent adenoviral vectors by a panel of drugs," *Cancer Gene Therapy*, vol. 10, no. 10, pp. 791–802, 2003.

[38] S. Bhaumik, "Advances in imaging gene-directed enzyme prodrug therapy," *Current Pharmaceutical Biotechnology*, vol. 12, no. 4, pp. 497–507, 2011.