nature biotechnology

Oncolytic virotherapy

Stephen J Russell[1], Kah-Whye Peng[1] & John C Bell[2,3]

Oncolytic virotherapy is an emerging treatment modality that uses replication-competent viruses to destroy cancers. Recent advances include preclinical proof of feasibility for a single-shot virotherapy cure, identification of drugs that accelerate intratumoral virus propagation, strategies to maximize the immunotherapeutic action of oncolytic viruses and clinical confirmation of a critical viremic threshold for vascular delivery and intratumoral virus replication. The primary clinical milestone has been completion of accrual in a phase 3 trial of intratumoral herpes simplex virus therapy using talimogene laherparepvec for metastatic melanoma. Key challenges for the field are to select 'winners' from a burgeoning number of oncolytic platforms and engineered derivatives, to transiently suppress but then unleash the power of the immune system to maximize both virus spread and anticancer immunity, to develop more meaningful preclinical virotherapy models and to manufacture viruses with orders-of-magnitude higher yields than is currently possible.

Oncolytic viruses are therapeutically useful viruses that selectively infect and damage cancerous tissues without causing harm to normal tissues[1]. Each virus has a specific cellular tropism that determines which tissues are preferentially infected, and hence what disease is caused. Rabies virus, for example, damages neurons, hepatitis B virus damages hepatocytes, HIV damages helper T lymphocytes and influenza virus damages airway epithelium. Many naturally occurring viruses have a preferential, although nonexclusive, tropism for tumors and tumor cells. This probably has more to do with tumor biology than with virus biology as most tumors have evolved not only to avoid immune detection and destruction but also to resist apoptosis and translational suppression, which are the key responses used by normal cells to limit a virus infection. Oncolytic viruses can kill infected cancer cells in many different ways, ranging from direct virus-mediated cytotoxicity through a variety of cytotoxic immune effector mechanisms. Conventional concepts of cell death—apoptosis, necrosis and autophagy—are generally inadequate to fully describe the complex cell-killing scenarios encountered in virotherapy. This is because the oncolytic virus typically takes over and controls the molecular cell death machinery of the infected cancer cell, allowing death to occur only after available cellular resources have been maximally exploited for the synthesis and assembly of new viruses[2]. In addition to the killing of infected cells, oncolytic viruses can mediate the killing of uninfected cancer cells by indirect mechanisms such as destruction of tumor blood vessels, amplification of specific anticancer immune responses or through specific activities of transgene-encoded proteins expressed from engineered viruses[1].

Specific targeting of cancer cells is the *sine qua non* for oncolytic virotherapy and can be achieved in several ways. Some viruses, such as the H1 autonomously replicating parvovirus, reovirus, Newcastle disease virus, mumps virus and Moloney leukemia virus, have a natural preference for cancer cells, whereas viruses such as measles, adenovirus, vesicular stomatitis virus (VSV), vaccinia and herpes simplex virus (HSV) can be adapted or engineered to make them cancer specific. Surface markers such as epidermal growth factor receptor, Her2-neu, folate receptor, prostate-specific membrane antigen and CD20, and nuclear transcription factors such as estrogen receptor, androgen receptor, GATA factors and hypoxia-inducible factor 1, that are expressed selectively by specific tumor cells can be targeted by their use as receptors for virus entry or as essential cofactors for viral gene expression[3,4]. Alternatively, oncolytic viruses can be engineered to exploit the defective antiviral defenses of tumor cells[5]. Normal cells respond to virus infection by downmodulating their metabolism and/or by undergoing apoptosis, thereby inhibiting virus propagation. Successful viruses use various strategies to combat these innate immune responses, but researchers can make them nonpathogenic by engineering or evolving them to incapacitate their immune combat proteins. Examples include the matrix protein of VSV, the NS1 protein of influenza virus, the C and V proteins of paramyxovirus family members, the HSV γ34.5 protein and the proteins encoded in the E1 and E3 regions of the adenovirus genome. Notably, as the apoptotic and antimetabolic responses of tumor cells are generally deficient, attenuated viruses with defective immune combat proteins can often propagate in tumor cells. An alternative way to 'target' viruses to cancer cells is to selectively eliminate their undesirable tropisms by engineering targets for brain-, liver- or muscle-specific microRNAs into their genomes so that the viral life cycle is selectively blocked in the relevant target tissue[6].

Here we provide a critical overview of the current state of the field of oncolytic virotherapy research, emphasizing what we consider the most important recent advances and the main challenges going forward. The review is divided into three sections. The first section summarizes the clinical oncolytic virotherapy experience to date and suggests that the approach has genuine promise but that its full potential has yet to be realized. The subsequent sections address the two key stages of a successful oncolytic virus treatment, both of which are hotbeds of preclinical research innovation: first, delivery of the virus to the tumor; and second, spread of the virus infection

[1]Department of Molecular Medicine, Mayo Clinic, Rochester, Minnesota, USA. [2]Center for Cancer Therapeutics, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada. [3]Jennerex Biotherapeutics, San Francisco, California. Correspondence should be addressed to S.J.R. (sjr@mayo.edu).

Published online 10 July 2012; doi:10.1038/nbt.2287

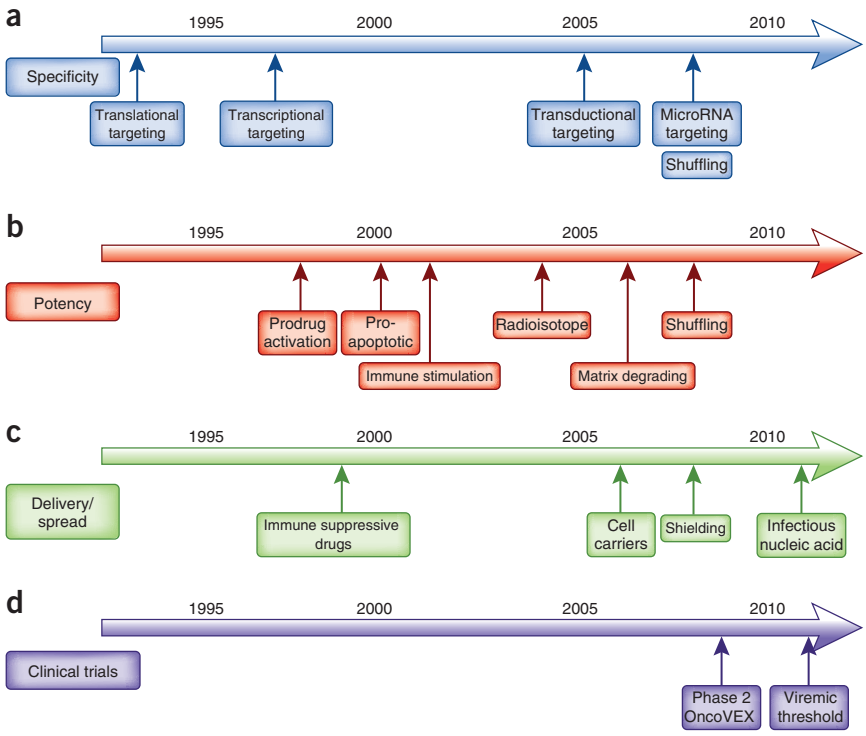

Figure 1 A timeline of milestones in the development of oncolytic virotherapy to improve virus specificity, potency, delivery and spread. (**a**) Notable advances in virotherapy specificity include translational targeting via engineering of a replication-competent HSV attenuated for neurovirulence for glioma treatment[10] (1991); transcriptional targeting of HSV using an albumin promoter-enhancer for hepatoma cells[161] or advenovirus using a prostate-specific antigen promoter for prostate cancer cells[162] (1997); transductional targeting of entry and cytopathic effects of oncolytic measles virus by display of single-chain antibody on the virus attachment protein (2005); microRNA targeting of picornavirus[27] and VSV[133] to control unwanted toxicity while retaining antitumor activity (2008); and DNA shuffling by mixing a pool of adenoviral serotypes and passaging the pools under conditions that invite recombination between serotypes to generate tumor-selective virus[163] (2008). (**b**) Notable advances in the enhancement of potency include prodrug activation in which an oncolytic adenovirus expressing cytosine deaminase and HSV-thymidine kinase have been designed to work in combination with 5-fluorocytosine and ganciclovir (1998); introduction of proapoptotic genes, such as the adenovirus death protein (ADP), into an oncolytic adenovirus to enhance its cytotoxicity[164] (2000); immune stimulation via an oncolytic HSV encoding IL-12 and GM-CSF to recruit T lymphocyte–mediated antitumor immune response[165] (2001); the inclusion of radioisotopes in virotherapy in the form of an oncolytic measles virus encoding NIS, which concentrates β-emitting (radiovirotherapy) and γ-emitting isotopes (imaging)[35] (2004); the use of matrix-degrading proteins, such as adenovirus encoding relaxin protein to enhance virus intratumoral spread[166] (2006); and DNA shuffling by mixing a pool adenoviral serotypes and passaging the pools under conditions that invite recombination between serotypes to generate more potent adenovirus ColoAd1 (ref. 163; 2008), (**c**) Notable advances in delivery and spread include immunosuppressive drugs, such as the addition of cyclophosphamide to combat innate and adaptive antiviral immunity, thereby enhancing intratumoral spread of HSV (1999); the introduction of cell carriers, such as cytokine-induced killer cells, to deliver oncolytic vaccinia virus to tumors, leading to synergistic antitumor activity[167] (2006); shielding approaches in which polymer coating and retargeting of an oncolytic adenovirus for ovarian cancer enhance viral pharmacokinetics[42] (2008); and delivery of oncolytic picornavirus using infectious nucleic acid (RNA) to successfully achieve sustained viremia and tumor regression (2011). (**d**) Notable clinical developments include the first demonstration of virotherapy activity in a phase 2 trial with intralesional injection of an oncolytic HSV, OncoVEX (talimogene laherparepvec), in melanoma patients (2009). Trial data indicate a 26% complete response (8 of 50), with durability in both injected and uninjected lesions including visceral sites[19]. OncoVEX is currently undergoing phase 3 evaluation. Intravenous delivery of JX-594, an oncolytic vaccinia virus, in patients with metastatic tumors has demonstrated the need for a viremic threshold to be reached for efficient virus delivery to tumors[29] (2011).

through the tumor. Optimizing the efficiency and accuracy of both of these critical processes will challenge the field for years to come, but there have been many recent developments, several of which are already being translated to determine whether they can improve clinical outcomes.

Because of space constraints, citations have been limited to key manuscripts published since 2007. However, in some cases we refer to seminal papers published before this time. Where multiple primary manuscripts address the same topic, we have cited review articles. We apologize to investigators whose work has not been cited and take full responsibility for these omissions.

Clinical development

The idea of using viruses to treat cancer first began to take hold in the 1950s, when tissue culture systems and rodent cancer models were originally developed[7]. Hundreds of cancer patients were treated with impure oncolytic virus preparations (even infected body fluids) administered by almost every imaginable route[8]. The viruses were usually arrested by the immune system and did not affect tumor growth, but sometimes infection took hold and tumors regressed, especially in immunosuppressed patients, although they frequently became sick or died when the infection spread to normal tissues. In a particularly promising study, from Osaka University, tumor regressions were reported in 37 of 90 terminal cancer patients, with a variety of tumor histologies, treated with a nonattenuated mumps virus[9]. But this work was not continued beyond the 1970s, and the strains of mumps virus used for the work have since been lost. The modern era of oncolytic virotherapy, in which virus genomes are engineered to enhance their antitumor specificity (milestones detailed in **Fig. 1**), began with a 1991 publication in which a thymidine kinase–negative HSV with attenuated neurovirulence was shown to be active in a murine glioblastoma model[10]. Since that first application of virus engineering to an oncolytic HSV, the pace of clinical activities has accelerated considerably, with many ongoing or completed trials using oncolytic viruses belonging to at least ten different virus families (**Table 1**) and a steady stream of new oncolytic viruses entering the clinical arena[11–13].

Overall, the clinical tolerability of oncolytic viruses has been excellent, even at today's highest feasible doses[14]. But future oncolytic virus trials will probably use higher doses as manufacturing yields are continually increasing owing to a variety of technical advances, such as cell-substrate optimization and the use of cell microcarriers and disposable wave bioreactors[15–17]. Hence, it may be premature

Table 1 Current and recently completed oncolytic virotherapy trials

Virus	Name	Modifications	Phase	Tumor	Route	Combination	Site	Status (PubMed reference)
Adenovirus	Oncorine (H101)	E1B-55k- E3-	2 3	SCCHN SCCHN	IT IT	Cisplatin Cisplatin	Multicenter Multicenter	Completed, PMID: 14693057 Completed, PMID: 15601557
	Onyx-015	E1B-55k-	1	Lung Mets	IV	–	Mutlicenter	Completed, PMID: 11420638
		E3B-	1	Glioma	Intracavity	–	Mutlicenter	Completed, PMID: 15509513
			1	Ovarian cancer	IP	–	Mutlicenter	Completed, PMID: 11896105
			1	SCCHN	IT	–	Multicenter	Completed, PMID: 10741699
			1	Solid tumors	IV	Enbrel	Mary Crowley	Completed, PMID: 17704755
			1	Sarcoma	IT	Mitomycin-C Dox, cisplatin	Mayo Clinic	Completed, PMID: 15647767
			1/2	PanCa	IT	Gemzar	UCLA	Completed, PMID: 12576418
			2	CRC	IV	–	Mutlicenter	Completed, PMID: 12697873
			2	Hepatobiliary	IT	–	Montefiore	Completed, PMID: 12576437
			2	CRC, PanCa	IA	–	Mutlicenter	Completed, PMID: 12414631
			2	SCCHN	IT	–	Multicenter	Completed, PMID: 11208818
			2	SCCHN	IT	Cisplatin, 5-FU	Multicenter	Completed, PMID: 10932224
			2	CRC	IV	5-FU/leucovorin	Stanford	Completed, PMID: 15803147
	CG7060	PSA control	1	Prostate cancer	IT	RT	Johns Hopkins	Completed, PMID: 11606381
	CG7870/CV787	Rat probasin-E1A	1/2	Prostate cancer	IV	–	Multicenter	Completed, PMID: 16690359
		hPSA-E1B E3+	1/2	Prostate cancer	IV	Docetaxel	Mary Crowley	Terminated, 2005
	CG0070	E2F-1, GM-CSF	2/3	Bladder cancer	Intracavity	–	UCSF	Not yet open, PMID: 16397056
	Telomelysin	hTERT	1	Solid tumors	IT	–	Mary Crowley	Completed, PMID: 19935775
	Ad5-CD/TKrep	CD/TK	1	Prostate cancer	IT	5-FC & GCV	Henry Ford, Detroit	Completed, PMID: 12208748
			1	Prostate cancer	IT	5-FC+GCV+RT	Henry Ford, Detroit	Completed, PMID: 14612551
	Ad5-D24-RGD	RGD, Delta-24	1	Ovarian cancer	IP	–	UAB	Completed, PMID: 20978148
			1	Glioma	IT	–	MD Andersen	Recruiting
			1/2	Glioma	IT	–	Erasmus Medical Center	Recruiting
	Ad5-SSTR/TK-RGD	SSTR, TK, RGD	1	Ovarian cancer	IP	GCV	UAB	Active, PMID: 16397056
	CGTG-102	Ad5/3, GM-CSF	1/2	Solid tumors	IT	–	Baylor	Not open, PMID: 20664527
		Delta-24	1	Solid tumors	IT/IV	Metronomic CTX	Docrates Hospital Helsinki	Recruiting
	INGN-007 (VRX-007)	wtE1a, ADP	1	Solid tumors	IT	–	Mary Crowley	Not open, PMID: 19197324
	ColoAd1	Ad3/11p	1/2	CRC, HCC		–	PsiOxus	Not open, PMID: 18560559
Coxsackie virus (CVA21)	CAVATAK	–	1	Melanoma	IT	–	Viralytics	Completed
			2	Melanoma	IT	–	Viralytics	Recruiting
			1	SCCHN	IT		Viralytics	Terminated
			1	Solid tumors	IV	–	Viralytics	Recruiting
Herpes simplex virus	Talimogene laher-parepvec (OncoVEX)	GM-CSF	1	Solid tumors	IT	–	Multicenter	Completed, PMID: 17121894
		ICP34.5(–)	2	Melanoma	IT	–	Multicenter	Completed, PMID: 19915919, 19884534
		ICP47(–)	3	Melanoma	IT	–	Multicenter	Active
		Us11 ↑	1/2	SCCHN	IT	RT, cisplatin	Multicenter	Completed, PMID: 20670951
	G207	ICP34.5(–), ICP6(–)	1/2	Glioma	IT	–	U of Alabama	Completed, PMID: 18957964, 10845725
		LacZ(+)	1	Glioma	IT	RT	U of Alabama	Completed
	G47Delta	From G207, ICP47–	1	Glioma	IT	–	Tokyo Hospital	Recruiting, PMID: 11353831
	HSV 1716 (Seprehvir)	ICP34.5(–)	1	Non-CNS solid tumors	IT	–	Cincinnati	Recruiting
			1	SCCHN	IT	–	U of Glasgow	Completed, PMID: 18615711
			1	Glioma	IT	–	U of Glasgow	Completed, PMID: 15334111, 11960316
			1	Melanoma	IT	–	U of Glasgow	11229673, 2001
			1	Mesothelioma	IP	–	UK	not active
	HF10	HSV-1 HF strain	1	Solid tumors	IT	–	Multicenter	Recruiting
			1	Pancreatic cancer	IT	–	Nagoya University	Completed, PMID: 21102422
			1	Breast cancer	IT	–	Nagoya University	Completed, PMID: 16865590
			1	SCCHN	IT	–	Nagoya University	Completed, PMID: 16923721
	NV1020		1	CRC liver mets	IA	–	MSKCC	Completed, PMID: 19018254
Measles virus (Edmonston)	MV-CEA	CEA	1	Ovarian cancer	IP	–	Mayo Clinic	Completed, PMID: 20103634
			1	Glioma	IT	–	Mayo Clinic	Recruiting
	MV-NIS	NIS	1	Myeloma	IV	CTX	Mayo Clinic	Recruiting
			1	Ovarian cancer	IP	–	Mayo Clinic	Recruiting
			1	Mesothelioma	IP	–	U of Minnesota/ Mayo Clinic	Recruiting
			1	SCCHN	IT	–	Mayo Clinic	Not open

(Continued)

Table 1 (Continued)

Virus	Name	Modifications	Phase	Tumor	Route	Combination	Site	Status (PubMed reference)
Newcastle disease virus	NDV-HUJ	–	1/2	Glioma	IV	–	Goldyne Savad Inst	Completed, PMID: 16257582
	PV701	–	1	Solid tumors	IV	–	Ottawa Hospital	Completed, PMID: 16638865
	MTH-68/H	–	2	Solid tumors	Inhalation	–	UCRI	Completed, PMID: 8275514
	NV1020		1	Solid tumors	IV	–	Multicenter	Completed, PMID: 11980996
Parvovirus	H-1PV	–	1/2	Glioma	IT/IV	–	University Hospital Heidelberg	Recruiting, PMID: 20299703
Poliovirus (Sabin)	PVS-RIPO	IRES	1	Glioma	IT	–	Duke	Recruiting, PMID: 20299272
Reovirus (Dearing)	Reolysin	–	1/2	Glioma	IT	–	Multicenter	Completed, PMID: 18253152
			1	Peritoneal cancer	IP	–	Ohio State	Recruiting
			1	Solid tumors	IV	–	Multicenter	Completed, PMID: 18981012
			1	Solid tumors	IV	CTX	Multicenter	Recruiting
			1	CRC	IV	FOLFIRI	Multicenter	Recruiting
			2	Sarcoma	IV	–	Multicenter	Completed
			2	Melanoma	IV	–	Multicenter	Suspended
			2	Ovarian, peritoneal cancer	IV	PTX	Mutlicenter	Recruiting
			2	Pancreatic cancer	IV	PTX, CBDCA	Multicenter	Recruiting
			2	SCCHN	IV	PTX, CBDCA	Multicenter	Not recruiting
			2	Melanoma	IV	PTX, CBDCA	U of Texas	Recruiting
			2	Pancreatic cancer	IV	Gemzar	U of Texas	Recruiting
			2	Lung cancer	IV	PTX, CBDCA	Multicenter	Recruiting
			3	SCCHN	IV	PTX, CBDCA	Multicenter	Recruiting
Seneca Valley virus	NTX-010		2	Small cell lung cancer	IV	–	NCCTG multicenter	Recruiting, PMID: 17971529
Retrovirus	Toca 511	CD	1/2	Glioma	IT	5-FC	Multicenter	Recruiting, PMID: 16257382
Vaccinia (Wyeth strain)	JX-594	GM-CSF	1	CRC	IV	–	South Korea	Recruiting
		TK(–)	1	Solid tumors	IV	–	Multicenter	Completed
			1	HCC	IT	–	Busan, South Korea	Completed, PMID: 18495536
			1	Pediatric solid tumors	IT	–	Cincinnnati	Recruiting
			1	Melanoma	IT	–	Busan, South Korea	Completed, PMID: 21772252
			1/2	Melanoma	IT	–	Multicenter	Completed, PMID: 10505851
			2	HCC	IT	–	Multicenter	Not recruiting, data analysis
			2B	HCC	IV	–	Multicenter	Recruiting
			1/2	CRC	IV/IT	Irinotecan	Multicenter	Recruiting
			2	CRC	IT	–	Ottawa Hospital	Not yet recruiting
Vaccinia (Western Reserve)	vvDD-CDSR	TK–, VGF– LacZ, CD Somatostatin R	1	Solid tumors	IT/IV	–	U of Pittsburgh	Recruiting, PMID: 15336655
Vaccinia (Lister)	GL-ONC1	Renilla luciferase	1	Solid tumors	IV	–	Royal Marsden	Recruiting, PMID: 21779374
	(GLV-h68)	GFP, β-gal	1/2	Peritoneal carcinomatosis	IP		University Hospital Tuebingen	Recruiting
		β-glucoronidase	1/2	SCCHN	IV	RT, cisplatin	Moores UCSD Cancer Center	Recruiting
Vesicular stomatitis virus (Indiana)	VSV-hIFNβ	IFN-β	1	HCC	IT	–	Mayo Clinic	Recruiting

A search was carried out on http://www.clinicaltrials.gov/ and the clinical trial database of the *Journal of Gene Medicine* (http://www.wiley.com/legacy/wileychi/genmed/clinical/). 5-FC: 5-fluorocytosine; 5-FU: 5-fluorouracil; ADP: Adenovirus death protein; β-gal: Beta galactosidase; Ca: Cancer; CBDCA: Carboplatin; CD: Cytosine deaminase; CEA: carcinoembryonic antigen; CNS: Central nervous system; CRC: colorectal cancer; CTX: Cyclophosphamide; Dox: Doxorubicin; FOLFIRI: 5-fluorouracil, leucovorin, irinotecan; GCV: Ganciclovir; Gemzar: Gemcitabine; GFP: green fluorescent protein; GM-CSF: granulocyte-macrophage colony-stimulating factor; HCC: hepatocellular carcinoma; HSV: herpes simplex virus; hTERT: human telomerase reverse transcriptase; ICP: infected cell protein; IFN: interferon; IP: intraperitoneal; IRES: internal ribosomal entry site; IT: intratumoral; IV: intravenous; Mets: metastases; MV: measles virus; NDV: Newcastle disease virus; NIS: sodium iodide symporter; PanCa: pancreatic cancer; PSA: prostate specific antigen; PTX: paclitaxel; RT: radiation; SCCHN: squamous cell carcinoma of the head and neck; Somatostatin R: stomatostatin receptor; SSTR: stomatostatin receptor; TK: thymidine kinase; UAB: University of Alabama Birmingham; UCSF: University of California, San Francisco; VGF: vaccinia growth factor; Wt: wild-type.

to judge whether effective oncolytic virotherapy will be devoid of serious toxicities at clinically effective doses. A safety risk specific to viral therapies is the concern that a virus might spread from a treated patient and mutate to regain its pathogenic potential[18]. However, although virus shedding has sometimes been documented in urine or respiratory secretions, oncolytic virus transmission to contacts and caregivers has not yet been seen[14].

Clinical efficacy. Evidence for the efficacy of single-agent oncolytic virotherapy comes from two recent phase 1/2 clinical trials, and is

supported by several positive anecdotal reports[7,19–21]. In one trial, talimogene laherparepvec (formerly named OncoVEX), an oncolytic HSV encoding granulocyte macrophage–colony stimulating factor (GM-CSF), was administered by direct intratumoral injection to patients with metastatic malignant melanoma and led to complete regression of injected and uninjected lesions in 8 of 50 treated patients[19]. This study remains the most compelling demonstration that intratumoral administration of an oncolytic virus can powerfully crossprime and amplify anticancer immunity. Perhaps because of its well-known susceptibility to immunotherapy, melanoma seems to be a particularly good target for oncolytic virotherapy, responding well not just to HSV-GM-CSF (OncoVEX) but also to vaccinia virus therapy[22]. In the second trial, an oncolytic vaccinia virus, JX594, which was also engineered to express GM-CSF, was administered intratumorally to patients with nonresectable hepatocellular carcinoma, leading to objective responses in three of ten evaluable patients[20].

Trials combining oncolytic viruses, such as reovirus, vaccinia and HSV, with drugs or radiation have shown a high frequency of tumor responses[21,23–25], but it is difficult to know whether the oncolytic viruses are contributing to these responses over and above the active anticancer drugs with which they are combined. Answering this critical question will require randomized phase 3 trials.

So far, clinical trials have not provided a clear demonstration that direct viral lysis of infected cells is an important mechanism of tumor destruction[14]. Thus, the oncolytic virotherapy paradigm (**Fig. 2**), whereby a systemically administered virus extravasates and spreads extensively at sites of tumor growth to cause tumor destruction, although well validated in animal models[11,26–28], remains to be proven in humans. A recent phase 1 clinical trial found that intravenously administered JX594 was recoverable from tumor biopsies only at a viremic threshold dose of $>10^9$ infectious units[29], indicating that extravasation of oncolytic viruses from tumor blood vessels to the tumor is a concentration-driven process and is detectable only above a threshold virus dose. Direct oncolytic tumor destruction may therefore be tightly linked to the dose of virus administered, which for many oncolytic viruses is limited primarily by manufacturing considerations.

Completed and ongoing clinical trials have led to additional insights. First, as trial results often fall short of hopes and expectations, it has become clear that more reliably predictive preclinical models are needed. More specifically, the field would benefit from orthotopic cancer models in immunocompetent animals that not only are susceptible to the oncolytic virus being evaluated but also mirror the human pathogenesis of the viral infection. Current models are often inadequate because they lack an immune system (cultured cells and human xenograft models) or are not susceptible to the virus in question, although exceptions do exist (for example, vaccinia).

Recent clinical experience has also revealed that it is feasible to monitor virus spread by following reporter-transgene expression, which is a useful source of pharmacokinetic data that are particularly helpful during early-stage clinical development. In rodent experiments, the progress of an oncolytic virus infection can be monitored by postmortem analysis of the biodistribution of virus-infected cells at multiple time points, but, in clinical studies, a lack of adequate monitoring has meant that little has been learned about why the outcomes of oncolytic virotherapy are inferior compared with results in rodents. To begin to address this issue, reporter genes have been engineered into oncolytic virus genomes to facilitate repetitive, noninvasive determination of the number and location of virus-infected cells in the human body[26,30]. In one example, we (S.J.R., K.W. and collaborators[31]) administered an oncolytic measles virus encoding the soluble extracellular domain of carcinoembryonic antigen intraperitoneally to patients with refractory ovarian cancer; monitoring of serum carcinoembryonic antigen suggested that the virus infected few tumor cells and was not substantially amplified *in vivo*[31]. Reporter genes compatible with radioactive tracers have also been tested in humans. Oncolytic HSVs are amenable to positron emission tomography imaging via the HSV thymidine kinase, which phosphorylates specific positron-emitting substrates, trapping them inside the cell, as shown in a clinical trial of HSV thymidine kinase gene therapy for glioblastoma[32]. However, clinical validation of this approach to track the spread of a replication-competent oncolytic virus is still awaited. In another strategy, the gene encoding the thyroidal sodium iodide symporter (NIS), which concentrates radioactive iodide, has been inserted into the genomes of several oncolytic viruses, such as adenovirus, measles, VSV, HSV and vaccinia. This approach was used preclinically in conjunction with various radioisotopes (^{125}I, ^{123}I, ^{124}I and $^{99m}TcO_4$) to monitor *in vivo* spread[33] and was recently validated in a clinical study in which intratumoral spread of an oncolytic adenovirus encoding NIS was monitored with $^{99m}TcO_4$-based single-photon emission computed tomography[34]. Using an approach known as radiovirotherapy, we (S.J.R., K.W. and collaborators[35]) have shown that it will also be feasible in the future to increase the potency of a NIS-expressing virus by administering ^{131}I, which delivers high-energy β particles, into the infected tumor[35]. Although currently available reporter-gene imaging technologies are helping to elucidate the spread of oncolytic viruses in the body, their inability to distinguish infected and uninfected cells is limiting. The development of more sensitive reporter-gene detection systems that are capable of resolving smaller numbers of infected cells is therefore of great importance. Also, as it is inconvenient to work with radioactive isotopes, the field could be further advanced by the development of nonradioactive reporter-gene detection systems, such as magnetic resonance imaging spectroscopy. In the long term, it is expected that noninvasive monitoring of the spread and elimination of the infection will become a routine part of oncolytic virus therapy.

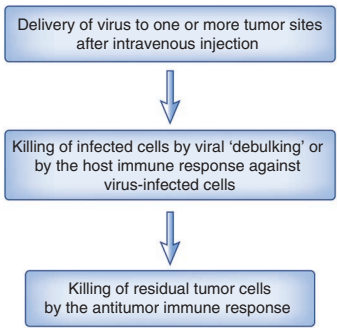

Figure 2 The oncolytic virotherapy paradigm.

Delivering oncolytic viruses to the tumor

Although several ongoing trials are emphasizing intratumoral delivery, systemic delivery will be required for treatment of metastatic cancer. The goal of systemic therapy is to exceed the 'viremic threshold' above which the virus nucleates a critical number of intratumoral infectious centers whose expansion and coalescence lead to tumor destruction. Current research is therefore focused on minimizing oncolytic virus sequestration in the liver and spleen, evading neutralization by serum factors, targeting viruses to the vascular endothelial cells lining tumor blood vessels and selectively enhancing vessel permeability (**Fig. 3**).

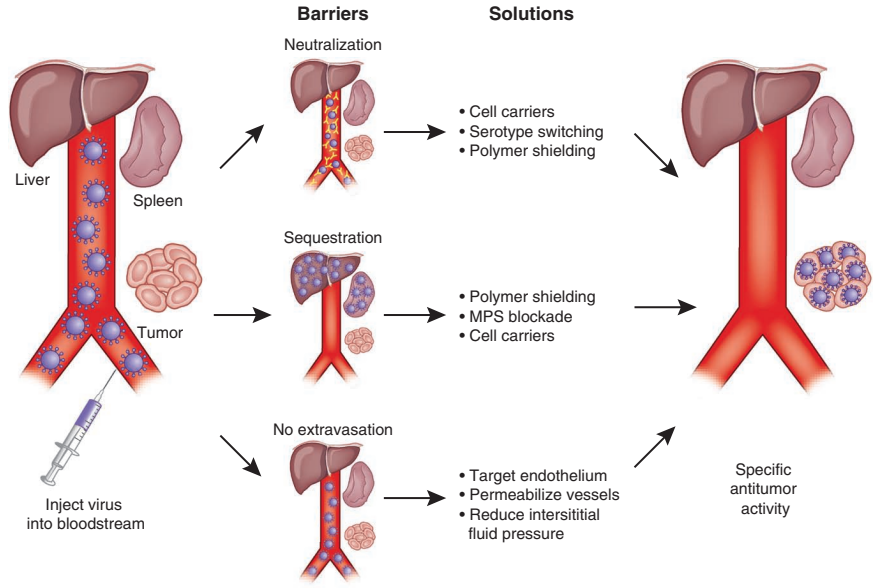

Figure 3 Barriers to efficient oncolytic virus delivery via the bloodstream (virus neutralization by serum factors, sequestration by the mononuclear phagocytic system and lack of extravasation) and solutions to circumvent them.

Minimizing sequestration in the liver and spleen. Intravenously administered viruses are rapidly cleared from the circulation through sequestration by the mononuclear phagocytic system (MPS) in the liver and spleen. Before clearance, they are typically coated (opsonized) with antibodies, complement, coagulation factors and/or other serum proteins that facilitate their recognition by splenic macrophages and hepatic Kupffer cells. These 'decorated' particles bind to receptors (for example, Fcγ receptors, complement receptor 1 or 3, or scavenger receptors) on macrophages and endothelial cells, leading to receptor-mediated phagocytosis and accelerated clearance from the circulation[36]. Some viruses, such as adenoviruses, can bind directly to scavenger receptors on Kupffer cells, inducing proinflammatory cytokines that can result in serious dose-limiting toxicities[37,38].

Strategies to minimize sequestration include chemical modification of viral coat proteins by conjugation of biocompatible polymers, such as polyethylene glycol (PEG) and *N*-[2-hydroxypropyl]methacrylamide (HPMA)[39,40]. Both PEG and HPMA are used clinically to prolong the circulation times of proteins and liposomes and to reduce off-target toxicities[41]. Polymer coating can destroy virus infectivity, which can be restored by re-engineering receptor-binding ligands onto the surface of the shielded particles[42]. For example, PEGylation of VSV glycoprotein–pseudotyped lentiviral vectors increases vector circulation half-life by five-fold and markedly inhibits complement inactivation[43]. PEGylated adenovirus 5 (Ad5) is cleared four-fold slower than unmodified Ad5 (ref. 44). The length of the PEG influences outcome; 20 kDa PEG, but not 5 kDa PEG, can detarget oncolytic Ad5 from Kupffer cells and hepatocytes without inducing liver enzymes[45]. HPMA-cloaked adenovirus vectors are also protected from neutralizing antibodies and have a prolonged circulatory half-life[46]. An alternative approach to minimize sequestration of viruses (for example, HSV) that are readily bound by IgM and complement proteins is to deplete these serum factors by pretreatment with cobra venom factor or cyclophosphamide[47–49].

Virus sequestration by the MPS is saturable[46]. Sequestration by the liver and spleen can therefore be inhibited either by preconditioning to saturate MPS scavenger receptors or by poisoning macrophages and endothelial cells. Predosing of mice with polyinosinic acid, which binds to scavenger receptors on endothelial cells or macrophages in the liver and spleen, can reduce MPS sequestration of adenoviruses[50]. Clodronate-loaded liposomes can also deplete liver Kupffer cells and splenic macrophages of mice. Oncolytic adenoviral therapy has been combined with clodronate liposomes for depletion of Kupffer cells to enhance therapeutic outcome[51,52]. Other MPS-blocking strategies include preadministration of gadolinium chloride or γ-globulins[44,53]. Gadolinium chloride prolonged the circulatory half-life of an Ad5 vector, with a 100-fold difference in blood concentrations at 60 min (ref. 53). Predosing with high doses of intravenous adenoviral particles is toxic to Kupffer cells, which decline substantially in number by 4 h, greatly reducing MPS clearance of a second dose[54,55].

Evading neutralization by serum factors. Many of the barriers viruses encounter after intravenous administration (for example, neutralizing antibodies, inactivation by complement and scavenging by Kupffer cells) can be overcome by hiding oncolytic viruses inside carrier cells. Two cell types have shown promise in preclinical models: tumor cell lines[56] and normal primary cells that can home to tumor beds[57]. Permissive tumor cells are easy to propagate and genetically modify, are productive virus factories *in vivo* and could in theory be used as an 'off the shelf' product. We (Liu, C., S.J.R. & K.W.P.)[58] have shown that lethally irradiated myeloma cells infected with an oncolytic measles virus are therapeutically potent when administered intravenously to myeloma-bearing mice with protective titers of antimeasles antibodies.

Mesenchymal stem cells[59] (MSCs) have been used both preclinically and in small clinical trials[60] to deliver oncolytic viruses to tumor beds. MSCs preferentially engraft into solid tumors[61], and we (K.W.P., S.J.R. and collaborators)[62] have used them recently to efficiently deliver oncolytic measles viruses to intraperitoneal ovarian cancer deposits in the presence of neutralizing antiviral antibodies.

Cellular carriers should ideally be combined with oncolytic viruses that will not kill the carrier before it has infiltrated into the tumor. Some viruses can piggyback on cells found normally in the circulation. Dendritic cells and T cells admixed with reovirus carry and deliver their oncolytic cargo, even in the face of neutralizing antibodies[57,63]. VSV and measles virus can be delivered to tumor beds by being loaded onto T cells; when bound to these cells, VSV particles are protected from neutralizing antibodies[64,65]. Technology for routine isolation of assorted white cells from blood products, which is widely available in medical centers, may facilitate clinical translation of carrier cell approaches.

Selectively increasing the permeability of tumor blood vessels. The enhanced permeability and retention effect, first described in 1986, suggests that the leaky vasculature of tumors can be exploited to enhance delivery of therapeutic macromolecules from the lumenal side of blood vessels into tumor tissues[66,67]. Leakiness is due to the presence of fenestrae (50–80 nm) and intercellular gaps between tumor endothelial cells (200–900 nm compared with 2–6 nm in normal

blood vessels), which mediate extravasation of macromolecules, viruses and nanoparticles[68–71]. However, poor lymphatic drainage and dense stromal tissue increase the interstitial fluid pressure in tumors, impeding virus extravasation and diffusion.

Vascular permeability can be increased by preadministration of interleukin 2 (IL-2), tumor necrosis factor-α, histamine or a bradykinin analog[67,70,72]. Giving chemotherapy can reduce the intratumoral interstitial fluid pressure by killing tumor cells, thereby enhancing extravasation without directly affecting vessel permeability[70]. In a recent study, a combination of vascular endothelial growth factor (VEGF) and metronomic doses of paclitaxel or cisplatin increased the vascular permeability of the tumor endothelium and improved the delivery of Sindbis vector to tumors[73]. Multiple injections of VEGF165 led to superior reovirus infection of proliferating tumor endothelium, thereby increasing therapeutic activity in a syngeneic B16 melanoma model[74]. Systemic IL-2 accompanied by depletion of regulatory T cells also enhanced the extravasation of oncolytic viruses in B16 metastases in the lungs of mice[75].

Targeting viruses to tumor vessel endothelium. In addition to being structurally different from normal vessels[70], the tumor vasculature is antigenically distinct[76–78]. Targets visible from the lumenal side include antigens overexpressed on tumor endothelial cells (for example, $\alpha_v\beta_3$ integrins, VEGF receptor 2, prostate-specific membrane antigen, urokinase plasminogen activator receptor, phosphatidylserine, E-selectin, vascular cell adhesion molecule, tissue factor, endosialin and endoglin (CD105)[77,78]. High-affinity protein or peptide ligands are available for targeting most of these endothelial markers. Other targets include structural elements that are exposed during vessel formation and remodeling; for example, laminin (targeted by L36 single-chain Fv (scFv)[79]) and fibronectin (targeted by the E19 scFv)[80]. Notably, most of the vascular targets mentioned above are not expressed exclusively on tumor blood vessel endothelium but are nevertheless potentially helpful to guide retargeted viruses preferentially to sites of tumor growth.

Chemical or genetic modifications to oncolytic viruses have been used to selectively target the tumor cell surface, detarget sensitive tissues or create dual-target viruses to enhance both vascular targeting and tumor infection (transductional targeting). For instance, we have genetically modified measles virus[81] to display a variety of polypeptide ligands on its surface, facilitating infection of tumor cells overexpressing the targeted receptor[3]. Polymer coating has been used to mask natural attachment proteins, redirecting virus infection by chemically coupling therapeutic antibodies (for example, cetuximab (Erbitux)) that bind tumor cells[82].

A scFv against E selectin was conjugated onto polymer-coated adenoviral particles to enhance their binding to activated endothelial cells in inflamed areas or in tumors[83]. Oncolytic measles viruses expressing vascular targeting peptides, the N-terminal fragment of urokinase plasminogen activator, or cyclic arginine-glycine-aspartate (RGD) and echistatin, which bind to $\alpha_v\beta_3$ integrin receptor, were shown in our laboratory to infect tumor vessel endothelial cells *in vivo*[84,85]. Echistatin binds the $\alpha_v\beta_3$ receptor with 1,000-fold higher affinity than cyclic RGD[86], and this is associated with enhanced ability of the virus to infect tumor vasculature. Recently, one of us (J.C.B. and collaborators)[87] reported that VSV can naturally interact with tumor blood vessel endothelium in CT26 colorectal tumors in Balb/c mice. By 24 h after intravenous infusion of VSV, vascular perfusion shut down at the core of the tumors and VSV antigen was detected in the blood vessels. Another strategy to improve transfer of oncolytic viruses from blood vessel lumen to tumor parenchyma is heterocellular fusion between endothelial cells and underlying tumor cells[88]. Adenoviral vectors encoding a fusogenic membrane glycoprotein driven by the human endothelial receptor tyrosine kinase promoter triggered fusion *in vivo* between endothelial cells and epithelial cells, facilitating transendothelial virus penetration[88].

Enhancing intratumoral spread of oncolytic viruses

Mammalian cells have evolved to resist virus infections (**Fig. 4**). A typical infection involves attacks on cellular defenses by viral gene products (virulence proteins), defensive parries by the host cell through the elaboration of antiviral proteins and further counterattacks by the virus. Viral virulence genes encode proteins that suppress host defense systems, facilitate virus spread between cells and usurp cell metabolic processes. Oncolytic viruses are selected or engineered to be attenuated in normal tissues, often by mutation or deletion of virus virulence genes[77,89]. Thus, an oncolytic virus entering a normal cell triggers the cellular antiviral response but cannot counterattack, so the infection is quickly eliminated. The antiviral response involves production of proteins that counteract the virus by acting directly against the virus[90,91], communicating with adjacent cells[92] or jump-starting apoptotic programs[93]. Type I interferons and their receptors are key players in this antiviral response, reprogramming the physiological properties of infected and surrounding cells, inducing cell cycle arrest, providing antiangiogenic signals, promoting apoptosis, inhibiting protein synthesis and activating the immune system.

In contrast to normal cells, the successful tumor cell has often eliminated or inactivated key gene products that have the dual role of controlling critical cell growth and death programs and of aiding in resisting virus infections[94]. Because of these tumor-specific mutations, oncolytic viruses—despite their defective virulence genes—can initiate productive infections in cancer cells. Occasionally, cancer cells are completely devoid of antiviral activity[89], but partial inactivation is more common, leading to only limited sensitivity to oncolytic virus therapy[95].

Promoting viral growth by genetic arming and chemical sensitizers. Incorporation of virulence genes from other virus strains or repair of previously attenuated or deleted virulence genes can overcome residual antiviral responses found in some tumors[95,96]. Although this could compromise the excellent safety profile of oncolytic viruses, the approach could be fine-tuned to enhance clinical oncolytic virus therapy. A related approach to improve oncolytic virus potency is to combine viruses with complementing virulence proteins[97]. An interferon-sensitive oncolytic VSV replicated efficiently in refractory tumor cells when they were co-infected with an

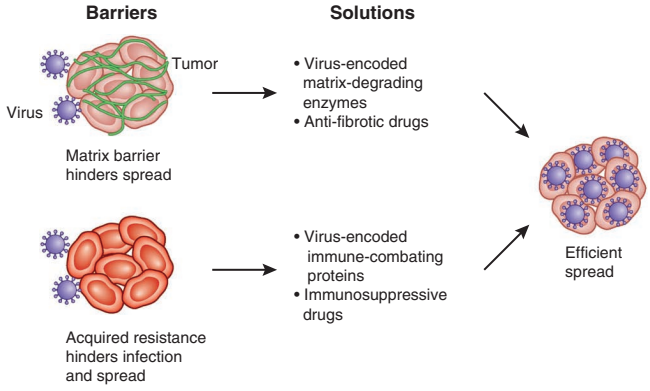

Figure 4 Factors constraining intratumoral virus spread (extracellular matrix and host innate or acquired immunity) and solutions to circumvent them.

oncolytic poxvirus encoding a secreted interferon antagonist. One of our groups (J.C.B. and collaborators)[97] achieved a similar 'ping pong' synergy effect using an engineered fusogenic VSV to accelerate intratumoral spread (through cell fusion) of an oncolytic poxvirus. Because VSV has an RNA genome and vaccinia has a DNA genome, an exchange of virulence genes leading to a pathogenic 'supervirus' seems improbable. Clinical development of poxviruses encoding an interferon antagonist (B18R protein) is currently underway[98], and two type I interferon–responsive oncolytic viruses (OncoVEX and Reolysin) are in advanced-stage trials. If approved as single agents, these viruses could be combined to increase efficacy.

Another tactic to neutralize residual antiviral activities in oncolytic virus–resistant cancers is through the use of small molecules. Several groups (including our own) have shown that histone deacetylase inhibitors can suppress the residual interferon responsiveness of tumor cells, thereby increasing oncolytic virus potency without compromising specificity[99–102]. One of our groups (J.C.B. and colleagues[103]) has identified additional compounds that enhance oncolytic virus growth in refractory tumor cells using high-throughput screening. The previously uncharacterized compound, 3,4-dichloro-5-phenyl-2,5-dihydrofuran-2-on, whose mechanism of action is under investigation, enhanced the growth of various oncolytic viruses on a spectrum of tumor cells by blunting their interferon responsiveness. Several chemically unrelated compounds were isolated in this screen, but their cellular targets have not yet been defined. A similar screen by another group[104] with an attenuated HSV lacking ribonucleotide reductase identified two molecules (dipyridamole and dilazep) that inhibit the cellular equilibrative nucleoside transporter 1, thereby inducing cellular ribonucleotide reductase.

Rapamycin potentiates the growth of several oncolytic viruses in rodent tumor models, mainly by disrupting the target of rapamycin complex-1 (TORC1)-dependent production of interferon and/or disrupting the phosphotidylinositol 3-kinase AKT (protein kinase B) pathway[105–107]. Cyclophosphamide also improves oncolytic virus efficacy through several mechanisms. It dampens the innate antiviral response, slows the generation of antioncolytic virus–neutralizing antibodies, may target regulatory T cells and may affect tumor vasculature, enhancing oncolytic virus extravasation[108–110]. Even so, combining drugs with viruses is not without risk and could promote off-target infections, compromising safety[109].

Improving virus spread in tumors. Some oncolytic viruses are particularly well equipped to spread within and between tumors. For instance, vaccinia virus generates multiple virus 'subspecies' adapted in different ways for efficient spread. The extracellular enveloped or 'cloaked' form facilitates widespread dissemination and to some extent avoidance of neutralizing antibodies[111], whereas the cell-associated form has an actin tail that propels the virus into adjacent tumor cells[112]. Other viruses, such as oncolytic measles, spread by fusing infected with uninfected cells[113,114]. Engineering a cell-fusion capacity into other virus platforms can improve therapy[115] but can also lead to increased unwanted pathology[116].

The movement of viruses through tumors can be impeded by dense intratumoral connective tissue[117,118]. Losartan (Cozaar), a US Food and Drug Administration–approved angiotensin II receptor antagonist and antihypertensive agent, enhanced the intratumoral spread of an oncolytic HSV by disrupting transforming growth factor-β1 (TGF-β1) signaling, which decreases collagen production, although several weeks of antifibrotic activity would be needed to alter clinical oncolytic virus outcomes[119]. Hyaluronan is a sulfated glycosaminoglycan and key component of the tumor extracellular matrix. Injecting hyaluronidase into tumors enhances the spread and efficacy of oncolytic adenoviruses[120]. A hyaluronidase-expressing oncolytic adenovirus showed improved spread and activity in a human melanoma xenograft model[121]. Lastly, damage caused to tumors by cytotoxic agents, radiation or apoptosis inducers can lead to creation of voids and channels that facilitate virus spread[118].

Engineering tumor selectivity into oncolytic virus backbones. Many of the earliest engineered oncolytic viruses were based on the adenovirus backbone and were designed to take advantage of 'tumor-specific' promoter elements[122]. For example, the human telomerase reverse transcriptase promoter is inactive in essentially all adult somatic tissues but is robustly expressed in cancer cells[104]. An alternative strategy is to use a promoter element that targets both the cancer and an expendable adult tissue (for example, prostate[123]). This approach has been extended to HSV[124] and more recently to replication-competent retroviral oncolytic vectors[13]. Expression profiling is providing new leads for promoters that could be used for oncolytic virus regulation[125]. Poxviruses are not amenable to transcriptional targeting because they replicate entirely in the cell cytoplasm and regulate their transcription independently of the host cell transcriptional machinery.

Transductional targeting (discussed above) can also be used to eliminate toxicities, particularly when the oncolytic virus binds a ubiquitous receptor. VSV was pseudotyped with the surface glycoprotein from a non-neurotropic lymphocytic choriomeningitis virus or retargeted measles virus, thereby eliminating its neurotoxicity without compromising its ability to infect and kill cancer cells[126,127]. Modification of the hypervariable loop of the adenovirus hexon protein ablated the ability of that virus to infect normal hepatocytes but not tumor cells[128].

Given the potential off-target effects of transcriptional and transductional targeting, other tropism-modifying strategies are of interest. A notable new strategy is the application of microRNA targeting to oncolytic viruses[129,130], which takes advantage of differential expression of certain microRNA species in tumor and normal tissues. Insertion of liver-specific microRNA binding sites in the 3′ untranslated region (UTR) of the gene encoding E1A of an oncolytic adenovirus eliminated its hepatotoxicity without destroying tumor cell–killing activity[130,131].

MicroRNA regulation of oncolytic virus tropism was first described in RNA viruses that cannot be controlled through transcriptional targeting[27,132,133]. Particularly notable are the results with coxsackievirus A21, a potent oncolytic virus in mice that also causes fatal myositis owing to off-target infection of normal muscle. We showed that inclusion of muscle-specific microRNA targets into the 3′ UTR of coxsackievirus A21 eliminates muscle toxicity but does not compromise anticancer activity[27]. A potential issue with this approach is that microRNA targets can mutate during oncolysis, so it may be prudent to use a second selectivity strategy to minimize the chances of toxic escape variants arising during therapy. An oncolytic adenovirus was regulated by both transcriptional targeting (telomerase promoter) and microRNA targeting of the gene encoding E1 (ref. 134). Dual targeting approaches may facilitate the generation of potent but highly specific oncolytic strains encoding wild-type virulence proteins. The positioning of microRNA targets is another critical determinant for their effectiveness in attenuating virus replication. Inclusion of microRNA targets in VSV can eliminate unwanted neurotoxicity in mice; however, it is successful only when positioned at the extreme end of the viral genome controlling the expression of the L or polymerase gene of the virus[132].

Oncolytic virus replication can also be targeted by regulation of viral protein translation. The potently oncolytic chimeric poliovirus,

PVS-RIPO, a live-attenuated poliovirus type 1 (Sabin) vaccine containing an internal ribosome entry site (IRES) element from human rhinovirus type 2, lacks neurotoxicity because translation from the inserted rhinovirus IRES is selectively blocked in neurons[135]. Translational control through the IRES element of another picornavirus, encephalomyocarditis virus, seems to have a role in its oncolytic specificity[136]. The concentration and activation state of eukaryotic initiation factor 4E (eIF4E) contribute to the initiation and progression of various cancers, and some viruses actively promote eIF4E activation[137]. Incorporation of complex 5′ UTRs responsive to cellular eIF4E has therefore been used to target HSV[124] and adenovirus[138].

Conditional manipulation of protein stability can also regulate oncolytic virus expression. Fusion of a 'destabilizing domain' was used to create chimeric proteins that are inherently unstable[139,140]. A cell-permeable synthetic small molecule ligand called Shield-1 ((S)-(R)-3-(3,4-dimethoxyphenyl)-1-(3-(2-morpholinoethoxy)phenyl)propyl 1-((S)-2-(3,4,5-trimethoxyphenyl)butanoyl)piperidine-2-carboxylate) can bind the destabilizing domain and reverse this instability, allowing regulated production of imaging proteins (for example, luciferase) or tumor necrosis factor-α in animal tumor models[139–141]. This provides a system for fine-tuned expression of therapeutic transgenes to control the spread of an oncolytic virus.

Controlling adaptive immunity and clearance of oncolytic viruses. The history of the oncolytic virus field suggests that immunosuppressed patients generally respond better to oncolytic virus therapy than those with an intact immune system and that this higher oncolytic activity is often associated with unacceptable toxicity[7]. Impairment of the adaptive antiviral immune response is therefore a double-edged sword, but can be used to our advantage provided that the virus is so specific for the tumor that it cannot damage normal tissues.

Virus-targeting technologies have now advanced to the point at which combining virotherapy with immunosuppressive drugs is an appealing approach for enhancing their antitumor activity. There are many immunosuppressive drugs to choose from in this regard, but cyclophosphamide is currently the most favored because it is potently toxic to both T and B lymphocytes, has direct antitumor activity, has been widely used since 1949 for both cancer therapy and immunosuppression and is reasonably priced[142]. Several preclinical studies have shown that cyclophosphamide can retard immune clearance of oncolytic viruses, enhance persistence of virus infection and prolong therapeutic efficacy[143]; the approach is now being evaluated in clinical trials.

Enhancing antitumor immunity. Immune evasion by tumors, which is one of the hallmarks of cancer, is an important target for new cancer therapeutics[144]. Tumors produce immunosuppressive cytokines (for example, TGF-β) and recruit immune inhibitory cells (for example, regulatory T cells), thereby paralyzing the antitumor immune response[145,146]. Oncolytic viruses may represent a unique strategy for combining tumor debulking activity (via direct tumor lysis and/or vascular attack) with potent activation of adaptive and innate immune responses[28]. Targeted infection of the tumor leads to a localized inflammatory response, triggering an immune storm directly within the malignancy and facilitating immune recognition of cancer-specific neoantigens[146]. As discussed above, work with the oncolytic vaccinia virus JX-594 and more recently with the oncolytic herpes virus talimogene laherparepvec, both of which are armed with GM-CSF, suggests that clinical benefit can be achieved when localized oncolytic activity is coupled with immune cell recruitment[19,146,147].

The idea of an oncolytic vaccine combining virus-mediated tumor destruction with immune recognition of tumor antigens is attractive but requires careful orchestration as the activated immune system may prematurely suppress therapeutic virus replication[148]. Even limited infection of distant lymph node metastases may lead to enhanced therapeutic benefit. An oncolytic rhabdovirus expressing a tumor antigen can robustly boost a primed antitumor immune response[149], but only if given systemically when the virus can access both the tumor and distant lymph tissues[146,149]. Recently, striking results were observed in tumor-bearing animals 'vaccinated' with an oncolytic rhabdovirus expressing a complex library of cDNAs encoding normal cellular antigens[150]. Although it is improbable that a library of viruses expressing thousands of unique sequences could become a therapeutic product, this work suggests that future oncolytics expressing a few carefully selected tumor antigens should be tested clinically. Toward that goal, three VSVs encoding melanoma-specific antigens that induce IL-17 recall responses were selected from a library of VSV-cDNA; when used in combination but not alone, they were as efficacious as the parental complete VSV-cDNA library[151]. Several groups are combining adoptive cell therapy with oncolytic viruses, reasoning that virus-mediated tumor cell destruction should enhance the activity of the transferred cells[146,152].

Which virus for which indication? Given that naturally occurring viruses have such widely differing structures, life cycles and tropisms, leading to many diverse clinical manifestations, it would seem logical that each oncolytic virus would be ideally suited to a specific malignancy. It is therefore somewhat surprising that there are so far few examples of this specific matching of a given oncolytic virus with a specific class of malignancy, and most of the oncolytic viruses currently in development show a relatively broad spectrum of antitumor activity, typically against both epithelial and hematological malignancies. Certain oncolytic viruses were initially developed with the expectation that they would be better suited to a given broad class of malignancy, but this has proven not to be the case. Thus, oncolytic adenoviruses were considered better suited for therapy of epithelial malignancies but show activity against hematologic cancers[153,154]; HSVs were developed originally for brain cancer therapy but show promise in various non–central nervous system tumors, including sarcomas and epithelial malignancies[154,155]; and measles viruses were first considered ideal for hematologic malignancies but also have broad-spectrum activity against epithelial malignancies and sarcomas[156].

When viruses are engineered to target specific cell surface receptors or nuclear transcription factors, their use is thereafter limited to tumors that express the relevant target, but so far there has been a preference for clinical translation of oncolytic viruses with broader-spectrum antitumor activity. This may be because safety concerns are more difficult to address for fully retargeted viruses that stringently target a single type of tumor. Although it may seem counterintuitive that a virus engineered to restrict its host range might have greater pathogenic potential than the parent virus, several examples indicate that loss of pathogenic potential (attenuation) is associated with broadening of virus host range[157,158]. Thus, assumptions about safety and host range must be tested experimentally in appropriate animal species to directly address this question.

Safety considerations are ever present in preclinical oncolytic virus studies and may also drive the choice of virus for a given indication. Different viruses have differing toxicities, and genetic manipulation of viruses may lead to unexpected toxicities, such as an instance in which insertion of the gene encoding IL-4 into a murine poxvirus led to 100% lethality in prevaccinated animals that had been completely immune to the wild-type virus[159]. Natural and engineered virus tropisms, virus mutability and capacity for evolution, immunomodulatory,

antiapoptotic and cytotoxic gene products, virus transmissibility, prevalence of antiviral immunity in the population and availability of drugs or antisera to eliminate unwanted or persistent infections are all important factors to be considered in the safety analysis of oncolytic viruses that are candidates for clinical translation.

Conclusions

Oncolytic viruses are structurally and biologically diverse, spreading through tumors and killing tumor cells by multiple mechanisms and with different kinetics. Because of their large size and immunogenicity, they are constrained by physical barriers and by host immunity, but they can also cross-prime and amplify antitumor immunity, serving as a cancer immunotherapy. Overall, the field has been slow to develop, but recent clinical trial data have been promising, and a first-in-class US approval is expected soon for talimogene laherparepvec, which is being tested in a randomized phase 3 clinical trial that has recently completed accrual. The phase 1/2 trial showed that, after intralesional injection to patients with metastatic malignant melanoma, this virus spreads locally, cross-priming the antimelanoma immune response, but does not spread systemically to distant sites of tumor growth. Thus, the approach primarily exploits the oncolytic virus as a tumor-debulking immunotherapy and does not clinically validate the 'oncolytic paradigm', in which systemic and intratumoral spread of infection leads to tumor debulking as a prelude to immune-mediated eradication of minimal residual disease. However, a more direct validation of the oncolytic paradigm may soon come from ongoing clinical trials testing intravascular oncolytic virus delivery in immunotherapy-resistant tumors using, for example, reovirus, vaccinia virus and measles virus. For the future, there is a long and growing list of new or improved versions of oncolytic viruses that have been ingeniously selected, engineered and honed for systemic therapy, several of which may, in the fullness of time, join the growing arsenal of clinically approved anticancer drugs.

Looking beyond the expected clinical approval of oncolytic viruses as single agents, there is enormous scope for the development of more complex protocols to achieve superior treatment outcomes. Preclinical studies provide a strong basis for this assertion, demonstrating many synergistic interactions that can overcome the various barriers constraining oncolytic viruses, such as the use of cell carriers to optimize virus delivery[62] or of immunosuppressive drugs[160] to enhance their intratumoral spread. A particularly interesting prospect is new drugs that can potently suppress the innate immune responses of virus-infected cells. Elucidation of the intracellular signaling pathways of innate immunity has been progressing rapidly in recent years, so the stage is set for this important area of drug discovery.

As the field comes closer to its lofty goal of a single-shot virotherapy cure for cancer[28], we will probably encounter considerable treatment-related toxicities. The minimal toxicity in clinical trials so far is often cited as a strength of the oncolytic virotherapy approach, but without rapid, destructive spread of intratumoral virus, which is the ultimate goal, it is hardly surprising that the treatment has seemed innocuous. With greater potency and more reliable efficacy, toxicity will surely follow, and hence we will need more stringent virus-targeting technology to ensure that the destructive power of these new agents is focused exclusively on the tumor.

The most important technical challenges that continue to captivate the oncolytic research community are optimization and enhancement of systemic virus delivery, intratumoral virus spread and cross-priming of anticancer immunity. However, harmonization of solutions to these problems is perhaps a greater challenge, although it is certainly achievable. Suppressing immunity may increase intratumoral spread, but it diminishes cross-priming of the anticancer immune response. Conversely, enhancing immunity may improve cross-priming, but the price paid is suppression of intratumoral virus spread, the basis of oncolytic tumor debulking. Many of the 'solutions' that have been developed so far have been analyzed in artificial model systems that cannot reveal the positive and negative consequences of a given modification to all aspects of the treatment. This points to another major challenge for the field, which is to develop better model systems that reliably mirror the human oncolytic virotherapy scenario. Mouse xenograft models lack a functional immune system, and immunocompetent mouse tumor models are frequently misleading because the viruses being tested behave differently in mice and humans. Thus, many oncolytic viruses cannot infect mouse cells, so they lack activity in syngeneic mouse models, whereas others preferentially infect mouse versus human cells, so their anticancer activity (and toxicity) is not transferrable to human trials. The use of oncolytic agents such as VSV or vaccinia virus, which can infect mouse and human cells with equal efficiency, is a potential solution to this problem. The development of transgenic mouse models susceptible to 'human-specific' viruses is also an important goal.

The biggest overall challenges facing the field now have less to do with the development of technology solutions to enhance virus delivery and spread than with how to get new viruses clinically tested. There are so many elegant solutions available for hypothetical problems that it can be demoralizing for scientists to see their engineering efforts lost in the morass. Clinical testing of each new virus modification is not realistic because of the enormous amount of work and expense required—manufacturing, pharmacology and toxicology testing, protocol development and regulatory approval—to move each new product into phase 1 trials. For example, a PubMed search over the past ten years for oncolytic adenoviruses shows ~1,000 publications on almost as many unique adenovirus configurations, representing multiple serotypes (for antibody evasion) with or without engineered fiber modifications (for transductional targeting), hexon modifications (to eliminate hepatic sequestration), polymeric coats (for shielding), gene deletions (for physiological targeting) and transgene insertions (to combat innate immunity, enhance adaptive immunity, promote spread, increase cytotoxicity or facilitate noninvasive monitoring). Each of these modifications can be classified as a new product, and the modifications are appearing so fast that an oncolytic virus that was state of the art a few years ago may today be considered archaic even before it has completed phase 2 clinical testing. The solution to this conundrum of technology developments outstripping our ability to test them in the clinic will most likely have to be a new drug development paradigm for the oncolytic virotherapy field in which iterative phase 1 equivalence clinical trials become standard practice. Unlike conventional drugs, which are typically perfected before they enter clinical testing, oncolytic viruses are more akin to motor cars with multiple component parts, each one constantly subject to improvement, refinement and perfection through engineering efforts. Iterative phase 1 equivalence trials may thus provide a mechanism whereby the steady stream of new engineering modifications that only slightly change the product specification without affecting its safety features can be accommodated into an evolving clinically approved product.

ACKNOWLEDGMENTS

S.J.R. and K.-W.P. acknowledge funding support from the Mayo Foundation, Mayo Clinic Comprehensive Cancer Center (CA15083), US National Institutes of Health and National Cancer Institute (CA100634, CA129966, CA118488, CA129193, CA136547 and CA136393), Richard M. Schulze Family Foundation, Al and Mary Agnes McQuinn and Minnesota Partnership for Biotechnology. J.C.B. is supported

by the Ontario Institute for Cancer Research, the Terry Fox Foundation and the Ottawa Regional Cancer Foundation.

COMPETING FINANCIAL INTERESTS

The authors declare competing financial interests: details are available in the online version of the paper.

Published online at http://www.nature.com/doifinder/10.1038/nbt.2287.
Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Russell, S.J. & Peng, K.W. Viruses as anticancer drugs. *Trends Pharmacol. Sci.* **28**, 326–333 (2007).
2. Virgin, S. Pathogenesis of viral infection. in *Fields Virology*, 5th edn, vol. 1 (eds. Knipe, D.M. & Howley, P.M. (Lippincott Williams & Wilkins, Philadelphia, 2007).
3. Cattaneo, R., Miest, T., Shashkova, E.V. & Barry, M.A. Reprogrammed viruses as cancer therapeutics: targeted, armed and shielded. *Nat. Rev. Microbiol.* **6**, 529–540 (2008).
4. Dorer, D.E. & Nettelbeck, D.M. Targeting cancer by transcriptional control in cancer gene therapy and viral oncolysis. *Adv. Drug Deliv. Rev.* **61**, 554–571 (2009).
5. Naik, S. & Russell, S.J. Engineering oncolytic viruses to exploit tumor specific defects in innate immune signaling pathways. *Expert Opin. Biol. Ther.* **9**, 1163–1176 (2009).
6. Kelly, E.J. & Russell, S.J. MicroRNAs and the regulation of vector tropism. *Mol. Ther.* **17**, 409–416 (2009).
7. Kelly, E. & Russell, S.J. History of oncolytic viruses: genesis to genetic engineering. *Mol. Ther.* **15**, 651–659 (2007).
8. Southam, C.M. Present status of oncolytic virus studies. *Trans. N.Y. Acad. Sci.* **22**, 657–673 (1960).
9. Asada, T. Treatment of human cancer with mumps virus. *Cancer* **34**, 1907–1928 (1974).
10. Martuza, R.L., Malick, A., Markert, J.M., Ruffner, K.L. & Coen, D.M. Experimental therapy of human glioma by means of a genetically engineered virus mutant. *Science* **252**, 854–856 (1991).
11. Au, G.G., Lindberg, A.M., Barry, R.D. & Shafren, D.R. Oncolysis of vascular malignant human melanoma tumors by coxsackievirus A21. *Int. J. Oncol.* **26**, 1471–1476 (2005).
12. Rudin, C.M. *et al.* Phase I clinical study of Seneca Valley virus (SVV-001), a replication-competent picornavirus, in advanced solid tumors with neuroendocrine features. *Clin. Cancer Res.* **17**, 888–895 (2011).
13. Tai, C.K. & Kasahara, N. Replication-competent retrovirus vectors for cancer gene therapy. *Front. Biosci.* **13**, 3083–3095 (2008).
14. Liu, T.C., Galanis, E. & Kirn, D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. *Nat. Clin. Pract. Oncol.* **4**, 101–117 (2007).
15. Schoofs, G. *et al.* A high-yielding serum-free, suspension cell culture process to manufacture recombinant adenoviral vectors for gene therapy. *Cytotechnology* **28**, 81–89 (1998).
16. Knop, D.R. & Harrell, H. Bioreactor production of recombinant herpes simplex virus vectors. *Biotechnol. Prog.* **23**, 715–721 (2007).
17. Lewis, J.A., Brown, E.L. & Duncan, P.A. Approaches to the release of a master cell bank of PER.C6 cells; a novel cell substrate for the manufacture of human vaccines. *Dev. Biol. (Basel)* **123**, 165–176, discussion 183–197 (2006).
18. Russell, S.J. Replicating vectors for cancer therapy: a question of strategy. *Semin. Cancer Biol.* **5**, 437–443 (1994).
19. Senzer, N.N. *et al.* Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. *J. Clin. Oncol.* **27**, 5763–5771 (2009).
20. Park, B.H. *et al.* Use of a targeted oncolytic poxvirus, JX-594, in patients with refractory primary or metastatic liver cancer: a phase I trial. *Lancet Oncol.* **9**, 533–542 (2008).
21. Eager, R.M. & Nemunaitis, J. Clinical development directions in oncolytic viral therapy. *Cancer Gene Ther.* **18**, 305–317 (2011).
22. Mastrangelo, M.J. *et al.* Intratumoral recombinant GM-CSF-encoding virus as gene therapy in patients with cutaneous melanoma. *Cancer Gene Ther.* **6**, 409–422 (1999).
23. Harrington, K.J. *et al.* Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. *Clin. Cancer Res.* **16**, 4005–4015 (2010).
24. Harrington, K.J., Vile, R.G., Melcher, A., Chester, J. & Pandha, H.S. Clinical trials with oncolytic reovirus: moving beyond phase I into combinations with standard therapeutics. *Cytokine Growth Factor Rev.* **21**, 91–98 (2010).
25. Heo, J. *et al.* Sequential therapy with JX-594, a targeted oncolytic poxvirus, followed by sorafenib in hepatocellular carcinoma: preclinical and clinical demonstration of combination efficacy. *Mol. Ther.* **19**, 1170–1179 (2011).
26. Peng, K.W., Facteau, S., Wegman, T., O'Kane, D. & Russell, S.J. Non-invasive *in vivo* monitoring of trackable viruses expressing soluble marker peptides. *Nat. Med.* **8**, 527–531 (2002).
27. Kelly, E.J., Hadac, E.M., Greiner, S. & Russell, S.J. Engineering microRNA responsiveness to decrease virus pathogenicity. *Nat. Med.* **14**, 1278–1283 (2008).
28. Naik, S. *et al.* Curative one-shot systemic virotherapy in murine myeloma. *Leukemia* published online, 10.1038/leu.2012.70 (19 March 2012).
29. Breitbach, C.J. *et al.* Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature* **477**, 99–102 (2011).
30. Serganova, I., Ponomarev, V. & Blasberg, R. Human reporter genes: potential use in clinical studies. *Nucl. Med. Biol.* **34**, 791–807 (2007).
31. Galanis, E. *et al.* Phase I trial of intraperitoneal administration of an oncolytic measles virus strain engineered to express carcinoembryonic antigen for recurrent ovarian cancer. *Cancer Res.* **70**, 875–882 (2010).
32. Jacobs, A. *et al.* Positron-emission tomography of vector-mediated gene expression in gene therapy for gliomas. *Lancet* **358**, 727–729 (2001).
33. Dingli, D., Russell, S.J. & Morris, J.C. III. *In vivo* imaging and tumor therapy with the sodium iodide symporter. *J. Cell. Biochem.* **90**, 1079–1086 (2003).
34. Barton, K.N. *et al.* Phase I study of noninvasive imaging of adenovirus-mediated gene expression in the human prostate. *Mol. Ther.* **16**, 1761–1769 (2008).
35. Dingli, D. *et al.* Image-guided radiovirotherapy for multiple myeloma using a recombinant measles virus expressing the thyroidal sodium iodide symporter. *Blood* **103**, 1641–1646 (2004).
36. Underhill, D.M. & Ozinsky, A. Phagocytosis of microbes: complexity in action. *Annu. Rev. Immunol.* **20**, 825–852 (2002).
37. Haisma, H.J. *et al.* Scavenger receptor A: a new route for adenovirus 5. *Mol. Pharm.* **6**, 366–374 (2009).
38. Bessis, N., GarciaCozar, F.J. & Boissier, M.C. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. *Gene Ther.* **11** (suppl. 1), S10–S17 (2004).
39. Fisher, K.D. & Seymour, L.W. HPMA copolymers for masking and retargeting of therapeutic viruses. *Adv. Drug Deliv. Rev.* **62**, 240–245 (2010).
40. Eto, Y., Yoshioka, Y., Mukai, Y., Okada, N. & Nakagawa, S. Development of PEGylated adenovirus vector with targeting ligand. *Int. J. Pharm.* **354**, 3–8 (2008).
41. Duncan, R. Polymer conjugates as anticancer nanomedicines. *Nat. Rev. Cancer* **6**, 688–701 (2006).
42. Morrison, J. *et al.* Virotherapy of ovarian cancer with polymer-cloaked adenovirus retargeted to the epidermal growth factor receptor. *Mol. Ther.* **16**, 244–251 (2008).
43. Croyle, M.A. *et al.* PEGylation of a vesicular stomatitis virus G pseudotyped lentivirus vector prevents inactivation in serum. *J. Virol.* **78**, 912–921 (2004).
44. Alemany, R., Suzuki, K. & Curiel, D.T. Blood clearance rates of adenovirus type 5 in mice. *J. Gen. Virol.* **81**, 2605–2609 (2000).
45. Doronin, K., Shashkova, E.V., May, S.M., Hofherr, S.E. & Barry, M.A. Chemical modification with high molecular weight polyethylene glycol reduces transduction of hepatocytes and increases efficacy of intravenously delivered oncolytic adenovirus. *Hum. Gene Ther.* **20**, 975–988 (2009).
46. Green, N.K. *et al.* Extended plasma circulation time and decreased toxicity of polymer-coated adenovirus. *Gene Ther.* **11**, 1256–1263 (2004).
47. Ikeda, K. *et al.* Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses. *Nat. Med.* **5**, 881–887 (1999).
48. Ikeda, K. *et al.* Complement depletion facilitates the infection of multiple brain tumors by an intravascular, replication-conditional herpes simplex virus mutant. *J. Virol.* **74**, 4765–4775 (2000).
49. Wakimoto, H. *et al.* The complement response against an oncolytic virus is species-specific in its activation pathways. *Mol. Ther.* **5**, 275–282 (2002).
50. Haisma, H.J. & Bellu, A.R. Pharmacological interventions for improving adenovirus usage in gene therapy. *Mol. Pharm.* **8**, 50–55 (2011).
51. Shashkova, E.V., Doronin, K., Senac, J.S. & Barry, M.A. Macrophage depletion combined with anticoagulant therapy increases therapeutic window of systemic treatment with oncolytic adenovirus. *Cancer Res.* **68**, 5896–5904 (2008).
52. Koski, A. *et al.* Systemic adenoviral gene delivery to orthotopic murine breast tumors with ablation of coagulation factors, thrombocytes and Kupffer cells. *J. Gene Med.* **11**, 966–977 (2009).
53. Ziegler, R.J. *et al.* Correction of the nonlinear dose response improves the viability of adenoviral vectors for gene therapy of Fabry disease. *Hum. Gene Ther.* **13**, 935–945 (2002).
54. Manickan, E. *et al.* Rapid Kupffer cell death after intravenous injection of adenovirus vectors. *Mol. Ther.* **13**, 108–117 (2006).
55. Tao, N. *et al.* Sequestration of adenoviral vector by Kupffer cells leads to a nonlinear dose response of transduction in liver. *Mol. Ther.* **3**, 28–35 (2001).
56. Power, A.T. & Bell, J.C. Taming the Trojan horse: optimizing dynamic carrier cell/oncolytic virus systems for cancer biotherapy. *Gene Ther.* **15**, 772–779 (2008).
57. Ilett, E.J. *et al.* Dendritic cells and T cells deliver oncolytic reovirus for tumour killing despite pre-existing anti-viral immunity. *Gene Ther.* **16**, 689–699 (2009).
58. Liu, C., Russell, S.J. & Peng, K.W. Systemic therapy of disseminated myeloma in passively immunized mice using measles virus-infected cell carriers. *Mol. Ther.* **18**, 1155–1164 (2010).
59. Dwyer, R.M., Khan, S., Barry, F.P., O'Brien, T. & Kerin, M.J. Advances in mesenchymal stem cell-mediated gene therapy for cancer. *Stem Cell Res. Ther.* **1**, 25 (2010).

60. García-Castro, J. *et al.* Treatment of metastatic neuroblastoma with systemic oncolytic virotherapy delivered by autologous mesenchymal stem cells: an exploratory study. *Cancer Gene Ther.* **17**, 476–483 (2010).
61. Ling, X. *et al.* Mesenchymal stem cells overexpressing IFN-β inhibit breast cancer growth and metastases through Stat3 signaling in a syngeneic tumor model. *Cancer Microenviron.* **3**, 83–95 (2010).
62. Mader, E.K. *et al.* Mesenchymal stem cell carriers protect oncolytic measles viruses from antibody neutralization in an orthotopic ovarian cancer therapy model. *Clin. Cancer Res.* **15**, 7246–7255 (2009).
63. Ilett, E.J. *et al.* Internalization of oncolytic reovirus by human dendritic cell carriers protects the virus from neutralization. *Clin. Cancer Res.* **17**, 2767–2776 (2011).
64. Qiao, J. *et al.* Loading of oncolytic vesicular stomatitis virus onto antigen-specific T cells enhances the efficacy of adoptive T-cell therapy of tumors. *Gene Ther.* **15**, 604–616 (2008).
65. Ong, H.T., Hasegawa, K., Dietz, A.B., Russell, S.J. & Peng, K.W. Evaluation of T cells as carriers for systemic measles virotherapy in the presence of antiviral antibodies. *Gene Ther.* **14**, 324–333 (2007).
66. Matsumura, Y. & Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **46**, 6387–6392 (1986).
67. Fang, J., Nakamura, H. & Maeda, H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. *Adv. Drug Deliv. Rev.* **63**, 136–151 (2011).
68. Hobbs, S.K. *et al.* Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc. Natl. Acad. Sci. USA* **95**, 4607–4612 (1998).
69. Hashizume, H. *et al.* Openings between defective endothelial cells explain tumor vessel leakiness. *Am. J. Pathol.* **156**, 1363–1380 (2000).
70. Jain, R.K. & Stylianopoulos, T. Delivering nanomedicine to solid tumors. *Nat. Rev. Clin. Oncol.* **7**, 653–664 (2010).
71. Fox, M.E., Szoka, F.C. & Frechet, J.M. Soluble polymer carriers for the treatment of cancer: the importance of molecular architecture. *Acc. Chem. Res.* **42**, 1141–1151 (2009).
72. Barnett, F.H. *et al.* Selective delivery of herpes virus vectors to experimental brain tumors using RMP-7. *Cancer Gene Ther.* **6**, 14–20 (1999).
73. Tseng, J.C., Granot, T., DiGiacomo, V., Levin, B. & Meruelo, D. Enhanced specific delivery and targeting of oncolytic Sindbis viral vectors by modulating vascular leakiness in tumor. *Cancer Gene Ther.* **17**, 244–255 (2010).
74. Kottke, T. *et al.* Antiangiogenic cancer therapy combined with oncolytic virotherapy leads to regression of established tumors in mice. *J. Clin. Invest.* **120**, 1551–1560 (2010).
75. Kottke, T. *et al.* T_{reg} depletion-enhanced IL-2 treatment facilitates therapy of established tumors using systemically delivered oncolytic virus. *Mol. Ther.* **16**, 1217–1226 (2008).
76. Driessen, W.H., Ozawa, M.G., Arap, W. & Pasqualini, R. Ligand-directed cancer gene therapy to angiogenic vasculature. *Adv. Genet.* **67**, 103–121 (2009).
77. Thorne, S.H. *et al.* Rational strain selection and engineering creates a broad-spectrum, systemically effective oncolytic poxvirus, JX-963. *J. Clin. Invest.* **117**, 3350–3358 (2007).
78. Neri, D. & Bicknell, R. Tumour vascular targeting. *Nat. Rev. Cancer* **5**, 436–446 (2005).
79. Sanz, L. *et al.* Single-chain antibody-based gene therapy: inhibition of tumor growth by in situ production of phage-derived human antibody fragments blocking functionally active sites of cell-associated matrices. *Gene Ther.* **9**, 1049–1053 (2002).
80. Palumbo, A. *et al.* A chemically modified antibody mediates complete eradication of tumours by selective disruption of tumour blood vessels. *Br. J. Cancer* **104**, 1106–1115 (2011).
81. Nakamura, T. *et al.* Rescue and propagation of fully retargeted oncolytic measles viruses. *Nat. Biotechnol.* **23**, 209–214 (2005).
82. Morrison, J. *et al.* Cetuximab retargeting of adenovirus via the epidermal growth factor receptor for treatment of intraperitoneal ovarian cancer. *Hum. Gene Ther.* **20**, 239–251 (2009).
83. Bachtarzi, H. *et al.* Targeting adenovirus gene delivery to activated tumour-associated vasculature via endothelial selectins. *J. Control. Release* **150**, 196–203 (2011).
84. Ong, H.T. *et al.* Intravascularly administered RGD-displaying measles viruses bind to and infect neovessel endothelial cells *in vivo*. *Mol. Ther.* **17**, 1012–1021 (2009).
85. Jing, Y. *et al.* Tumor and vascular targeting of a novel oncolytic measles virus retargeted against the urokinase receptor. *Cancer Res.* **69**, 1459–1468 (2009).
86. Kumar, C.C. *et al.* Biochemical characterization of the binding of echistatin to integrin αvβ3 receptor. *J. Pharmacol. Exp. Ther.* **283**, 843–853 (1997).
87. Breitbach, C.J. *et al.* Targeting tumor vasculature with an oncolytic virus. *Mol. Ther.* **19**, 886–894 (2011).
88. Chen, H.H. *et al.* Active adenoviral vascular penetration by targeted formation of heterocellular endothelial-epithelial syncytia. *Mol. Ther.* **19**, 67–75 (2011).
89. Stojdl, D.F. *et al.* VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. *Cancer Cell* **4**, 263–275 (2003).
90. Schoggins, J.W. *et al.* A diverse range of gene products are effectors of the type I interferon antiviral response. *Nature* **472**, 481–485 (2011).
91. Haller, O., Kochs, G. & Weber, F. Interferon, Mx, and viral countermeasures. *Cytokine Growth Factor Rev.* **18**, 425–433 (2007).
92. Vandevenne, P., Sadzot-Delvaux, C. & Piette, J. Innate immune response and viral interference strategies developed by human herpesviruses. *Biochem. Pharmacol.* **80**, 1955–1972 (2010).
93. Lu, M.Y. & Liao, F. Interferon-stimulated gene ISG12b2 is localized to the inner mitochondrial membrane and mediates virus-induced cell death. *Cell Death Differ.* **18**, 925–936 (2011).
94. Stojdl, D.F. *et al.* Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat. Med.* **6**, 821–825 (2000).
95. Haralambieva, I. *et al.* Engineering oncolytic measles virus to circumvent the intracellular innate immune response. *Mol. Ther.* **15**, 588–597 (2007).
96. Altomonte, J. *et al.* Exponential enhancement of oncolytic vesicular stomatitis virus potency by vector-mediated suppression of inflammatory responses *in vivo*. *Mol. Ther.* **16**, 146–153 (2008).
97. Le Boeuf, F. *et al.* Synergistic interaction between oncolytic viruses augments tumor killing. *Mol. Ther.* **18**, 888–895 (2010).
98. Kirn, D.H. & Thorne, S.H. Targeted and armed oncolytic poxviruses: a novel multi-mechanistic therapeutic class for cancer. *Nat. Rev. Cancer* **9**, 64–71 (2009).
99. Chang, H.M. *et al.* Induction of interferon-stimulated gene expression and antiviral responses require protein deacetylase activity. *Proc. Natl. Acad. Sci. USA* **101**, 9578–9583 (2004).
100. Nguyên, T.L. *et al.* Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis. *Proc. Natl. Acad. Sci. USA* **105**, 14981–14986 (2008).
101. Otsuki, A. *et al.* Histone deacetylase inhibitors augment antitumor efficacy of herpes-based oncolytic viruses. *Mol. Ther.* **16**, 1546–1555 (2008).
102. MacTavish, H. *et al.* Enhancement of vaccinia virus based oncolysis with histone deacetylase inhibitors. *PLoS ONE* **5**, e14462 (2010).
103. Diallo, J.S. *et al.* A high-throughput pharmacoviral approach identifies novel oncolytic virus sensitizers. *Mol. Ther.* **18**, 1123–1129 (2010).
104. Passer, B.J. *et al.* Identification of the ENT1 antagonists dipyridamole and dilazep as amplifiers of oncolytic herpes simplex virus-1 replication. *Cancer Res.* **70**, 3890–3895 (2010).
105. Alain, T. *et al.* Vesicular stomatitis virus oncolysis is potentiated by impairing mTORC1-dependent type I IFN production. *Proc. Natl. Acad. Sci. USA* **107**, 1576–1581 (2010).
106. Lun, X. *et al.* Myxoma virus virotherapy for glioma in immunocompetent animal models: optimizing administration routes and synergy with rapamycin. *Cancer Res.* **70**, 598–608 (2010).
107. Lun, X.Q. *et al.* Targeting human medulloblastoma: oncolytic virotherapy with myxoma virus is enhanced by rapamycin. *Cancer Res.* **67**, 8818–8827 (2007).
108. Qiao, J. *et al.* Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus. *Clin. Cancer Res.* **14**, 259–269 (2008).
109. Kottke, T. *et al.* Improved systemic delivery of oncolytic reovirus to established tumors using preconditioning with cyclophosphamide-mediated T_{reg} modulation and interleukin-2. *Clin. Cancer Res.* **15**, 561–569 (2009).
110. Kurozumi, K. *et al.* Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy. *J. Natl. Cancer Inst.* **99**, 1768–1781 (2007).
111. Kirn, D.H., Wang, Y., Liang, W., Contag, C.H. & Thorne, S.H. Enhancing poxvirus oncolytic effects through increased spread and immune evasion. *Cancer Res.* **68**, 2071–2075 (2008).
112. Reeves, P.M. *et al.* Variola and monkeypox viruses utilize conserved mechanisms of virion motility and release that depend on abl and SRC family tyrosine kinases. *J. Virol.* **85**, 21–31 (2011).
113. Hoffmann, D. & Wildner, O. Enhanced killing of pancreatic cancer cells by expression of fusogenic membrane glycoproteins in combination with chemotherapy. *Mol. Cancer Ther.* **5**, 2013–2022 (2006).
114. Patel, B. *et al.* Differential cytopathology and kinetics of measles oncolysis in two primary B-cell malignancies provides mechanistic insights. *Mol. Ther.* **19**, 1034–1040 (2011).
115. Israyelyan, A. *et al.* Herpes simplex virus type-1(HSV-1) oncolytic and highly fusogenic mutants carrying the NV1020 genomic deletion effectively inhibit primary and metastatic tumors in mice. *Virol. J.* **5**, 68 (2008).
116. Brown, C.W. *et al.* The p14 FAST protein of reptilian reovirus increases vesicular stomatitis virus neuropathogenesis. *J. Virol.* **83**, 552–561 (2009).
117. Sauthoff, H. *et al.* Intratumoral spread of wild-type adenovirus is limited after local injection of human xenograft tumors: virus persists and spreads systemically at late time points. *Hum. Gene Ther.* **14**, 425–433 (2003).
118. Yun, C.O. Overcoming the extracellular matrix barrier to improve intratumoral spread and therapeutic potential of oncolytic virotherapy. *Curr. Opin. Mol. Ther.* **10**, 356–361 (2008).
119. Diop-Frimpong, B., Chauhan, V.P., Krane, S., Boucher, Y. & Jain, R.K. Losartan inhibits collagen I synthesis and improves the distribution and efficacy of nanotherapeutics in tumors. *Proc. Natl. Acad. Sci. USA* **108**, 2909–2914 (2011).
120. Ganesh, S., Gonzalez-Edick, M., Gibbons, D., Van Roey, M. & Jooss, K. Intratumoral coadministration of hyaluronidase enzyme and oncolytic adenoviruses enhances virus potency in metastatic tumor models. *Clin. Cancer Res.* **14**, 3933–3941 (2008).
121. Guedan, S. *et al.* Hyaluronidase expression by an oncolytic adenovirus enhances its intratumoral spread and suppresses tumor growth. *Mol. Ther.* **18**, 1275–1283 (2010).

122. Toth, K., Dhar, D. & Wold, W.S. Oncolytic (replication-competent) adenoviruses as anticancer agents. *Expert Opin. Biol. Ther.* **10**, 353–368 (2010).
123. Muthana, M. *et al.* Use of macrophages to target therapeutic adenovirus to human prostate tumors. *Cancer Res.* **71**, 1805–1815 (2011).
124. Lee, C.Y.F. *et al.* Transcriptional and translational dual-regulated oncolytic herpes simplex virus type 1 for targeting prostate tumors. *Mol. Ther.* **18**, 929–935 (2010).
125. Foka, P. *et al.* Novel tumour-specific promoters for transcriptional targeting of hepatocellular carcinoma by herpes simplex virus vectors. *J. Gene Med.* **12**, 956–967 (2010).
126. Muik, A. *et al.* Pseudotyping vesicular stomatitis virus with lymphocytic choriomeningitis virus glycoproteins enhances infectivity for glioma cells and minimizes neurotropism. *J. Virol.* **85**, 5679–5684 (2011).
127. Ayala Breton, C., Barber, G.N., Russell, S. & Peng, K.W. Retargeting vesicular stomatitis virus using measles virus envelope glycoproteins. *Hum. Gene Ther.* **23**, 484–491 (2012).
128. Shashkova, E.V., May, S.M., Doronin, K. & Barry, M.A. Expanded anticancer therapeutic window of hexon-modified oncolytic adenovirus. *Mol. Ther.* **17**, 2121–2130 (2009).
129. Leber, M.F. *et al.* MicroRNA-sensitive oncolytic measles viruses for cancer-specific vector tropism. *Mol. Ther.* **19**, 1097–1106 (2011).
130. Cawood, R., Wong, S.L., Di, Y., Baban, D.F. & Seymour, L.W. MicroRNA controlled adenovirus mediates anti-cancer efficacy without affecting endogenous microRNA activity. *PLoS ONE* **6**, e16152 (2011).
131. Cawood, R. *et al.* Use of tissue-specific microRNA to control pathology of wild-type adenovirus without attenuation of its ability to kill cancer cells. *PLoS Pathog.* **5**, e1000440 (2009).
132. Kelly, E.J., Nace, R., Barber, G.N. & Russell, S.J. Attenuation of vesicular stomatitis virus encephalitis through microRNA targeting. *J. Virol.* **84**, 1550–1562 (2010).
133. Edge, R.E. *et al.* A let-7 microRNA-sensitive vesicular stomatitis virus demonstrates tumor-specific replication. *Mol. Ther.* **16**, 1437–1443 (2008).
134. Sugio, K. *et al.* Enhanced safety profiles of the telomerase-specific replication-competent adenovirus by incorporation of normal cell-specific microRNA-targeted sequences. *Clin. Cancer Res.* **17**, 2807–2818 (2011).
135. Yang, X. *et al.* Evaluation of IRES-mediated, cell-type-specific cytotoxicity of poliovirus using a colorimetric cell proliferation assay. *J. Virol. Methods* **155**, 44–54 (2009).
136. Roos, F.C. *et al.* Oncolytic targeting of renal cell carcinoma via encephalomyocarditis virus. *EMBO Mol. Med.* **2**, 275–288 (2010).
137. Oliere, S. *et al.* Vesicular stomatitis virus oncolysis of T lymphocytes requires cell cycle entry and translation initiation. *J. Virol.* **82**, 5735–5749 (2008).
138. Stoff-Khalili, M.A. *et al.* Cancer-specific targeting of a conditionally replicative adenovirus using mRNA translational control. *Breast Cancer Res. Treat.* **108**, 43–55 (2008).
139. Banaszynski, L.A., Sellmyer, M.A., Contag, C.H., Wandless, T.J. & Thorne, S.H. Chemical control of protein stability and function in living mice. *Nat. Med.* **14**, 1123–1127 (2008).
140. Glass, M., Busche, A., Wagner, K., Messerle, M. & Borst, E.M. Conditional and reversible disruption of essential herpesvirus proteins. *Nat. Methods* **6**, 577–579 (2009).
141. Banaszynski, L.A., Chen, L.C., Maynard-Smith, L.A., Ooi, A.G. & Wandless, T.J. A rapid, reversible, and tunable method to regulate protein function in living cells using synthetic small molecules. *Cell* **126**, 995–1004 (2006).
142. Stepkowski, S.M. Molecular targets for existing and novel immunosuppressive drugs. *Expert Rev. Mol. Med.* **2**, 1–23 (2000).
143. Chiocca, E.A. The host response to cancer virotherapy. *Curr. Opin. Mol. Ther.* **10**, 38–45 (2008).
144. Hanahan, D. & Weinberg, R.A. Hallmarks of cancer: the next generation. *Cell* **144**, 646–674 (2011).
145. Yang, L., Pang, Y. & Moses, H.L. TGF-β and immune cells: an important regulatory axis in the tumor microenvironment and progression. *Trends Immunol.* **31**, 220–227 (2010).
146. Melcher, A., Parato, K., Rooney, C.M. & Bell, J.C. Thunder and lightning: immunotherapy and oncolytic viruses collide. *Mol. Ther.* **6**, 1008–1016 (2011).
147. Mastrangelo, M.J., Maguire, H.C. & Lattime, E.C. Intralesional vaccinia/GM-CSF recombinant virus in the treatment of metastatic melanoma. *Adv. Exp. Med. Biol.* **465**, 391–400 (2000).
148. Diaz, R.M. *et al.* Oncolytic immunovirotherapy for melanoma using vesicular stomatitis virus. *Cancer Res.* **67**, 2840–2848 (2007).
149. Bridle, B.W., Hanson, S. & Lichty, B.D. Combining oncolytic virotherapy and tumour vaccination. *Cytokine Growth Factor Rev.* **21**, 143–148 (2010).
150. Kottke, T. *et al.* Broad antigenic coverage induced by vaccination with virus-based cDNA libraries cures established tumors. *Nat. Med.* **17**, 854–859 (2011).
151. Pulido, J. *et al.* Using virally expressed melanoma cDNA libraries to identify tumor-associated antigens that cure melanoma. *Nat. Biotechnol.* **30**, 337–343 (2012).
152. Kottke, T. *et al.* Use of biological therapy to enhance both virotherapy and adoptive T-cell therapy for cancer. *Mol. Ther.* **16**, 1910–1918 (2008).
153. Senac, J.S. *et al.* Infection and killing of multiple myeloma by adenoviruses. *Hum. Gene Ther.* **21**, 179–190 (2010).
154. Zhang, X., Zhao, L., Hang, Z., Guo, H. & Zhang, M. Evaluation of HSV-1 and adenovirus vector-mediated infection, replication and cytotoxicity in lymphoma cell lines. *Oncol. Rep.* **26**, 637–644 (2011).
155. Kanai, R., Wakimoto, H., Cheema, T. & Rabkin, S.D. Oncolytic herpes simplex virus vectors and chemotherapy: are combinatorial strategies more effective for cancer? *Future Oncol.* **6**, 619–634 (2010).
156. Russell, S.J. & Peng, K.W. Measles virus for cancer therapy. *Curr. Top. Microbiol. Immunol.* **330**, 213–241 (2009).
157. Byrnes, A.P. & Griffin, D.E. Large-plaque mutants of Sindbis virus show reduced binding to heparan sulfate, heightened viremia, and slower clearance from the circulation. *J. Virol.* **74**, 644–651 (2000).
158. Lee, P., Knight, R., Smit, J.M., Wilschut, J. & Griffin, D.E. A single mutation in the E2 glycoprotein important for neurovirulence influences binding of sindbis virus to neuroblastoma cells. *J. Virol.* **76**, 6302–6310 (2002).
159. Chen, N. *et al.* Poxvirus interleukin-4 expression overcomes inherent resistance and vaccine-induced immunity: pathogenesis, prophylaxis, and antiviral therapy. *Virology* **409**, 328–337 (2011).
160. Peng, K.W. *et al.* Using clinically approved cyclophosphamide regimens to control the humoral immune response to oncolytic viruses. *Gene Ther.* published, online doi:10.1038/gt.2012.31 (5 April 2012).
161. Miyatake, S., Iyer, A., Martuza, R.L. & Rabkin, S.D. Transcriptional targeting of herpes simplex virus for cell-specific replication. *J. Virol.* **71**, 5124–5132 (1997).
162. Rodriguez, R. *et al.* Prostate attenuated replication competent adenovirus (ARCA) CN706: a selective cytotoxic for prostate-specific antigen-positive prostate cancer cells. *Cancer Res.* **57**, 2559–2563 (1997).
163. Kuhn, I. *et al.* Directed evolution generates a novel oncolytic virus for the treatment of colon cancer. *PLoS ONE* **3**, e2409 (2008).
164. Doronin, K. *et al.* Tumor-specific, replication-competent adenovirus vectors overexpressing the adenovirus death protein. *J. Virol.* **74**, 6147–6155 (2000).
165. Wong, R.J. *et al.* Cytokine gene transfer enhances herpes oncolytic therapy in murine squamous cell carcinoma. *Hum. Gene Ther.* **12**, 253–265 (2001).
166. Kim, J.H. *et al.* Relaxin expression from tumor-targeting adenoviruses and its intratumoral spread, apoptosis induction, and efficacy. *J. Natl. Cancer Inst.* **98**, 1482–1493 (2006).
167. Thorne, S.H., Negrin, R.S. & Contag, C.H. Synergistic antitumor effects of immune cell-viral biotherapy. *Science* **311**, 1780–1784 (2006).