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Review

Vesicular stomatitis virus as a flexible platform for oncolytic virotherapy against cancer

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Oncolytic virus (OV) therapy is an emerging anti-cancer approach that utilizes viruses to preferentially infect and kill cancer cells, while not harming healthy cells. Vesicular stomatitis virus (VSV) is a prototypic non-segmented, negative-strand RNA virus with inherent OV qualities. Antiviral responses induced by type I interferon pathways are believed to be impaired in most cancer cells, making them more susceptible to VSV than normal cells. Several other factors make VSV a promising OV candidate for clinical use, including its well-studied biology, a small, easily manipulated genome, relative independence of a receptor or cell cycle, cytoplasmic replication without risk of host-cell transformation, and lack of pre-existing immunity in humans. Moreover, various VSV-based recombinant viruses have been engineered via reverse genetics to improve oncoselectivity, safety, oncotoxicity and stimulation of tumour-specific immunity. Alternative delivery methods are also being studied to minimize premature immune clearance of VSV. OV treatment as a monotherapy is being explored, although many studies have employed VSV in combination with radiotherapy, chemotherapy or other OVs. Preclinical studies with various cancers have demonstrated that VSV is a promising OV; as a result, a human clinical trial using VSV is currently in progress.

Introduction

Oncolytic virus (OV) therapy is an emerging anti-cancer approach that utilizes viruses to preferentially infect and kill cancer cells, while not harming healthy cells. Since 1893, scientists have observed tumour regression after virus infection (Kelly & Russell, 2007). However, research in the field has occurred mostly in the last 15 years, when almost every major group of animal viruses has been tested for OV potential, and impressive preclinical successes have been reported (Hammill *et al.*, 2010). As a result, the adenovirus H101 was approved for clinical use in China in 2006 (Garber, 2006), and three other OVs based on vaccinia virus, herpes simplex virus and reovirus are currently in late-phase clinical trials and could soon be approved in the USA (Hammill *et al.*, 2010).

This review focuses on vesicular stomatitis virus (VSV) as a promising OV, beginning with the inherent qualities of wild-type (WT) VSV. As with any OV, VSV has limitations that must be overcome for future clinical success. Compared with other OVs, VSV is advantageous due to a combination of several factors, including its well-studied biology, relative independence of a receptor or cell cycle, ability to infect a wide range of laboratory cell lines and to produce very high virus yields, cytoplasmic replication without risk of host-cell transformation, a small, easily manipulated genome, and lack of pre-existing immunity in humans. In the last 10 years, a great number of recombinant VSVs (rVSVs) have been generated via reverse genetics, with the goal of generating more potent OVs that work synergistically with host immunity and/or other therapies to reduce or eliminate tumour burden. To facilitate the comparison of these recombinant viruses, we have organized them in Table 1 based on their modifications and a problem that they were designed to address. Similarly, this review will summarize previous studies based on their attempts to improve oncoselectivity, safety and oncotoxicity; to minimize premature immune clearance; and/or to induce or stimulate tumour-specific immunity. As the most effective therapies are likely to involve combinational regimens of VSV and chemotherapy, radiotherapy or even other OVs, these approaches are also discussed.

VSV biology

In this section we will review VSV biology with respect to its OV potential. VSV is a prototypic non-segmented, negative-sense RNA virus (order *Mononegavirales*, family *Rhabdoviridae*) and one of the best-studied animal viruses. Two major WT VSV serotypes, Indiana and New Jersey (VSV_{IN} and VSV_{NJ}), are endemic to much of Central and South America and parts of the USA (Lyles & Rupprecht, 2007). Natural hosts include horses, cattle, pigs and a range of other mammals and their insect vectors. Among livestock, WT VSV outbreaks occur seasonally and most infections are non-lethal, manifesting as fever and blister-like lesions of the oral cavity, feet and teats (Drolet *et al.*, 2009; Hansen *et al.*, 1985). In general, pre-existing immunity to VSV in human

Table 1. VSV recombinants used as oncolytic agents against cancer

Recombinant VSV	Virus description	Reference(s)	Designed to improve:				
			Oncoselectivity	Safety	Oncotoxicity	VSV survival	Tumour immunity
WT and miscellaneous							
WT VSV ('Rose lab')	The parental rWT VSV for most VSV-based OVs. The L gene and the N-terminal 49 residues of the N gene are derived from the Mudd-Summers strain, the rest is from the San Juan strain (both Indiana serotype)	Lawson *et al.* (1995)					
VSV-WT-XN2 (or XN1)	Derivative of rWT VSV ('Rose lab'). Generated using pVSV-XN2 (or pVSV-XN1), a full-length VSV plasmid containing unique *Xho*I and *Nhe*I sites flanked by VSV transcription start and stop signals between G and L genes. pVSV-XN2 (or pVSV-XN1) is commonly used to generate recombinant VSVs encoding an extra gene	Schnell *et al.* (1996)					
WT VSV ('Wertz lab')	Alternative rWT VSV. The N, P, M and L genes originate from the San Juan strain; G gene from the Orsay strain (both Indiana serotype). Rarely used in OV studies	Whelan *et al.* (1995)					
VSV-WT-GFP, -RFP, -Luc, -LacZ	WT VSV encoding reporter genes (between G and L) to track virus infection. Based on pVSV-XN2. Toxicity similar to VSV-WT	Fernandez *et al.* (2002), Wu *et al.* (2008)					
VSV-G/GFP	GFP sequence fused to VSV G gene is inserted between the WT G and L genes (in addition to WT G). Toxicity similar to that of VSV-WT	Dalton & Rose (2001)					
VSV-rp30	Derivative of VSV-G/GFP. Generated by positive selection on glioblastoma cells and contains two silent mutations and two missense mutations, one in P and one in L. 'rp30' indicates 30 repeated passages	Wollmann *et al.* (2005)	X	X	X		
VSV-p1-GFP, VSV-p1-RFP	VSV expressing GFP or red fluorescent protein (RFP or dsRed) reporter gene at position 1. Attenuated because all VSV genes are moved downward, to positions 2–6. Safe and still effective as an OV	Wollmann *et al.* (2010)	X	X			
VSV-dG-GFP (or RFP) (replication-defective)	Similar to VSV-p1-GFP or VSV-p1-RFP described above, but with the G gene deleted. Cannot generate a second round of infection. Poor ability to kill tumour cells	Wollmann *et al.* (2010)	X	X			
VSV-ΔP, -ΔL, -ΔG (semi-replication-competent)	Each virus cannot replicate alone because of one VSV gene deleted, but when viruses co-infect, they show good replication, safety and oncolysis (especially the combination of VSVΔG/VSVΔL). VSVΔP and VSVΔL contain dsRed in place of the corresponding viral gene. VSVΔG contains GFP gene in place of G	Muik *et al.* (2012)		X			
M mutants							
VSV-M51R	The M51R mutation was introduced into M	Kopecky *et al.* (2001)	X	X			
VSV-ΔM51, VSV-ΔM51-GFP, - RFP, -FLuc, -Luc, - LacZ	The ΔM51 mutation was introduced into M. In addition, some recombinants encode a reporter gene between the G and L	Stojdl *et al.* (2003), Power & Bell (2007), Wu *et al.* (2008)	X	X			
VSV-*M_{mut}	VSV with a single mutation or combination of mutations at the following M positions: M33A, M51R, V221F and S226R	Hoffmann *et al.* (2010)	X	X			

Table 1. cont.

Recombinant VSV	Virus description	Reference(s)	Designed to improve:				
			Oncoselectivity	Safety	Oncotoxicity	VSV survival	Tumour immunity
VSV-M6PY >A4-R34E and other M mutants	The M51R mutation was introduced into the M gene, and, in addition, the mutations in the PSAP motif (residues 37–40) of M	Irie *et al.* (2007)	X	X			
VSV-M(mut)	VSV M residues 52–54 are mutated from DTY to AAA. M(mut) cannot block nuclear mRNA export	Heiber & Barber (2011)	X	X			
G mutants							
VSV-G5, -G5R, -G6, -G6R	VSV-expressing mutant G with amino acid substitutions at various positions (between residues 100 and 471). Triggers type I IFN secretion as the M51R, but inhibits cellular transcription and host protein translation like WT	Janelle *et al.* (2011)	X	X			
VSV-CT1	The cytoplasmic tail of the G protein was truncated from 29 to 1 aa. Decreased neuropathology, but marginal oncolytic efficacy	Ozduman *et al.* (2009), Wollmann *et al.* (2010)	X	X			
VSV-CT9-M51	The cytoplasmic tail of VSV-G was reduced from 29 to 9 aa, also has ΔM51 mutation. Attenuated neurotoxicity and good OV abilities	Ozduman *et al.* (2009), Wollmann *et al.* (2010)	X	X			
Foreign glycoproteins							
VSV-DV/F(L289A) (same as rVSV-F)	VSV expressing the NDV fusion protein gene between G and L. The L289A mutation in this protein allows it to induce syncytia alone (without NDV HN protein)	Ebert *et al.* (2004)	X	X	X		
VSV-S-GP	VSV with the native G gene deleted and replaced with a modified glycoprotein protein (GP) from Sindbis virus (SV). Also expressing mouse GM-CSF and GFP (between SV GP and VSV L). The modified GP protein recognizes the Her2 receptor, which is overexpressed on many breast cancer cells	Bergman *et al.* (2007)	X	X	X		
VSV-ΔG-SV5-F	VSV G gene is replaced with the fusogenic simian parainfluenza virus 5 fusion protein (SV5-F) gene	Chang *et al.* (2010)		X	X		
VSV-FAST, VSV-(ΔM51)-FAST	VSV or VSV-MΔ51 expressing the p14 FAST protein of reptilian reovirus (between VSV G and L)	Brown *et al.* (2009)	X	X	X		
VSV-LCMV-GP (replication-defective)	VSV lacking the G gene was pseudotyped with the non-neurotropic glycoprotein of LMCV	Muik *et al.* (2011)		X			
VSV-H/F, -αEGFR, -αFR,-αPSMA (replication-defective)	VSV lacking the G gene was pseudotyped with the MV F and H displaying single-chain antibodies (scFv) specific for epidermal growth factor receptor, folate receptor, or prostate membrane-specific antigen. Retargeted VSV to cells that expressed the targeted receptor	Ayala-Breton *et al.* (2012)	X	X	X		X
microRNA targets							
VSV-let-7wt	The let-7 microRNA targets are inserted into the 3′-UTR of VSV M	Edge *et al.* (2008)	X	X			

Table 1. cont.

Recombinant VSV	Virus description	Reference(s)	Designed to improve:				
			Oncoselectivity	Safety	Oncotoxicity	VSV survival	Tumour immunity
VSV-124, -125, -128, -134 (M or L mRNA)	VSV recombinants with neuron-specific microRNA (miR-124, 125, 128 or 134) targets inserted in the 3′-UTR of VSV M or L mRNA	Kelly *et al.* (2010)	X	X			
Cancer suppressors							
VSV-mp53, VSV -M(mut)-mp53	VSV [WT or M(mut)] expressing the murine p53 gene. M(mut) has residues 52–54 of the M protein changed from DTY to AAA	Heiber & Barber (2011)	X	X	X		X
Suicide genes							
VSV-C : U	VSV expressing *E. coli* CD/UPRT, catalysing the modification of 5-fluorocytosine into chemotherapeutic 5-FU	Porosnicu *et al.* (2003)	X		X		
VSV-C	VSV-MΔ51 expressing CD/UPRT	Leveille *et al.* (2011b)	X	X	X		
VSV-(MΔ51)-NIS	VSV-MΔ51 expressing the human NIS gene (for 'radiovirotherapy' with ^{131}I)	Goel *et al.* (2007)	X	X	X		
VSV-TK	VSV expressing TK; can improve oncolysis if used with non-toxic prodrug ganciclovir	Fernandez *et al.* (2002)	X		X		
Immunomodulation							
VSV-mIFNβ, -hIFNβ, VSV-rIFNβ	VSV expressing the murine (m), human (h) or rat (r) IFN-β gene	Obuchi *et al.* (2003), Jenks *et al.* (2010)	X	X	X		X
VSV-IL4	VSV expressing IL-4	Fernandez *et al.* (2002)			X		X
VSV-IL12	VSV expressing IL-12	Shin *et al.* (2007a)			X		X
VSV-IL23	VSV expressing IL-23. Significantly attenuated in the CNS, but effective OV	Miller *et al.* (2010)	X	X	X		X
VSV-IL28	VSV expressing IL-28, a member of the type III IFN (IFN-λ) family	Wongthida *et al.* (2010)	X	X	X		X
VSV-opt.hIL-15	VSV-MΔ51 expressing a highly secreted version of human IL-15	Stephenson *et al.* (2012)	X	X	X		X
VSV-CD40L	VSV expressing CD40L, a member of the tumour necrosis factor (TNF) family of cell-surface molecules	Galivo *et al.* (2010)			X		X
VSV-Flt3L	VSV-MΔ51 expressing the soluble form of the human Flt3L, a growth factor activating DCs	Leveille *et al.* (2011a)	X	X	X		X
VSV/hDCT	VSV-MΔ51 expressing hDCT	Boudreau *et al.* (2009)	X	X	X		X
VSV-hgp100	VSV expressing hgp100, an altered self-TAA against which tolerance is well-established in C57BL/6 mice	Wongthida *et al.* (2011b)			X		X
VSV-ova	VSV expressing chicken ovalbumin (for B16ova cancer model)	Diaz *et al.* (2007)			X		X
VSV-gG	VSV expressing EHV-1 glycoprotein G, a broad-spectrum viral chemokine-binding protein	Altomonte *et al.* (2008b)			X	X	
VSV-UL141	VSV expressing a secreted form of the human cytomegalovirus UL141 protein, known to inhibit the function of NK cells by blocking the ligand of NK cell-activating receptors	Altomonte *et al.* (2009)			X	X	
VSV-(Δ51)-M3	VSV-MΔ51 expressing the murine gammaherpesvirus-68 chemokine-binding protein M3	Wu *et al.* (2008)	X	X	X	X	

populations is very low, and VSV infection in humans is generally asymptomatic and limited to agricultural and laboratory workers (Lyles & Rupprecht, 2007). Only one case of WT VSV_{IN}-mediated encephalitis in humans has been reported (Quiroz *et al.*, 1988).

It is important to mention that VSV pathogenesis in natural hosts depends on the virus serotype. Thus, using a reverse-genetics approach, VSV_{IN} and VSV_{NJ} recombinants encoding a heterogeneous glycoprotein (G protein) were generated. The study demonstrated that the VSV G protein is a determinant of higher virulence of VSV_{NJ} than of VSV_{IN} in swine (Martinez *et al.*, 2003).

VSV neurotoxicity has been studied extensively in different rodent systems. In principle, VSV can cause neurotoxicity in mice or rats when administered intracranially (Dal Canto *et al.*, 1976), intranasally (Plakhov *et al.*, 1995), intravascularly (Shinozaki *et al.*, 2005) and intraperitoneally (Schellekens *et al.*, 1984). Neurotoxicity following intranasal VSV infection (WT or non-attenuated VSV recombinants; Table 1) is very efficient and has been studied extensively. When administered intranasally, WT or non-attenuated VSV recombinants replicate rapidly in the nasal epithelium, spread to olfactory neurons, then move retrograde axonally to the brain, where they replicate and cause neuropathogenesis (Bi *et al.*, 1995; Reiss *et al.*, 1998; van den Pol *et al.*, 2002). Following infection of the central nervous system (CNS), the onset of encephalitis was shown to be T-cell-independent as it is seen in athymic mice, and WT VSV neuropathology appears to be more related to the cytopathological nature of VSV infection rather than to T-cell-mediated mechanisms (Frei *et al.*, 1989; Huneycutt *et al.*, 1993). Both innate (nitric oxide produced by neurons and glial cells) and adaptive (expression of MHC molecules and T-cell infiltration) immunity are required for clearance of VSV from the CNS (Bi *et al.*, 1995). Our own research demonstrated that WT VSV can infect microglia and astrocytes *in vitro* and *in vivo*, and suggests that infection of glial cells results in the production of inflammatory cytokines that may facilitate encephalitis (Chauhan *et al.*, 2010; Furr *et al.*, 2008, 2010). VSV-mediated encephalitis has also been observed in non-human primates (NHPs) and will be discussed in the 'Improving VSV oncoselectivity and safety' section, where we will describe attenuated VSV recombinants that lack neurotoxicity.

The 11 kb VSV genome encodes five genes encoding the nucleocapsid (N) protein, phosphoprotein (P), matrix (M) protein, G protein and large polymerase (L) (Fig. 1). At approximately 185×75 nm, the VSV virion is enveloped and bullet-shaped, and contains all five virus-encoded proteins (Ge *et al.*, 2010; Lyles & Rupprecht, 2007), as well as a number of host proteins (Moerdyk-Schauwecker *et al.*, 2011). The VSV G protein enables VSV to infect most, if not all, mammalian cell types. To date, no definitive cell-surface receptor has been identified for the VSV G protein, with binding attributed to negatively charged membrane lipids (Lyles & Rupprecht, 2007). Following attachment, VSV enters a cell through actin- and clathrin-dependent endocytosis (Cureton *et al.*, 2010). Once internalized, endosomal acidification mediates G protein conformational changes that facilitate fusion of the viral envelope with the endosomal membrane, releasing the VSV ribonucleoprotein core into the cytoplasm (Stanifer *et al.*, 2011).

The VSV RNA genome is encapsidated tightly by the N protein, forming a nuclease-resistant nucleocapsid, which serves as a template for the viral RNA-dependent RNA polymerase. The VSV polymerase complex consists of the L and P proteins and is carried in virions from cell to cell (Lyles & Rupprecht, 2007). Initiation of transcription of downstream genes occurs as the polymerase pauses at each intergenic region before reinitiation, resulting in a quantitative gradient of mRNAs with N>M>P>G>L (Ball *et al.*, 1999). Ongoing translation of these mRNAs is required for VSV genome replication. VSV is known for its fast replication, and assembly of enveloped progeny virions begins around the same time as secondary transcription, approximately 2–3 h post-infection (Lyles & Rupprecht, 2007). VSV infection culminates in cellular death through apoptosis (Gaddy & Lyles, 2005).

In infected cells, VSV mRNAs are translated preferentially. It remains unclear what allows the translation of VSV mRNA but inhibits the translation of cellular mRNA, as viral and host mRNA are structurally similar (5′-capped and 3′-polyadenylated) (Lyles & Rupprecht, 2007). Previous studies suggest that this effect is independent of VSV mRNA *cis*-acting sequences. Instead, alterations of the translational machinery in VSV-infected cells, such as dephosphorylation of the cap-binding subunit eIF4E (Connor & Lyles, 2002), may favour newly produced mRNAs (Whitlow *et al.*, 2006, 2008). A recent study suggests that the VSV WT M protein also plays a role in the preferential translation of VSV mRNAs, with M protein residue D125 being critical (Mire & Whitt, 2011). VSV secondary RNA synthesis was shown to occur predominantly in cytoplasmic inclusions and that viral mRNAs produced at the inclusions are transported away in a microtubule-dependent manner, which facilitates their translation (Heinrich *et al.*, 2010). This mechanism may contribute to the preferential translation of viral mRNAs in VSV-infected cells. In addition, although replication of VSV is believed to be generally cell-cycle-independent, VSV infection of primary T-lymphocytes was shown to depend on the cell-cycle transition from the G_0 to the G_1 phase, characterized by robust ribogenesis and mRNA translation, illustrating the dependence of VSV on robust mRNA translation machinery (Oliere *et al.*, 2008).

Cellular responses to VSV infection are multi-pronged and are initiated by pattern-recognition receptors. Toll-like receptor (TLR) 4, TLR3 and TLR7 induce expression of type I interferons (IFNs) after sensing VSV proteins or genome (Georgel *et al.*, 2007; Rieder & Conzelmann, 2009). A novel TLR13 may also induce type I IFNs (Shi *et al.*, 2011). Retinoic acid-inducible gene I (RIG-I)-like receptors are found in the cytoplasm of most cells. RIG-I stimulates mitochondrial antiviral signalling (MAVS/IPS-1/VISA/Cardif),

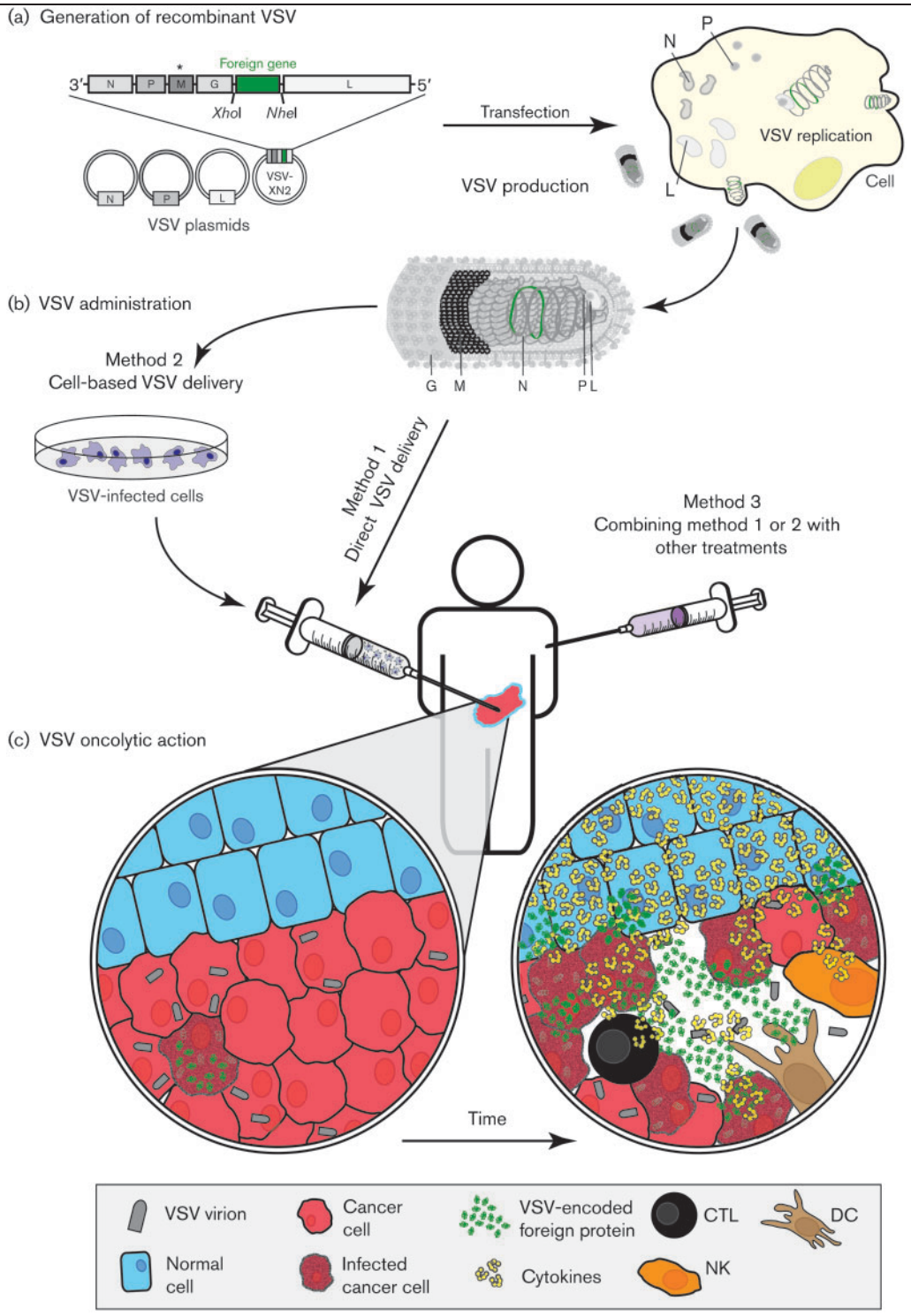

Fig. 1. Scheme of VSV-based OV therapy. (a) Reverse genetics allows generation of a recombinant VSV encoding a foreign gene of interest between the VSV G and L genes. The asterisk above M indicates M protein mutation(s) resulting in VSV attenuation in normal cells. Plasmids encoding VSV replication machinery and the modified genome are co-transfected into a cell line, and complete virions are produced and amplified using good manufacturing practices. (b) For evaluation of oncolytic efficacy, VSV can be administered directly, via cell-based delivery, or in combination with other treatments (chemotherapy, radiotherapy or other OVs). (c) In infected cells, VSV recombinants may express a foreign gene that facilitates killing of the adjacent uninfected cancer cells (e.g. suicide-gene approach or immunostimulation). Innate antiviral responses and other mechanisms prevent cell death in normal cells. Ideally, stimulation of innate and adaptive immune cells by VSV and/or the foreign gene product should lead to tumour-specific immune responses, including memory responses that prevent cancer recurrence.

which induces type I IFN production (Hornung *et al.*, 2006). IFN molecules act in an autocrine or paracrine manner to upregulate expression of IFN-stimulated genes (ISGs) in the infected cell and in proximal uninfected cells. Several ISGs, including MxA (Staeheli & Pavlovic, 1991), PKR (Krishnamoorthy *et al.*, 2008), PCBP1/PCBP2 (Dinh *et al.*, 2011), PML (Chelbi-Alix *et al.*, 1998), IFITM2/3 (Weidner *et al.*, 2010) and tetherin (BST-2) (Sarojini *et al.*, 2011), have been reported to inhibit VSV replication directly.

The ability of VSV to suppress cellular responses to infection is required for continued replication. The M protein can localize to the nuclear envelope, where it interacts with Rae1 and Nup98 to inhibit nucleocytoplasmic trafficking of cellular mRNAs and small nuclear RNAs (Petersen *et al.*, 2000). VSV M mutants with defects in Rae1–Nup98 binding are unable to inhibit mRNA nuclear export (Faria *et al.*, 2005). Interestingly, a study by Chakraborty *et al.* (2009) showed that interaction of the VSV M protein with the Rae1–Nup98 complex during mitosis causes spindle abnormalities and triggers cell death during metaphase. The M protein may also inhibit host gene expression via inhibition of cellular mRNA synthesis (Yuan *et al.*, 2001). In a susceptible host, M protein inhibition of cellular gene expression allows VSV to complete its replication cycle and to produce high titres of progeny before the initially infected cell mounts any significant antiviral response. This is the major mechanism by which VSV evades the host IFN system (Rieder & Conzelmann, 2009).

It is important to note that recent advances in VSV biology are possible thanks to the methods established to recover infectious recombinant virus from cDNA, so-called reverse-genetics systems (Lawson *et al.*, 1995; Whelan *et al.*, 1995). Reverse genetics enables the use of genetic engineering of VSV to study all aspects of the virus life cycle and to create rVSVs better suited for vaccine and oncolytic applications (Finke & Conzelmann, 2005). In a typical reverse-genetics approach, mammalian cells expressing bacteriophage T7 are transfected with a plasmid mixture containing VSV N, P, L and full-length genomic plasmid containing the desired modifications (Fig. 1, Table 1). Most VSV-based OVs contain VSV sequences similar to the recombinant WT (rWT) VSV_{IN}, designated rWT VSV ('Rose lab') in Table 1. This particular rWT VSV, generated in Jack Rose's laboratory (Lawson *et al.*, 1995), has the L gene and the N-terminal 49 residues of the N gene derived from the Mudd-Summers strain; the rest is from the San Juan strain (both strains are of the Indiana serotype). Although most VSV-based recombinants used in OV applications have VSV sequences similar to that of this rWT VSV, we refer readers to original papers for specific details of each 'WT VSV' used in those studies.

Improving VSV oncoselectivity and safety

The type I IFN-associated antiviral potential of a cell is the key determinant of VSV oncoselectivity. VSV cannot distinguish non-malignant 'normal' cells from cancer cells based on differential receptor expression or cell-cycle state. The relative independence of VSV on a receptor can be an advantage when targeting cancer cells. For instance, not all cancer cells express sufficient coxsackievirus and adenovirus receptor for efficient entry of adenovirus 5-based OVs (Pearson *et al.*, 1999). Although normal cells can be infected by VSV, they sense virus infection and produce, secrete and respond to type I IFNs to impede virus replication by inducing an antiviral state in the cell. In many cancer cells, VSV oncoselectivity is based largely on defective or reduced type I IFN responses (Barber, 2004; Lichty *et al.*, 2004; Stojdl *et al.*, 2000, 2003). In many cancer cells, specific genes associated with type I IFN responses are downregulated or functionally inactive (Balachandran & Barber, 2004; Marozin *et al.*, 2008, 2010; Moussavi *et al.*, 2010; Zhang *et al.*, 2010). In addition, IFN signalling can be inhibited by MEK/ERK signalling, a cascade often upregulated in cancer cells (Noser *et al.*, 2007). Abrogation of IFN signalling in cancer cells can also be caused by epigenetic silencing of IFN-responsive transcription factors IRF7 or IRF5 (Li & Tainsky, 2011). In addition to defective IFN signalling, continuously proliferating cancer cells often have abnormal translation machinery that favours VSV replication (Oliere *et al.*, 2008). Several cellular proteins, including PKR, eIF2β, eIF4E, AKT and NFAR1/2, have been shown to play a role in mRNA translation as a determinant of VSV oncoselectivity (Balachandran & Barber, 2007; Barber, 2005; Harashima *et al.*, 2010).

However, some cancer cells do not have these defects and resist VSV infection like normal cells (Naik & Russell, 2009; Stojdl *et al.*, 2000). This includes some mesotheliomas (Saloura *et al.*, 2010), melanomas (Linge *et al.*, 1995; Wong *et al.*, 1997), lymphomas (Sun *et al.*, 1998), bladder cancers (Matin *et al.*, 2001), renal cancers (Pfeffer *et al.*, 1996) and possibly others (Stojdl *et al.*, 2003). Our own research showed that several human pancreatic ductal adenocarcinoma cancer cell lines resistant to VSV infection are sensitive to IFN-α treatment and capable of secreting IFN-β following VSV infection (Murphy *et al.*, 2012). Defects in the IFN pathway are not surprising, considering that IFN responses generally create conditions unfavourable for tumour formation, as they are anti-proliferative, anti-angiogenic and pro-apoptotic (Wang *et al.*, 2011).

Understanding the mechanisms of VSV oncoselectivity is important for creating new, safe OVs designed for selective replication in cancer cells. While high sensitivity to IFN would eventually stop VSV replication and dissemination in healthy tissues of an immunocompetent host, VSV replication in an immunocompromised cancer patient requires additional safety measures to minimize its potential neurovirulence.

As described in the 'VSV biology' section above, WT VSV can cause severe neurotoxicity in rodents, especially when administered intracranially or intranasally. Therefore, the development of any clinical application involving replication-competent VSV vectors requires understanding of

potential VSV neuropathogenesis in humans and appropriate attenuation of VSV to remove it. Many relevant studies analysed WT (or rWT) VSV and VSV recombinants with regard to their potential as vaccine vectors, and these studies are relevant to the applications of VSV recombinants as oncolytic agents. In fact, many oncolytic VSV recombinants were originally developed as vaccine vectors, and will be discussed later in this section. Some approaches, such as the rearrangement of VSV resulting in its attenuation, have yet to be applied to OV therapy, but will probably be explored in the future (Flanagan *et al.*, 2001).

In NHP infection models, which resemble human disease pathogenesis more closely, intranasal or intramuscular injection of WT (or rWT) VSV and VSV recombinants caused no clinically adverse signs (Egan *et al.*, 2004; Johnson *et al.*, 2007; Rose *et al.*, 2001). However, intrathalamic administration can result in severe neurological disease (Johnson *et al.*, 2007). In this study, when WT VSV, rWT VSV and two rVSV-HIV (human immunodeficiency virus) vectors were administered intranasally to NHPs, there was no evidence of VSV spread to CNS tissues. However, macaques inoculated intrathalamically with WT VSV developed severe neurological disease. Interestingly, rWT VSV was attenuated significantly compared with WT VSV, and all of the macaques in the rVSV-HIV vector groups showed no clinical signs of disease. The attenuation of rWT VSV (compared with WT VSV) was probably due to spontaneous mutations generated during the reverse-genetics process or due to sequence differences between WT VSV and rWT VSV (Table 1). The attenuation of rVSV-HIV (compared with rWT VSV) was probably due to the presence of the additional gene (HIV Gag) and the CT1 mutation (described later in this review). With regard to OV therapy, a recent study tested VSV-IFNβ on rhesus macaques via intrahepatic injection; no neurological signs were observed at any time point (Jenks *et al.*, 2010). As a result, a phase I clinical trial using VSV-IFNβ is currently in progress to evaluate the safety of intratumoral administration of VSV-IFNβ to human patients with hepatocellular carcinoma (ClinicalTrials.gov, 2012, trial ID NCT01628640; http://clinicaltrials.gov/ct2/show/NCT01628640).

Currently, at least eight approaches have been shown to improve VSV oncoselectivity and neurotropism safety without compromising its oncolytic abilities: (i) mutating the VSV M protein; (ii) VSV-directed IFN-β expression; (iii) attenuation of VSV through disruption of normal gene order; (iv) mutating the VSV G protein; (v) introducing targets for microRNA from normal cells into the VSV genome; (vi) pseudotyping VSV; (vii) experimental adaptation of VSV to cancer cells; and (viii) using semi-replicative VSV.

Employing VSVs encoding a mutated M protein, which are unable to evade antiviral innate responses in normal cells, is possibly the most common approach to improve both oncoselectivity and safety of VSV (Table 1). Such VSV mutants retain their oncotoxicity in cancer cells defective in their antiviral responses. Most studies use VSV M recombinants containing a mutation or deletion of the methionine residue at position 51 of the M protein (Black *et al.*, 1993; Coulon *et al.*, 1990). Alternatively, an M mutant with residues 52–54 mutated from DTY to AAA has been used (Heiber & Barber, 2011). These mutations prevent the M protein from binding to the Rae1–Nup98 mRNA export complex and inhibiting cellular gene expression in normal cells, and thus provide enhanced safety, including no neurotoxicity *in vivo*. Even safer VSVs have been generated by additional M modifications within the PSAP region (residues 33–44) (Irie *et al.*, 2007). It is important to note that inactivation of the ability of the M protein to inhibit cellular gene expression is a strategic advantage not only for safety reasons (e.g. normal cells can produce type I IFN and ISGs), but also when cellular gene expression is desirable (e.g. for tumour antigen presentation).

The oncoselectivity and safety of VSV are greatly improved in VSVs encoding mouse, rat or human IFN-β (which are species-specific), and one is being used in an ongoing clinical trial to evaluate VSV-IFNβ in human patients with hepatocellular carcinoma (ClinicalTrials.gov, 2012, trial ID NCT01628640; http://clinicaltrials.gov/ct2/show/NCT01628640). IFN-β stimulates innate immune responses in normal cells, but not in cancers with defective type I IFN signalling (Jenks *et al.*, 2010; Obuchi *et al.*, 2003; Saloura *et al.*, 2010). In addition to enhanced oncoselectivity and safety, VSV-directed IFN-β expression also generates desirable immunostimulation of the tumour microenvironment (discussed later).

Theoretically, any significant attenuation of VSV can improve oncoselectivity and safety. This approach has been used to generate VSV-p1-GFP and similar recombinants with a foreign gene inserted in position 1 of the VSV genome (before the N gene). While a typical insertion of a foreign gene between the VSV G and L genes affects VSV replication only marginally (L polymerase mRNA can be downregulated without dramatic consequences for VSV fitness), insertions at position 1 negatively affect expression of all VSV genes. The resulting VSV-p1-GFP lacks neurotoxicity, but retains good oncolytic abilities in an intracranial human glioblastoma tumour xenograft mouse model (Wollmann *et al.*, 2010).

WT VSV can also be attenuated by mutations in the G protein (Table 1). CT1 and CT9 mutants have the cytoplasmic tail of G truncated by removal of residues 1–29 and 9–29, respectively (Ozduman *et al.*, 2009). The best oncolytic ability and safety was shown for VSV-CT9-M51, which combined the CT9 and M51 mutations in a mouse model of human neuroblastoma (Ozduman *et al.*, 2009; Wollmann *et al.*, 2010). Another study, examining four VSVs with point mutations in the G protein against a variety of cancer cell lines, showed that mutant VSV-G6R (E238G substitution in the G protein) is as efficient as WT VSV at cell killing and inhibition of cellular transcription and host protein translation. Surprisingly, VSV-G6R triggers type I IFN secretion as efficiently as a VSV M51 mutant (Janelle *et al.*, 2011).

Altered expression of microRNAs in cancer cells can also be exploited to increase oncoselectivity and safety of VSV. A recombinant containing the highly conserved let-7 microRNA target sequence in the M mRNA 3′-UTR resulted in attenuation via lower M expression in normal cells that express high levels of let-7, but not in cancer cells that express low levels of let-7 *in vitro* and *in vivo*, and caused no neurotoxicity after intranasal virus infection of mice (Edge *et al.*, 2008). Insertion of neuronal miR125 targets into VSV, particularly the L mRNA 3′-UTR, reduced neurotoxicity even when virus was injected intracranially into mice, while retaining oncolytic abilities (Kelly *et al.*, 2010).

VSV neurotropism can also be inhibited through a pseudotyping approach (Table 1). Pseudotyped VSV-LCMV-GP virions, containing the non-neurotropic envelope glycoprotein of lymphocytic choriomeningitis virus (LCMV) instead of the VSV G protein, showed enhanced infectivity of malignant glioma cells while sparing primary human and rat neurons (Muik *et al.*, 2011). Although only replication-defective viruses were used, this proof-of-principle study demonstrated that VSV-LCMV-GP has a better therapeutic window than VSV, especially for clinical applications targeting brain cancers.

While the approaches described above were designed to prevent VSV replication in normal cells, several studies designed VSVs specifically to target cancer cells. One approach used VSV-S-GP, where a modified glycoprotein from Sindbis virus replaced VSV G (Bergman *et al.*, 2007; Gao *et al.*, 2006). The modified glycoprotein was designed to specifically recognize the Her2 receptor, which is overexpressed on many breast cancer cells. This approach successfully targeted and eliminated Her2-expressing tumours in mice *in vivo*. In a separate study, replication-defective VSV was pseudotyped with measles virus (MV) fusion and haemagglutinin glycoproteins displaying single-chain antibodies to target and infect cells expressing epidermal growth factor receptor, folate receptor or prostate membrane-specific antigen (Ayala-Breton *et al.*, 2012) in human tumour xenografts in mice.

VSV oncoselectivity can be increased via adaptation to cancer cells using serial passages. This approach successfully adapted VSV-S-GP (described above) to a murine mammary tumour cell line expressing the Her2 receptor (Gao *et al.*, 2006). In a separate study, VSV-rp30 was generated by passaging VSV-G/GFP (Table 1) 30 times on glioblastoma cells (Wollmann *et al.*, 2005). VSV-rp30 contains two silent mutations and two missense mutations, one in P and one in L.

Whilst oncolytic virotherapy is based predominantly on replication-competent viruses, some studies have examined replication-defective viruses, which do not produce infectious progeny, similar to those employed in most standard gene-therapy studies (Galivo *et al.*, 2010). While it is unlikely that replication-defective recombinants could be as effective as replicative VSVs (unless used mainly to deliver anti-cancer genes, induce adaptive immunity, etc.), so-called semi-replicative viruses have been generated and tested. Two *trans*-complementing recombinants, VSV*ΔG and VSVΔL-dsRed, lack the VSV G and L genes, respectively, and are non-replicative alone (Muik *et al.*, 2012). However, co-infection of a cell with the two recombinants results in production and spread of non-replicative progeny. The VSVΔG/VSVΔL-dsRed combination was as potent as WT VSV *in vitro* and induced long-term glioblastoma tumour regression in mice *in vivo* without neurotoxicity.

There are several options for treating VSV-resistant cancer cells. Pre-screening cells against an array of VSVs or other OVs could identify the best OV for treating a particular tumour. OV therapy can also be combined with chemical inhibitors to overcome VSV resistance. For example, the mammalian target of rapamycin stimulates type I IFN production via phosphorylation of its effectors. Using rapamycin, the inhibitor of this protein, in combination with VSVΔM51 increased survival of immunocompetent rats with malignant gliomas (Alain *et al.*, 2010). Histone deacetylase inhibitors influence epigenetic changes within cells, ultimately altering gene expression and affecting antiviral responses. Indeed, these inhibitors reversibly compromise host antiviral responses in multiple cancer cell lines and allow enhanced spread of VSV that correlates with inhibited IFN responses and VSV-mediated oncolysis in cancer cells (Nguyên *et al.*, 2008).

The resistance of cancer cells to VSV can also be overcome using a combination of VSV with other OVs, e.g. the double-deleted vaccinia virus (VV). The deletions restrict VV to cells that overexpress transcription factor E2F and have activated epithelial growth factor receptor pathways, a common cancer cell signature. Expression of the VV-encoded B18R protein antagonizes the innate cellular antiviral response to allow more robust VSVΔM51 replication in colon cancer xenografts in mice (Le Boeuf *et al.*, 2010). Furthermore, a recent analysis showed that previous infection of cervical carcinoma cancer cells with human papillomavirus (HPV) improved VSV infection and killing, compared with cervical carcinomas not infected with HPV (Le Boeuf *et al.*, 2012). HPV can inhibit IFN signalling, possibly creating a more hospitable environment for VSV.

Increased oncotoxicity

The ultimate goal of any successful OV therapy is the selective killing of cancer cells. There are at least seven approaches that have been shown to improve the oncolytic abilities ('oncotoxicity') of VSV independent of the immune system (as discussed in the last section): (i) combination of VSV with chemical agents; (ii) viral expression of tumour-suppressor genes; (iii) viral expression of 'suicide genes'; (iv) syncytium induction; (v) radiovirotherapy; (vi) combining VSV with tumour embolization; and (vii) combining VSV with anti-angiogenic agents.

VSV kills infected cells by inducing apoptosis via the mitochondrial (intrinsic) or death receptor (extrinsic)

pathway, or both (Cary *et al.*, 2011; Gaddy & Lyles, 2005, 2007; Sharif-Askari *et al.*, 2007). The mechanisms of induction can be cell-type-specific, and many cancer cells inhibit apoptosis to allow prolonged proliferation (Hamacher *et al.*, 2008; Hanahan & Weinberg, 2011). WT VSV induces apoptosis primarily via the intrinsic pathway, while recombinants with an M51 mutation induce apoptosis primarily via the extrinsic pathway, although this is not absolute (Cary *et al.*, 2011; Gaddy & Lyles, 2005). Understanding the interplay between VSV and cellular apoptotic mechanisms may be critical for developing and selecting OV treatment. Overexpression of the anti-apoptotic B-cell lymphoma 2 (BCL-2) protein was shown to impair VSV-mediated oncolysis, while this resistance was reversed when VSV was combined with obatoclax, a small-molecule BCL-2 inhibitor (Samuel *et al.*, 2010). Another BCL-2 inhibitor, EM20-25, rendered apoptosis-resistant cancer cells susceptible to VSV-induced apoptosis (Tumilasci *et al.*, 2008). In another study, infection by WT VSV (but not VSV with the M51 mutation) increased degradation of an anti-apoptotic myeloid cell leukaemia 1 protein (Mcl-1) that contributes to chemotherapy resistance (Schache *et al.*, 2009). This VSV-mediated Mcl-1 degradation sensitized apoptosis-resistant cancer cells to doxorubicin, an approved chemotherapeutic. This combined 'chemovirotherapy' had an enhanced therapeutic effect compared with each treatment alone in mice (Schache *et al.*, 2009).

The oncotoxicity of VSV can be enhanced by the expression of functional tumour-suppressor genes in cancer cells, e.g. VSV-M(mut)-mp53, which encodes p53 in addition to the mutated M protein and induces potent anti-tumour responses in mice (Heiber & Barber, 2011). This study showed that VSV-M(mut)-mp53 retained the selective ability to lyse cancer cells and also directed expression of high levels of functional p53. Importantly, VSV-M(mut)-mp53 showed improved safety when attenuated *in vivo* due to the activation of innate immune genes (such as type I IFNs) by p53, and induced enhanced adaptive tumour-specific immune responses.

One limitation of a standard OV therapy is that oncotoxicity is normally limited to virus-infected cancer cells. To address this issue, several approaches aim to increase the bystander effect and to kill uninfected cancer cells. One approach uses VSV expressing so-called 'suicide genes'. These genes catalyse conversion of a non-toxic prodrug into a toxic form that, in addition to its toxicity in infected cancer cells, can diffuse to neighbouring uninfected cancer cells through gap junctions. Administration of VSV expressing the herpesvirus thymidine kinase (TK) protein to mice in combination with the prodrug ganciclovir exerted a great oncolytic effect through TK/ganciclovir-mediated apoptosis, enhanced the bystander effect and induced tumour-specific immune responses in breast or melanoma tumours in mice (Fernandez *et al.*, 2002). Cytosine deaminase (CD) catalyses the conversion of 5-fluorocytosine (5-FC) to the commonly used chemotherapeutic drug 5-fluorouracil (5-FU), while uracil phosphoribosyltransferase (UPRT) converts 5-FU into the active 5-fluoro-UMP form. Intratumoral inoculation with VSV expressing these two proteins (VSV-C : U) followed by 5-FC administration improved tumour regression significantly compared with VSV or 5-FU alone, and activated tumour-specific immune responses against lymphoma or mammary carcinoma in mice (Porosnicu *et al.*, 2003). This approach was further optimized by combining the M51 mutation with CD/UPRT expression (VSV-C) (Leveille *et al.*, 2011b).

One critical limitation of OVs is their relatively poor penetration and spread within tumour masses. Several studies have attempted to address this problem through the generation of VSVs that spread by forming giant, multinuclear cells called syncytia. While VSV is generally not fusogenic, several fusogenic recombinants have been generated and showed promising results in different cancer models. VSV-NDV/F(L289A) (later designated rVSV-F) encodes the Newcastle disease virus (NDV) fusion protein gene with an L289A mutation to allow syncytium formation in the absence of the NDV haemagglutinin–neuraminidase (HN) protein (Altomonte *et al.*, 2008b; Ebert *et al.*, 2004; Shin *et al.*, 2007b). VSV-ΔG-SV5-F expresses the simian parainfluenza virus F protein (Chang *et al.*, 2010) and VSV/FAST virus expresses the p14 FAST protein of reptilian reovirus (Brown *et al.*, 2009). Adding fusogenic genes to the VSV genome should be done with caution as VSV/FAST with WT M showed dramatically increased neuropathology in mice, although VSV M51 expressing p14 remained attenuated (Brown *et al.*, 2009).

One of the most elegant approaches to increase VSV oncotoxicity is based on a combination of viro- and radiotherapy ('radiovirotherapy'). VSV-MΔ51-NIS expresses a human sodium iodide symporter (NIS) protein that mediates high-concentration iodide uptake and, in mice, has a synergistic effect with iodine. VSV-NIS in combination with iodine-123 allows sensitive monitoring of infection, while iodine-131 is used for treatment of radiosensitive tumours (Goel *et al.*, 2007; Naik *et al.*, 2012).

VSV-based OV therapy of liver cancers can be improved significantly when combined with tumour embolization ('viroembolization'), the blocking of arterial blood flow in the liver, thereby prolonging exposure of tumour cells to the therapeutic agent. When VSV was administered to rats in combination with degradable starch microspheres, an embolic agent currently used clinically for liver tumours, massive tumour necrosis and substantially prolonged survival were observed in test animals compared with monotherapy with either VSV or the embolic agent alone (Altomonte *et al.*, 2008a).

Finally, VSV oncotoxicity can be improved by targeting tumour vasculature. A recent study has shown the ability of VSV to target tumour vasculature and angiogenesis when administered subcutaneously to mice with colon adenocarcinoma (Breitbach *et al.*, 2011). The use of anti-angiogenic vascular endothelial growth factor 165 inhibitor combined with VSV led to increased tumour regression

and improved virus titre and dissemination, even within tumours that previously supported poor VSV replication (Kottke *et al.*, 2010).

Preventing premature clearance of VSV

Safe virotherapy ultimately requires clearance of the OV from the body. Unfortunately, those same mechanisms can eliminate the OVs prematurely, before they complete their task. Prior to initiation of infection, circulating antibodies (Abs), non-specific host proteins or complement proteins can neutralize virus particles. Virus sequestration to certain organs can also result in ineffective OV therapy. Several approaches have been developed to protect VSV-based OVs from premature clearance, including: (i) physical delivery methods hiding/masking virus from Abs, other host components or immune cells; (ii) VSVs expressing genes favouring VSV survival; and (iii) combination of VSV with chemicals favouring VSV survival.

Various cell-based methods to deliver OVs to tumours via carrier cells have been reviewed by Nakashima *et al.* (2010) and Power & Bell (2008). With regard to VSV, murine OT-I $CD8^+$ T-cells, specific for an epitope of the ovalbumin antigen (Ag), were infected *ex vivo* with VSV and delivered to established B16-OVA melanoma tumours in the lungs of immunocompetent mice (Kottke *et al.*, 2008a; Qiao *et al.*, 2008a). These virus 'Trojan horses' demonstrated significantly improved therapy compared with VSV or T-cells alone. Importantly, this therapy was effective even in mice with pre-existing Abs against VSV, indicating that therapy with virus-loaded T-cells may be useful even in patients with pre-existing immunity to VSV (Kottke *et al.*, 2008a). A new approach called aptamer-facilitated virus immunoshielding (AptaVISH) uses aptamer technology to mask OVs from their respective neutralizing Abs, and is currently in development for several OVs, including VSV (Labib *et al.*, 2012).

While these studies physically hide or mask VSV from the immune system, other approaches have attempted to modulate the immune system environment to favour virus survival. For instance, VSV-gG expresses the equine herpesvirus (EHV)-1 glycoprotein G, a broad-spectrum chemokine-binding protein. Addition of EHV-1 G increases the oncolytic potency of VSV due to substantial suppression of host antiviral inflammatory responses in rats (Altomonte *et al.*, 2008b). Similarly, VSV-M51-M3 expresses the murine gammaherpesvirus-68 M3 protein, which binds a broad range of chemokines and reduces the inflammatory response and NK and neutrophil accumulation in lesions in rats (Wu *et al.*, 2008). Furthermore, recombinant VSV-UL141 expressing UL141, which downregulates NK cell-activating ligand CD155, inhibited NK-cell recruitment in rats (Altomonte *et al.*, 2009).

Additional studies investigated the prevention of Ab-mediated VSV neutralization by combined administration of VSV and cyclophosphamide (CPA). CPA enhances delivery of OVs through reductions in levels of neutralizing Abs, suppression of innate immune effectors (Ikeda *et al.*, 2000; Qiao *et al.*, 2008b), depletion of number of Tregs (Kottke *et al.*, 2008b) and activation of immune cells (Ghiringhelli *et al.*, 2007). While a single dose of CPA has been shown to be insufficient to control primary anti-VSV immune responses in animal models, a clinically approved multi-dose CPA regimen suppressed antiviral Ab responses against VSV, even in mice with pre-existing Abs against VSV (Peng *et al.*, 2012). However, a recent study surprisingly showed that the combination of CPA and VSV was less effective than CPA alone, despite increased intratumoral VSV titres (Willmon *et al.*, 2011). This study suggests that CPA-mediated oncotherapy is dependent upon both CD4 T-cell and NK-cell activation, which are suppressed upon VSV infection, and serves as a warning of unforeseen consequences of experimental therapies involving immune modulation.

Inducing tumour-specific immunity

Fully effective OV therapy may require the activation of tumour-specific adaptive immune responses (Melcher *et al.*, 2011). Although all VSVs have immunostimulatory abilities, in this section we focus on VSV-based OVs designed specifically to induce tumour-specific immune responses.

A number of tested VSVs encode immunostimulatory host genes (Table 1), including interleukin (IL)-4 (Fernandez *et al.*, 2002), IL-12 (Shin *et al.*, 2007a), IL-15 (Stephenson *et al.*, 2012), IL-23 (Miller *et al.*, 2009, 2010), type III IFN-λ (also called IL-28) (Wongthida *et al.*, 2010), Fms-like tyrosine kinase 3 ligand (Flt3L) (Leveille *et al.*, 2011a) and CD40L (Galivo *et al.*, 2010) (Table 1). Interestingly, a study utilizing VSV-CD40L with or without VSV G indicated that therapeutic success may not depend on progressive rounds of VSV replication, as non-replicative VSV-CD40L-ΔG was equally as effective as fully replication-competent VSV-CD40L in mice. This result illustrates that tumour-specific immune responses could play a dominant role, at least in the employed experimental system (Galivo *et al.*, 2010). Some interleukins provide not only immunostimulation but also improved safety. For example, the incorporation of IL-23 into the VSV genome stimulated NK and CD4 cells and enhanced nitric oxide production in the CNS, aiding viral clearance from neurons (Miller *et al.*, 2009, 2010).

While these approaches stimulated the immune system and often resulted in tumour-specific memory responses, several studies investigated whether VSV can be designed specifically to facilitate the presentation of tumour-associated antigen (TAA) to immune cells. In a proof-of-principle study, the VSV-OVA virus was generated to express the chicken ovalbumin (ova) gene (Diaz *et al.*, 2007). Injection of VSV-OVA into established B16-OVA tumours increased the number of ova-specific T-cells significantly compared with VSV-GFP (Wongthida *et al.*, 2011b). VSV expressing an altered version of the murine self-TAA gp100 was able to stimulate gp100-specific T-cells

despite pre-existing immune tolerance. Although tumour reduction was not improved significantly compared with VSV-GFP, combining VSV-hgp100 infection with adoptive transfer of naïve gp100-specific T-cells improved efficacy greatly, suggesting the potential of this treatment strategy (Wongthida *et al.*, 2011b).

Dendritic cells (DCs) have the ability to activate Ag-specific T-cells and NK cells. While DCs do not support robust VSV replication, they can be infected *ex vivo*, then used to mount a specific anti-tumour response. DCs infected with VSV encoding human melanoma-associated Ag dopachrome tautomerase (hDCT) endogenously expressed by B16-OVA cells, or luciferase tagged with the immunodominant class-I epitope SIINFEKL, were able to mature and produce pro-inflammatory cytokines (Boudreau *et al.*, 2009). When mice with metastatic tumours received DC-VSV/hDCT, tumour growth was controlled by both NK and $CD8^+$ T-cells (Boudreau *et al.*, 2009). In an even more sophisticated approach, a combination of an adenovirus and VSV both expressing hDCT were used sequentially. The adenovirus pre-immunization of *in vivo* murine DCT tumours did not prevent intratumoral VSV infection. Furthermore, this treatment resulted in reduced VSV replication in normal cells and a shift in immune activation from viral Ags to TAAs (Bridle *et al.*, 2010).

These approaches may be useful if a specific TAA is stably expressed, but, in most cancer types, TAA expression is variable, transient and often unknown for individual tumours. In a new approach, a VSV-cDNA library was used to identify TAAs capable of inducing enhanced tumour-specific immunity (Pulido *et al.*, 2012). The screen identified three viruses encoding putative TAAs, and their therapeutic effect against B16 murine melanoma tumours was reconstituted *in vivo* when these viruses were used together.

A fine balance between antiviral and anti-cancer responses is probably needed for effective OV therapy using VSV. For example, TLR signalling through myeloid differentiation primary response gene 88 (MyD88) activates specific antiviral immune responses that inhibit virus replication within the tumour, but also induces critical anti-cancer responses; a recent study has shown that VSV anti-tumour therapy in the B16-OVA mouse model depends on antiviral signalling through MyD88 (Wongthida *et al.*, 2011a). Finally, while the majority of studies have demonstrated the desirable immunomodulation of the tumour microenvironment following VSV infection to favour tumour rejection, the opposite situation can also occur. A recent study demonstrated that VSV infection can negatively affect surface expression of immunostimulatory NKG2D-ligand, allowing viruses to escape immune recognition by NK cells, but negatively affecting anti-tumour immune responses (Jensen *et al.*, 2011).

Concluding remarks/future directions

While significant advances have been made in the use of VSV as an OV, room for improvement still remains, along with many challenges to be addressed. The creation of recombinants has improved the OV qualities of VSV, but it is important to be mindful of biosafety, especially with recombinants designed to evade host antiviral responses. Future recombinants will need to address the visualization of VSV infection and spread *in vivo*. Imaging of VSV-GFP or VSV-luciferase is widely used in rodent models, but requires levels of expression that may not be attainable in human clinical studies. Whilst VSV-NIS in combination with iodine-123 allows sensitive monitoring of infection, it is mostly applicable to studies involving radioisotopes (Goel *et al.*, 2007; Naik *et al.*, 2012).

To prevent cancer recurrence, successful OV should not only penetrate tumours, but also kill cancer stem cells. Whilst studied extensively in other viruses, few have looked at this issue with regard to VSV (Cripe *et al.*, 2009; Friedman *et al.*, 2012). To provide the best virus for clinical use, the 'perfect' therapeutic oncolytic VSV should cause no neurotoxicity, retain WT-like oncolytic ability, be easily adaptable to target specific cancer types, and induce immune memory. Development of good manufacturing practices is necessary to ensure that the VSVs are grown in the most appropriate cell line, free of contaminants and optimized for clinical use (Ausubel *et al.*, 2011; Diallo *et al.*, 2012). A recent study using VSV-IFNβ demonstrated successfully that systemic delivery in immunocompetent mice destroyed disseminated myeloma (Naik *et al.*, 2012). Currently, a phase I clinical trial using VSV-IFNβ is in progress (ClinicalTrials.gov, 2012, trial ID NCT01628640; http://clinicaltrials.gov/ct2/show/NCT01628640). The study aims to evaluate the safety of intratumoral administration of VSV-IFNβ to human patients with hepatocellular carcinoma refractory or intolerant to sorafenib, a standard agent for systemic chemotherapy. Expected to finish in 2013, the outcome will help to determine future maximum tolerated dose, assess tumour-response rate and look at immune responses, and will pave the way for future research and clinical trials.

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References

Alain, T., Lun, X., Martineau, Y., Sean, P., Pulendran, B., Petroulakis, E., Zemp, F. J., Lemay, C. G., Roy, D. & other authors (2010). Vesicular stomatitis virus oncolysis is potentiated by impairing mTORC1-dependent type I IFN production. *Proc Natl Acad Sci U S A* **107**, 1576–1581.

Altomonte, J., Braren, R., Schulz, S., Marozin, S., Rummeny, E. J., Schmid, R. M. & Ebert, O. (2008a). Synergistic antitumor effects of transarterial viroembolization for multifocal hepatocellular carcinoma in rats. *Hepatology* **48**, 1864–1873.

Altomonte, J., Wu, L., Chen, L., Meseck, M., Ebert, O., Garcia-Sastre, A., Fallon, J. & Woo, S. L. (2008b). Exponential enhancement of oncolytic vesicular stomatitis virus potency by vector-mediated suppression of inflammatory responses *in vivo*. *Mol Ther* **16**, 146–153.

Altomonte, J., Wu, L., Meseck, M., Chen, L., Ebert, O., Garcia-Sastre, A., Fallon, J., Mandeli, J. & Woo, S. L. (2009). Enhanced oncolytic potency of vesicular stomatitis virus through vector-mediated inhibition of NK and NKT cells. *Cancer Gene Ther* **16**, 266–278.

Ausubel, L. J., Meseck, M., Derecho, I., Lopez, P., Knoblauch, C., McMahon, R., Anderson, J., Dunphy, N., Quezada, V. & other authors (2011). Current good manufacturing practice production of an oncolytic recombinant vesicular stomatitis viral vector for cancer treatment. *Hum Gene Ther* **22**, 489–497.

Ayala-Breton, C., Barber, G. N., Russell, S. J. & Peng, K. W. (2012). Retargeting vesicular stomatitis virus using measles virus envelope glycoproteins. *Hum Gene Ther* **23**, 484–491.

Balachandran, S. & Barber, G. N. (2004). Defective translational control facilitates vesicular stomatitis virus oncolysis. *Cancer Cell* **5**, 51–65.

Balachandran, S. & Barber, G. N. (2007). PKR in innate immunity, cancer, and viral oncolysis. *Methods Mol Biol* **383**, 277–301.

Ball, L. A., Pringle, C. R., Flanagan, B., Perepelitsa, V. P. & Wertz, G. W. (1999). Phenotypic consequences of rearranging the P, M, and G genes of vesicular stomatitis virus. *J Virol* **73**, 4705–4712.

Barber, G. N. (2004). Vesicular stomatitis virus as an oncolytic vector. *Viral Immunol* **17**, 516–527.

Barber, G. N. (2005). VSV-tumor selective replication and protein translation. *Oncogene* **24**, 7710–7719.

Bergman, I., Griffin, J. A., Gao, Y. & Whitaker-Dowling, P. (2007). Treatment of implanted mammary tumors with recombinant vesicular stomatitis virus targeted to Her2/neu. *Int J Cancer* **121**, 425–430.

Bi, Z., Barna, M., Komatsu, T. & Reiss, C. S. (1995). Vesicular stomatitis virus infection of the central nervous system activates both innate and acquired immunity. *J Virol* **69**, 6466–6472.

Black, B. L., Rhodes, R. B., McKenzie, M. & Lyles, D. S. (1993). The role of vesicular stomatitis virus matrix protein in inhibition of host-directed gene expression is genetically separable from its function in virus assembly. *J Virol* **67**, 4814–4821.

Boudreau, J. E., Bridle, B. W., Stephenson, K. B., Jenkins, K. M., Brunellière, J., Bramson, J. L., Lichty, B. D. & Wan, Y. (2009). Recombinant vesicular stomatitis virus transduction of dendritic cells enhances their ability to prime innate and adaptive antitumor immunity. *Mol Ther* **17**, 1465–1472.

Breitbach, C. J., De Silva, N. S., Falls, T. J., Aladl, U., Evgin, L., Paterson, J., Sun, Y. Y., Roy, D. G., Rintoul, J. L. & other authors (2011). Targeting tumor vasculature with an oncolytic virus. *Mol Ther* **19**, 886–894.

Bridle, B. W., Stephenson, K. B., Boudreau, J. E., Koshy, S., Kazdhan, N., Pullenayegum, E., Brunellière, J., Bramson, J. L., Lichty, B. D. & Wan, Y. (2010). Potentiating cancer immunotherapy using an oncolytic virus. *Mol Ther* **18**, 1430–1439.

Brown, C. W., Stephenson, K. B., Hanson, S., Kucharczyk, M., Duncan, R., Bell, J. C. & Lichty, B. D. (2009). The p14 FAST protein of reptilian reovirus increases vesicular stomatitis virus neuropathogenesis. *J Virol* **83**, 552–561.

Cary, Z. D., Willingham, M. C. & Lyles, D. S. (2011). Oncolytic vesicular stomatitis virus induces apoptosis in U87 glioblastoma cells by a type II death receptor mechanism and induces cell death and tumor clearance *in vivo*. *J Virol* **85**, 5708–5717.

Chakraborty, P., Seemann, J., Mishra, R. K., Wei, J. H., Weil, L., Nussenzveig, D. R., Heiber, J., Barber, G. N., Dasso, M. & Fontoura, B. M. (2009). Vesicular stomatitis virus inhibits mitotic progression and triggers cell death. *EMBO Rep* **10**, 1154–1160.

Chang, G., Xu, S., Watanabe, M., Jayakar, H. R., Whitt, M. A. & Gingrich, J. R. (2010). Enhanced oncolytic activity of vesicular stomatitis virus encoding SV5-F protein against prostate cancer. *J Urol* **183**, 1611–1618.

Chauhan, V. S., Furr, S. R., Sterka, D. G., Jr, Nelson, D. A., Moerdyk-Schauwecker, M., Marriott, I. & Grdzelishvili, V. Z. (2010). Vesicular stomatitis virus infects resident cells of the central nervous system and induces replication-dependent inflammatory responses. *Virology* **400**, 187–196.

Chelbi-Alix, M. K., Quignon, F., Pelicano, L., Koken, M. H. & de Thé, H. (1998). Resistance to virus infection conferred by the interferon-induced promyelocytic leukemia protein. *J Virol* **72**, 1043–1051.

Connor, J. H. & Lyles, D. S. (2002). Vesicular stomatitis virus infection alters the eIF4F translation initiation complex and causes dephosphorylation of the eIF4E binding protein 4E-BP1. *J Virol* **76**, 10177–10187.

Coulon, P., Deutsch, V., Lafay, F., Martinet-Edelist, C., Wyers, F., Herman, R. C. & Flamand, A. (1990). Genetic evidence for multiple functions of the matrix protein of vesicular stomatitis virus. *J Gen Virol* **71**, 991–996.

Cripe, T. P., Wang, P. Y., Marcato, P., Mahller, Y. Y. & Lee, P. W. (2009). Targeting cancer-initiating cells with oncolytic viruses. *Mol Ther* **17**, 1677–1682.

Cureton, D. K., Massol, R. H., Whelan, S. P. & Kirchhausen, T. (2010). The length of vesicular stomatitis virus particles dictates a need for actin assembly during clathrin-dependent endocytosis. *PLoS Pathog* **6**, e1001127.

Dal Canto, M. C., Rabinowitz, S. G. & Johnson, T. C. (1976). Status spongiosus resulting from intracerebral infection of mice with temperature-sensitive mutants of vesicular stomatitis virus. *Br J Exp Pathol* **57**, 321–330.

Dalton, K. P. & Rose, J. K. (2001). Vesicular stomatitis virus glycoprotein containing the entire green fluorescent protein on its cytoplasmic domain is incorporated efficiently into virus particles. *Virology* **279**, 414–421.

Diallo, J. S., Vähä-Koskela, M., Le Boeuf, F. & Bell, J. (2012). Propagation, purification, and in vivo testing of oncolytic vesicular stomatitis virus strains. *Methods Mol Biol* **797**, 127–140.

Diaz, R. M., Galivo, F., Kottke, T., Wongthida, P., Qiao, J., Thompson, J., Valdes, M., Barber, G. & Vile, R. G. (2007). Oncolytic immunovirotherapy for melanoma using vesicular stomatitis virus. *Cancer Res* **67**, 2840–2848.

Dinh, P. X., Beura, L. K., Panda, D., Das, A. & Pattnaik, A. K. (2011). Antagonistic effects of cellular poly(C) binding proteins on vesicular stomatitis virus gene expression. *J Virol* **85**, 9459–9471.

Drolet, B. S., Stuart, M. A. & Derner, J. D. (2009). Infection of *Melanoplus sanguinipes* grasshoppers following ingestion of rangeland plant species harboring vesicular stomatitis virus. *Appl Environ Microbiol* **75**, 3029–3033.

Ebert, O., Shinozaki, K., Kournioti, C., Park, M. S., García-Sastre, A. & Woo, S. L. (2004). Syncytia induction enhances the oncolytic potential of vesicular stomatitis virus in virotherapy for cancer. *Cancer Res* **64**, 3265–3270.

Edge, R. E., Falls, T. J., Brown, C. W., Lichty, B. D., Atkins, H. & Bell, J. C. (2008). A let-7 microRNA-sensitive vesicular stomatitis virus demonstrates tumor-specific replication. *Mol Ther* **16**, 1437–1443.

Egan, M. A., Chong, S. Y., Rose, N. F., Megati, S., Lopez, K. J., Schadeck, E. B., Johnson, J. E., Masood, A., Piacente, P. & other authors (2004). Immunogenicity of attenuated vesicular stomatitis virus vectors expressing HIV type 1 Env and SIV Gag proteins:

comparison of intranasal and intramuscular vaccination routes. *AIDS Res Hum Retroviruses* **20**, 989–1004.

Faria, P. A., Chakraborty, P., Levay, A., Barber, G. N., Ezelle, H. J., Enninga, J., Arana, C., van Deursen, J. & Fontoura, B. M. (2005). VSV disrupts the Rae1/mrnp41 mRNA nuclear export pathway. *Mol Cell* **17**, 93–102.

Fernandez, M., Porosnicu, M., Markovic, D. & Barber, G. N. (2002). Genetically engineered vesicular stomatitis virus in gene therapy: application for treatment of malignant disease. *J Virol* **76**, 895–904.

Finke, S. & Conzelmann, K. K. (2005). Recombinant rhabdoviruses: vectors for vaccine development and gene therapy. *Curr Top Microbiol Immunol* **292**, 165–200.

Flanagan, E. B., Zamparo, J. M., Ball, L. A., Rodriguez, L. L. & Wertz, G. W. (2001). Rearrangement of the genes of vesicular stomatitis virus eliminates clinical disease in the natural host: new strategy for vaccine development. *J Virol* **75**, 6107–6114.

Frei, K., Malipiero, U. V., Leist, T. P., Zinkernagel, R. M., Schwab, M. E. & Fontana, A. (1989). On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. *Eur J Immunol* **19**, 689–694.

Friedman, G. K., Cassady, K. A., Beierle, E. A., Markert, J. M. & Gillespie, G. Y. (2012). Targeting pediatric cancer stem cells with oncolytic virotherapy. *Pediatr Res* **71**, 500–510.

Furr, S. R., Chauhan, V. S., Sterka, D., Jr, Grdzelishvili, V. Z. & Marriott, I. (2008). Characterization of retinoic acid-inducible gene-I expression in primary murine glia following exposure to vesicular stomatitis virus. *J Neurovirol* **14**, 503–513.

Furr, S. R., Moerdyk-Schauwecker, M., Grdzelishvili, V. Z. & Marriott, I. (2010). RIG-I mediates nonsegmented negative-sense RNA virus-induced inflammatory immune responses of primary human astrocytes. *Glia* **58**, 1620–1629.

Gaddy, D. F. & Lyles, D. S. (2005). Vesicular stomatitis viruses expressing wild-type or mutant M proteins activate apoptosis through distinct pathways. *J Virol* **79**, 4170–4179.

Gaddy, D. F. & Lyles, D. S. (2007). Oncolytic vesicular stomatitis virus induces apoptosis via signaling through PKR, Fas, and Daxx. *J Virol* **81**, 2792–2804.

Galivo, F., Diaz, R. M., Thanarajasingam, U., Jevremovic, D., Wongthida, P., Thompson, J., Kottke, T., Barber, G. N., Melcher, A. & Vile, R. G. (2010). Interference of CD40L-mediated tumor immunotherapy by oncolytic vesicular stomatitis virus. *Hum Gene Ther* **21**, 439–450.

Gao, Y., Whitaker-Dowling, P., Watkins, S. C., Griffin, J. A. & Bergman, I. (2006). Rapid adaptation of a recombinant vesicular stomatitis virus to a targeted cell line. *J Virol* **80**, 8603–8612.

Garber, K. (2006). China approves world's first oncolytic virus therapy for cancer treatment. *J Natl Cancer Inst* **98**, 298–300.

Ge, P., Tsao, J., Schein, S., Green, T. J., Luo, M. & Zhou, Z. H. (2010). Cryo-EM model of the bullet-shaped vesicular stomatitis virus. *Science* **327**, 689–693.

Georgel, P., Jiang, Z., Kunz, S., Janssen, E., Mols, J., Hoebe, K., Bahram, S., Oldstone, M. B. & Beutler, B. (2007). Vesicular stomatitis virus glycoprotein G activates a specific antiviral Toll-like receptor 4-dependent pathway. *Virology* **362**, 304–313.

Ghiringhelli, F., Menard, C., Puig, P. E., Ladoire, S., Roux, S., Martin, F., Solary, E., Le Cesne, A., Zitvogel, L. & Chauffert, B. (2007). Metronomic cyclophosphamide regimen selectively depletes $CD4^+CD25^+$ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother* **56**, 641–648.

Goel, A., Carlson, S. K., Classic, K. L., Greiner, S., Naik, S., Power, A. T., Bell, J. C. & Russell, S. J. (2007). Radioiodide imaging and radiovirotherapy of multiple myeloma using VSV(Δ51)-NIS, an attenuated vesicular stomatitis virus encoding the sodium iodide symporter gene. *Blood* **110**, 2342–2350.

Hamacher, R., Schmid, R. M., Saur, D. & Schneider, G. (2008). Apoptotic pathways in pancreatic ductal adenocarcinoma. *Mol Cancer* **7**, 64.

Hammill, A. M., Conner, J. & Cripe, T. P. (2010). Oncolytic virotherapy reaches adolescence. *Pediatr Blood Cancer* **55**, 1253–1263.

Hanahan, D. & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell* **144**, 646–674.

Hansen, D. E., Thurmond, M. C. & Thorburn, M. (1985). Factors associated with the spread of clinical vesicular stomatitis in California dairy cattle. *Am J Vet Res* **46**, 789–795.

Harashima, A., Guettouche, T. & Barber, G. N. (2010). Phosphorylation of the NFAR proteins by the dsRNA-dependent protein kinase PKR constitutes a novel mechanism of translational regulation and cellular defense. *Genes Dev* **24**, 2640–2653.

Heiber, J. F. & Barber, G. N. (2011). Vesicular stomatitis virus expressing tumor suppressor p53 is a highly attenuated, potent oncolytic agent. *J Virol* **85**, 10440–10450.

Heinrich, B. S., Cureton, D. K., Rahmeh, A. A. & Whelan, S. P. (2010). Protein expression redirects vesicular stomatitis virus RNA synthesis to cytoplasmic inclusions. *PLoS Pathog* **6**, e1000958.

Hoffmann, M., Wu, Y. J., Gerber, M., Berger-Rentsch, M., Heimrich, B., Schwemmle, M. & Zimmer, G. (2010). Fusion-active glycoprotein G mediates the cytotoxicity of vesicular stomatitis virus M mutants lacking host shut-off activity. *J Gen Virol* **91**, 2782–2793.

Hornung, V., Ellegast, J., Kim, S., Brzózka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K. K. & other authors (2006). 5′-Triphosphate RNA is the ligand for RIG-I. *Science* **314**, 994–997.

Huneycutt, B. S., Bi, Z., Aoki, C. J. & Reiss, C. S. (1993). Central neuropathogenesis of vesicular stomatitis virus infection of immunodeficient mice. *J Virol* **67**, 6698–6706.

Ikeda, K., Wakimoto, H., Ichikawa, T., Jhung, S., Hochberg, F. H., Louis, D. N. & Chiocca, E. A. (2000). Complement depletion facilitates the infection of multiple brain tumors by an intravascular, replication-conditional herpes simplex virus mutant. *J Virol* **74**, 4765–4775.

Irie, T., Carnero, E., Okumura, A., García-Sastre, A. & Harty, R. N. (2007). Modifications of the PSAP region of the matrix protein lead to attenuation of vesicular stomatitis virus *in vitro* and *in vivo*. *J Gen Virol* **88**, 2559–2567.

Janelle, V., Brassard, F., Lapierre, P., Lamarre, A. & Poliquin, L. (2011). Mutations in the glycoprotein of vesicular stomatitis virus affect cytopathogenicity: potential for oncolytic virotherapy. *J Virol* **85**, 6513–6520.

Jenks, N., Myers, R., Greiner, S. M., Thompson, J., Mader, E. K., Greenslade, A., Griesmann, G. E., Federspiel, M. J., Rakela, J. & other authors (2010). Safety studies on intrahepatic or intratumoral injection of oncolytic vesicular stomatitis virus expressing interferon-β in rodents and nonhuman primates. *Hum Gene Ther* **21**, 451–462.

Jensen, H., Andresen, L., Nielsen, J., Christensen, J. P. & Skov, S. (2011). Vesicular stomatitis virus infection promotes immune evasion by preventing NKG2D-ligand surface expression. *PLoS ONE* **6**, e23023.

Johnson, J. E., Nasar, F., Coleman, J. W., Price, R. E., Javadian, A., Draper, K., Lee, M., Reilly, P. A., Clarke, D. K. & other authors (2007). Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates. *Virology* **360**, 36–49.

Kelly, E. & Russell, S. J. (2007). History of oncolytic viruses: genesis to genetic engineering. *Mol Ther* **15**, 651–659.

Kelly, E. J., Nace, R., Barber, G. N. & Russell, S. J. (2010). Attenuation of vesicular stomatitis virus encephalitis through microRNA targeting. *J Virol* **84**, 1550–1562.

Kopecky, S. A., Willingham, M. C. & Lyles, D. S. (2001). Matrix protein and another viral component contribute to induction of apoptosis in cells infected with vesicular stomatitis virus. *J Virol* **75**, 12169–12181.

Kottke, T., Diaz, R. M., Kaluza, K., Pulido, J., Galivo, F., Wongthida, P., Thompson, J., Willmon, C., Barber, G. N. & other authors (2008a). Use of biological therapy to enhance both virotherapy and adoptive T-cell therapy for cancer. *Mol Ther* **16**, 1910–1918.

Kottke, T., Galivo, F., Wongthida, P., Diaz, R. M., Thompson, J., Jevremovic, D., Barber, G. N., Hall, G., Chester, J. & other authors (2008b). Treg depletion-enhanced IL-2 treatment facilitates therapy of established tumors using systemically delivered oncolytic virus. *Mol Ther* **16**, 1217–1226.

Kottke, T., Hall, G., Pulido, J., Diaz, R. M., Thompson, J., Chong, H., Selby, P., Coffey, M., Pandha, H. & other authors (2010). Antiangiogenic cancer therapy combined with oncolytic virotherapy leads to regression of established tumors in mice. *J Clin Invest* **120**, 1551–1560.

Krishnamoorthy, J., Mounir, Z., Raven, J. F. & Koromilas, A. E. (2008). The eIF2α kinases inhibit vesicular stomatitis virus replication independently of eIF2alpha phosphorylation. *Cell Cycle* **7**, 2346–2351.

Labib, M., Zamay, A. S., Muharemagic, D., Chechik, A., Bell, J. C. & Berezovski, M. V. (2012). Electrochemical sensing of aptamer-facilitated virus immunoshielding. *Anal Chem* **84**, 1677–1686.

Lawson, N. D., Stillman, E. A., Whitt, M. A. & Rose, J. K. (1995). Recombinant vesicular stomatitis viruses from DNA. *Proc Natl Acad Sci U S A* **92**, 4477–4481.

Le Boeuf, F., Diallo, J. S., McCart, J. A., Thorne, S., Falls, T., Stanford, M., Kanji, F., Auer, R., Brown, C. W. & other authors (2010). Synergistic interaction between oncolytic viruses augments tumor killing. *Mol Ther* **18**, 888–895.

Le Boeuf, F., Niknejad, N., Wang, J., Auer, R., Weberpals, J. I., Bell, J. C. & Dimitroulakos, J. (2012). Sensitivity of cervical carcinoma cells to vesicular stomatitis virus-induced oncolysis: potential role of human papilloma virus infection. *Int J Cancer* **131**, E204–E215.

Leveille, S., Goulet, M. L., Lichty, B. D. & Hiscott, J. (2011a). Vesicular stomatitis virus oncolytic treatment interferes with tumor-associated dendritic cell functions and abrogates tumor antigen presentation. *J Virol* **85**, 12160–12169.

Leveille, S., Samuel, S., Goulet, M. L. & Hiscott, J. (2011b). Enhancing VSV oncolytic activity with an improved cytosine deaminase suicide gene strategy. *Cancer Gene Ther* **18**, 435–443.

Li, Q. & Tainsky, M. A. (2011). Epigenetic silencing of IRF7 and/or IRF5 in lung cancer cells leads to increased sensitivity to oncolytic viruses. *PLoS ONE* **6**, e28683.

Lichty, B. D., Power, A. T., Stojdl, D. F. & Bell, J. C. (2004). Vesicular stomatitis virus: re-inventing the bullet. *Trends Mol Med* **10**, 210–216.

Linge, C., Gewert, D., Rossmann, C., Bishop, J. A. & Crowe, J. S. (1995). Interferon system defects in human malignant melanoma. *Cancer Res* **55**, 4099–4104.

Lyles, D. S. & Rupprecht, C. E. (2007). *Rhabdoviridae.* In *Fields Virology*, 5th edn, pp. 1363–1408. Edited by D. M. Knipe & P. M. Howley. Philadelphia, PA: Lippincott Williams & Wilkins.

Marozin, S., Altomonte, J., Stadler, F., Thasler, W. E., Schmid, R. M. & Ebert, O. (2008). Inhibition of the IFN-β response in hepatocellular carcinoma by alternative spliced isoform of IFN regulatory factor-3. *Mol Ther* **16**, 1789–1797.

Marozin, S., De Toni, E. N., Rizzani, A., Altomonte, J., Junger, A., Schneider, G., Thasler, W. E., Kato, N., Schmid, R. M. & Ebert, O. (2010). Cell cycle progression or translation control is not essential for vesicular stomatitis virus oncolysis of hepatocellular carcinoma. *PLoS ONE* **5**, e10988.

Martinez, I., Rodriguez, L. L., Jimenez, C., Pauszek, S. J. & Wertz, G. W. (2003). Vesicular stomatitis virus glycoprotein is a determinant of pathogenesis in swine, a natural host. *J Virol* **77**, 8039–8047.

Matin, S. F., Rackley, R. R., Sadhukhan, P. C., Kim, M. S., Novick, A. C. & Bandyopadhyay, S. K. (2001). Impaired α-interferon signaling in transitional cell carcinoma: lack of p48 expression in 5637 cells. *Cancer Res* **61**, 2261–2266.

Melcher, A., Parato, K., Rooney, C. M. & Bell, J. C. (2011). Thunder and lightning: immunotherapy and oncolytic viruses collide. *Mol Ther* **19**, 1008–1016.

Miller, J., Bidula, S. M., Jensen, T. M. & Reiss, C. S. (2009). Cytokine-modified VSV is attenuated for neural pathology, but is both highly immunogenic and oncolytic. *Int J Infereron Cytokine Mediator Res* **1**, 15–32.

Miller, J. M., Bidula, S. M., Jensen, T. M. & Reiss, C. S. (2010). Vesicular stomatitis virus modified with single chain IL-23 exhibits oncolytic activity against tumor cells *in vitro* and *in vivo*. *Int J Infereron Cytokine Mediator Res* **2010**, 63–72.

Mire, C. E. & Whitt, M. A. (2011). The protease-sensitive loop of the vesicular stomatitis virus matrix protein is involved in virus assembly and protein translation. *Virology* **416**, 16–25.

Moerdyk-Schauwecker, M., Destephanis, D., Hastie, E. & Grdzelishvili, V. Z. (2011). Detecting protein–protein interactions in vesicular stomatitis virus using a cytoplasmic yeast two hybrid system. *J Virol Methods* **173**, 203–212.

Moussavi, M., Fazli, L., Tearle, H., Guo, Y., Cox, M., Bell, J., Ong, C., Jia, W. & Rennie, P. S. (2010). Oncolysis of prostate cancers induced by vesicular stomatitis virus in PTEN knockout mice. *Cancer Res* **70**, 1367–1376.

Muik, A., Kneiske, I., Werbizki, M., Wilflingseder, D., Giroglou, T., Ebert, O., Kraft, A., Dietrich, U., Zimmer, G. & other authors (2011). Pseudotyping vesicular stomatitis virus with lymphocytic choriomeningitis virus glycoproteins enhances infectivity for glioma cells and minimizes neurotropism. *J Virol* **85**, 5679–5684.

Muik, A., Dold, C., Geiß, Y., Volk, A., Werbizki, M., Dietrich, U. & von Laer, D. (2012). Semireplication-competent vesicular stomatitis virus as a novel platform for oncolytic virotherapy. *J Mol Med (Berl)* **90**, 959–970.

Murphy, A. M., Besmer, D. M., Moerdyk-Schauwecker, M., Moestl, N., Ornelles, D. A., Mukherjee, P. & Grdzelishvili, V. Z. (2012). Vesicular stomatitis virus as an oncolytic agent against pancreatic ductal adenocarcinoma. *J Virol* **86**, 3073–3087.

Naik, S. & Russell, S. J. (2009). Engineering oncolytic viruses to exploit tumor specific defects in innate immune signaling pathways. *Expert Opin Biol Ther* **9**, 1163–1176.

Naik, S., Nace, R., Federspiel, M. J., Barber, G. N., Peng, K. W. & Russell, S. J. (2012). Curative one-shot systemic virotherapy in murine myeloma. *Leukemia* **26**, 1870–1878.

Nakashima, H., Kaur, B. & Chiocca, E. A. (2010). Directing systemic oncolytic viral delivery to tumors via carrier cells. *Cytokine Growth Factor Rev* **21**, 119–126.

Nguyên, T. L., Abdelbary, H., Arguello, M., Breitbach, C., Leveille, S., Diallo, J. S., Yasmeen, A., Bismar, T. A., Kirn, D. & other authors (2008). Chemical targeting of the innate antiviral response by histone deacetylase inhibitors renders refractory cancers sensitive to viral oncolysis. *Proc Natl Acad Sci U S A* **105**, 14981–14986.

Noser, J. A., Mael, A. A., Sakuma, R., Ohmine, S., Marcato, P., Lee, P. W. & Ikeda, Y. (2007). The RAS/Raf1/MEK/ERK signaling pathway facilitates VSV-mediated oncolysis: implication for the defective interferon response in cancer cells. *Mol Ther* **15**, 1531–1536.

Obuchi, M., Fernandez, M. & Barber, G. N. (2003). Development of recombinant vesicular stomatitis viruses that exploit defects in host defense to augment specific oncolytic activity. *J Virol* **77**, 8843–8856.

Oliere, S., Arguello, M., Mesplede, T., Tumilasci, V., Nakhaei, P., Stojdl, D., Sonenberg, N., Bell, J. & Hiscott, J. (2008). Vesicular stomatitis virus oncolysis of T lymphocytes requires cell cycle entry and translation initiation. *J Virol* **82**, 5735–5749.

Ozduman, K., Wollmann, G., Ahmadi, S. A. & van den Pol, A. N. (2009). Peripheral immunization blocks lethal actions of vesicular stomatitis virus within the brain. *J Virol* **83**, 11540–11549.

Pearson, A. S., Koch, P. E., Atkinson, N., Xiong, M., Finberg, R. W., Roth, J. A. & Fang, B. (1999). Factors limiting adenovirus-mediated gene transfer into human lung and pancreatic cancer cell lines. *Clin Cancer Res* **5**, 4208–4213.

Peng, K. W., Myers, R., Greenslade, A., Mader, E., Greiner, S., Federspiel, M. J., Dispenzieri, A. & Russell, S. J. (2012). Using clinically approved cyclophosphamide regimens to control the humoral immune response to oncolytic viruses. *Gene Ther* (in press). doi:10.1038/gt.2012.31

Petersen, J. M., Her, L. S., Varvel, V., Lund, E. & Dahlberg, J. E. (2000). The matrix protein of vesicular stomatitis virus inhibits nucleocytoplasmic transport when it is in the nucleus and associated with nuclear pore complexes. *Mol Cell Biol* **20**, 8590–8601.

Pfeffer, L. M., Wang, C., Constantinescu, S. N., Croze, E., Blatt, L. M., Albino, A. P. & Nanus, D. M. (1996). Human renal cancers resistant to IFN's antiproliferative action exhibit sensitivity to IFN's gene-inducing and antiviral actions. *J Urol* **156**, 1867–1871.

Plakhov, I. V., Arlund, E. E., Aoki, C. & Reiss, C. S. (1995). The earliest events in vesicular stomatitis virus infection of the murine olfactory neuroepithelium and entry of the central nervous system. *Virology* **209**, 257–262.

Porosnicu, M., Mian, A. & Barber, G. N. (2003). The oncolytic effect of recombinant vesicular stomatitis virus is enhanced by expression of the fusion cytosine deaminase/uracil phosphoribosyltransferase suicide gene. *Cancer Res* **63**, 8366–8376.

Power, A. T. & Bell, J. C. (2007). Cell-based delivery of oncolytic viruses: a new strategic alliance for a biological strike against cancer. *Mol Ther* **15**, 660–665.

Power, A. T. & Bell, J. C. (2008). Taming the Trojan horse: optimizing dynamic carrier cell/oncolytic virus systems for cancer biotherapy. *Gene Ther* **15**, 772–779.

Pulido, J., Kottke, T., Thompson, J., Galivo, F., Wongthida, P., Diaz, R. M., Rommelfanger, D., Ilett, E., Pease, L. & other authors (2012). Using virally expressed melanoma cDNA libraries to identify tumor-associated antigens that cure melanoma. *Nat Biotechnol* **30**, 337–343.

Qiao, J., Wang, H., Kottke, T., Diaz, R. M., Willmon, C., Hudacek, A., Thompson, J., Parato, K., Bell, J. & other authors (2008a). 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.

Qiao, J., Wang, H., Kottke, T., White, C., Twigger, K., Diaz, R. M., Thompson, J., Selby, P., de Bono, J. & other authors (2008b). Cyclophosphamide facilitates antitumor efficacy against subcutaneous tumors following intravenous delivery of reovirus. *Clin Cancer Res* **14**, 259–269.

Quiroz, E., Moreno, N., Peralta, P. H. & Tesh, R. B. (1988). A human case of encephalitis associated with vesicular stomatitis virus (Indiana serotype) infection. *Am J Trop Med Hyg* **39**, 312–314.

Reiss, C. S., Plakhov, I. V. & Komatsu, T. (1998). Viral replication in olfactory receptor neurons and entry into the olfactory bulb and brain. *Ann N Y Acad Sci* **855**, 751–761.

Rieder, M. & Conzelmann, K. K. (2009). Rhabdovirus evasion of the interferon system. *J Interferon Cytokine Res* **29**, 499–510.

Rose, N. F., Marx, P. A., Luckay, A., Nixon, D. F., Moretto, W. J., Donahoe, S. M., Montefiori, D., Roberts, A., Buonocore, L. & Rose, J. K. (2001). An effective AIDS vaccine based on live attenuated vesicular stomatitis virus recombinants. *Cell* **106**, 539–549.

Saloura, V., Wang, L. C., Fridlender, Z. G., Sun, J., Cheng, G., Kapoor, V., Sterman, D. H., Harty, R. N., Okumura, A. & other authors (2010). Evaluation of an attenuated vesicular stomatitis virus vector expressing interferon-beta for use in malignant pleural mesothelioma: heterogeneity in interferon responsiveness defines potential efficacy. *Hum Gene Ther* **21**, 51–64.

Samuel, S., Tumilasci, V. F., Oliere, S., Nguyên, T. L., Shamy, A., Bell, J. & Hiscott, J. (2010). VSV oncolysis in combination with the BCL-2 inhibitor obatoclax overcomes apoptosis resistance in chronic lymphocytic leukemia. *Mol Ther* **18**, 2094–2103.

Sarojini, S., Theofanis, T. & Reiss, C. S. (2011). Interferon-induced tetherin restricts vesicular stomatitis virus release in neurons. *DNA Cell Biol* **30**, 965–974.

Schache, P., Gürlevik, E., Strüver, N., Woller, N., Malek, N., Zender, L., Manns, M., Wirth, T., Kühnel, F. & Kubicka, S. (2009). VSV virotherapy improves chemotherapy by triggering apoptosis due to proteasomal degradation of Mcl-1. *Gene Ther* **16**, 849–861.

Schellekens, H., Smiers-de Vreede, E., de Reus, A. & Dijkema, R. (1984). Antiviral activity of interferon in rats and the effect of immune suppression. *J Gen Virol* **65**, 391–396.

Schnell, M. J., Buonocore, L., Kretzschmar, E., Johnson, E. & Rose, J. K. (1996). Foreign glycoproteins expressed from recombinant vesicular stomatitis viruses are incorporated efficiently into virus particles. *Proc Natl Acad Sci U S A* **93**, 11359–11365.

Sharif-Askari, E., Nakhaei, P., Oliere, S., Tumilasci, V., Hernandez, E., Wilkinson, P., Lin, R., Bell, J. & Hiscott, J. (2007). Bax-dependent mitochondrial membrane permeabilization enhances IRF3-mediated innate immune response during VSV infection. *Virology* **365**, 20–33.

Shi, Z., Cai, Z., Sanchez, A., Zhang, T., Wen, S., Wang, J., Yang, J., Fu, S. & Zhang, D. (2011). A novel Toll-like receptor that recognizes vesicular stomatitis virus. *J Biol Chem* **286**, 4517–4524.

Shin, E. J., Wanna, G. B., Choi, B., Aguila, D., III, Ebert, O., Genden, E. M. & Woo, S. L. (2007a). Interleukin-12 expression enhances vesicular stomatitis virus oncolytic therapy in murine squamous cell carcinoma. *Laryngoscope* **117**, 210–214.

Shin, E. J., Chang, J. I., Choi, B., Wanna, G., Ebert, O., Genden, E. M. & Woo, S. L. (2007b). Fusogenic vesicular stomatitis virus for the treatment of head and neck squamous carcinomas. *Otolaryngol Head Neck Surg* **136**, 811–817.

Shinozaki, K., Ebert, O., Suriawinata, A., Thung, S. N. & Woo, S. L. (2005). Prophylactic alpha interferon treatment increases the therapeutic index of oncolytic vesicular stomatitis virus virotherapy for advanced hepatocellular carcinoma in immune-competent rats. *J Virol* **79**, 13705–13713.

Staeheli, P. & Pavlovic, J. (1991). Inhibition of vesicular stomatitis virus mRNA synthesis by human MxA protein. *J Virol* **65**, 4498–4501.

Stanifer, M. L., Cureton, D. K. & Whelan, S. P. (2011). A recombinant vesicular stomatitis virus bearing a lethal mutation in the glycoprotein gene uncovers a second site suppressor that restores fusion. *J Virol* **85**, 8105–8115.

Stephenson, K. B., Barra, N. G., Davies, E., Ashkar, A. A. & Lichty, B. D. (2012). Expressing human interleukin-15 from oncolytic

vesicular stomatitis virus improves survival in a murine metastatic colon adenocarcinoma model through the enhancement of anti-tumor immunity. *Cancer Gene Ther* **19**, 238–246.

Stojdl, D. F., Lichty, B., Knowles, S., Marius, R., Atkins, H., Sonenberg, N. & Bell, J. C. (2000). Exploiting tumor-specific defects in the interferon pathway with a previously unknown oncolytic virus. *Nat Med* **6**, 821–825.

Stojdl, D. F., Lichty, B. D., tenOever, B. R., Paterson, J. M., Power, A. T., Knowles, S., Marius, R., Reynard, J., Poliquin, L. & other authors (2003). VSV strains with defects in their ability to shutdown innate immunity are potent systemic anti-cancer agents. *Cancer Cell* **4**, 263–275.

Sun, W. H., Pabon, C., Alsayed, Y., Huang, P. P., Jandeska, S., Uddin, S., Platanias, L. C. & Rosen, S. T. (1998). Interferon-α resistance in a cutaneous T-cell lymphoma cell line is associated with lack of STAT1 expression. *Blood* **91**, 570–576.

Tumilasci, V. F., Olière, S., Nguyên, T. L., Shamy, A., Bell, J. & Hiscott, J. (2008). Targeting the apoptotic pathway with BCL-2 inhibitors sensitizes primary chronic lymphocytic leukemia cells to vesicular stomatitis virus-induced oncolysis. *J Virol* **82**, 8487–8499.

van den Pol, A. N., Dalton, K. P. & Rose, J. K. (2002). Relative neurotropism of a recombinant rhabdovirus expressing a green fluorescent envelope glycoprotein. *J Virol* **76**, 1309–1327.

Wang, B. X., Rahbar, R. & Fish, E. N. (2011). Interferon: current status and future prospects in cancer therapy. *J Interferon Cytokine Res* **31**, 545–552.

Weidner, J. M., Jiang, D., Pan, X. B., Chang, J., Block, T. M. & Guo, J. T. (2010). Interferon-induced cell membrane proteins, IFITM3 and tetherin, inhibit vesicular stomatitis virus infection via distinct mechanisms. *J Virol* **84**, 12646–12657.

Whelan, S. P., Ball, L. A., Barr, J. N. & Wertz, G. T. (1995). Efficient recovery of infectious vesicular stomatitis virus entirely from cDNA clones. *Proc Natl Acad Sci U S A* **92**, 8388–8392.

Whitlow, Z. W., Connor, J. H. & Lyles, D. S. (2006). Preferential translation of vesicular stomatitis virus mRNAs is conferred by transcription from the viral genome. *J Virol* **80**, 11733–11742.

Whitlow, Z. W., Connor, J. H. & Lyles, D. S. (2008). New mRNAs are preferentially translated during vesicular stomatitis virus infection. *J Virol* **82**, 2286–2294.

Willmon, C., Diaz, R. M., Wongthida, P., Galivo, F., Kottke, T., Thompson, J., Albelda, S., Harrington, K., Melcher, A. & Vile, R. (2011). Vesicular stomatitis virus-induced immune suppressor cells generate antagonism between intratumoral oncolytic virus and cyclophosphamide. *Mol Ther* **19**, 140–149.

Wollmann, G., Tattersall, P. & van den Pol, A. N. (2005). Targeting human glioblastoma cells: comparison of nine viruses with oncolytic potential. *J Virol* **79**, 6005–6022.

Wollmann, G., Rogulin, V., Simon, I., Rose, J. K. & van den Pol, A. N. (2010). Some attenuated variants of vesicular stomatitis virus show enhanced oncolytic activity against human glioblastoma cells relative to normal brain cells. *J Virol* **84**, 1563–1573.

Wong, L. H., Krauer, K. G., Hatzinisiriou, I., Estcourt, M. J., Hersey, P., Tam, N. D., Edmondson, S., Devenish, R. J. & Ralph, S. J. (1997). Interferon-resistant human melanoma cells are deficient in ISGF3 components, STAT1, STAT2, and p48-ISGF3γ. *J Biol Chem* **272**, 28779–28785.

Wongthida, P., Diaz, R. M., Galivo, F., Kottke, T., Thompson, J., Pulido, J., Pavelko, K., Pease, L., Melcher, A. & Vile, R. (2010). Type III IFN interleukin-28 mediates the antitumor efficacy of oncolytic virus VSV in immune-competent mouse models of cancer. *Cancer Res* **70**, 4539–4549.

Wongthida, P., Diaz, R. M., Galivo, F., Kottke, T., Thompson, J., Melcher, A. & Vile, R. (2011a). VSV oncolytic virotherapy in the B16 model depends upon intact MyD88 signaling. *Mol Ther* **19**, 150–158.

Wongthida, P., Diaz, R. M., Pulido, C., Rommelfanger, D., Galivo, F., Kaluza, K., Kottke, T., Thompson, J., Melcher, A. & Vile, R. (2011b). Activating systemic T-cell immunity against self tumor antigens to support oncolytic virotherapy with vesicular stomatitis virus. *Hum Gene Ther* **22**, 1343–1353.

Wu, L., Huang, T. G., Meseck, M., Altomonte, J., Ebert, O., Shinozaki, K., García-Sastre, A., Fallon, J., Mandeli, J. & Woo, S. L. (2008). rVSV(MΔ51)-M3 is an effective and safe oncolytic virus for cancer therapy. *Hum Gene Ther* **19**, 635–647.

Yuan, H., Puckett, S. & Lyles, D. S. (2001). Inhibition of host transcription by vesicular stomatitis virus involves a novel mechanism that is independent of phosphorylation of TATA-binding protein (TBP) or association of TBP with TBP-associated factor subunits. *J Virol* **75**, 4453–4458.

Zhang, K. X., Matsui, Y., Hadaschik, B. A., Lee, C., Jia, W., Bell, J. C., Fazli, L., So, A. I. & Rennie, P. S. (2010). Down-regulation of type I interferon receptor sensitizes bladder cancer cells to vesicular stomatitis virus-induced cell death. *Int J Cancer* **127**, 830–838.