doi:10.1038/mt.2013.207

Panorama From the Oncolytic Virotherapy Summit

7th International Summit on Oncolytic Viral Therapeutics

Quebec, Canada, 15–18 June 2013

Jonathan G Pol[1], Monique Marguerie[2], Rozanne Arulanandam[2], John C Bell[2] and Brian D Lichty[1]

In June, the historical heart of Quebec City welcomed the 7th International Summit on Oncolytic Viral Therapeutics for an excellent opportunity to witness the growth of the field, in both size and maturity. Efforts are being made to better characterize interactions between oncolytic viruses (OVs) and host components, both inside and outside the tumor bed, with their relative contribution to the overall therapeutic efficacy. New approaches in improving OV delivery, tumor selectivity, spreading, and killing continue to flourish. Clinical evaluations, ongoing and upcoming, have never been this numerous. The present review draws on some of the unpublished and recently published findings reported during the meeting.

Oncolytic virotherapy appears to be a promising contender for the treatment of neoplasms that fail to respond to approved therapies. Encouraging responses with minimal side effects have already been reported against cancers considered to be incurable, such as glioblastoma multiforme (GBM). With a 15-month median survival and a 5-year survival rate of <10% under standard care, it is the most aggressive primary brain malignancy. Several OVs have demonstrated a tolerable safety profile in phase I trials against GBM. At this summit, related efficacy data were unveiled. It was reported that 52% of the 24 patients who received a single intratumoral injection of the adenovirus (Ad) DNX-2401 as first-line therapy showed stabilization or partial or complete regression of their disease. Six patients are still alive, one reaching 4 years posttreatment (Frank Tufaro, DNAtrix). Clinical activity and benefits have also been reported in GBM patients treated with the replicating lentivirus Toca511 (in combination with the prodrug TocaFC) (Doug Jolly, Tocagen) or with the poliovirus PVS-RIPO (Matthias Gromeier, Duke University). Both viruses were administered locally, as a first-line treatment or as a second-line therapy in combination with resection, respectively. Results are very promising with many complete responses so far. Impressively, three of the seven patients who received the PVS-RIPO so far have already displayed complete response.

Three viral oncolytics have reached phases IIB–III of clinical evaluation, giving hope for a first OV approval in Western countries: the modified vaccinia virus (VACV) Pexa-vec/JX-594 (Caroline Breitbach, Jennerex), the wild-type reovirus Reolysin, and the engineered herpes simplex virus (HSV) T-VEC.[1] Results from the phase III OPTiM trial, involving T-VEC for the treatment of unresected stages IIIb/c and IV melanoma, were presented. T-VEC, an oncolytic HSV-1 expressing granulocyte–macrophage colony-stimulating factor (GM-CSF), was administered into primary or nodal lesions, while the control arm received subcutaneous injections of GM-CSF. The primary end point was met, with a durable response (objective response lasting 6 months or more) observed in 16.3% of patients treated with T-VEC vs. 2.1% in the GM-CSF cohort. The difference was even more striking when only stage IIIb/c patients were considered: 33% vs. 0%, respectively. Several secondary end points were also met, including overall response (26.4% vs. 5.7%). Overall survival analysis was not finalized but showed a trend in favor of T-VEC.

Because of tumor evasion and metastasis, efficient treatment of advanced-stage neoplasms will probably require systemic delivery of the oncolytic agent. Through this route, OVs must overcome numerous barriers to reach the tumor, such as antibody neutralization, macrophage capture, and off-target tissue adsorption. Two years ago, Pexa-Vec/JX-594 first demonstrated antitumor activity following a single intravenous injection in 12 of 22 patients with advanced treatment-refractory solid tumors.[2] Ever since, several OVs—including Reolysin, the Ad chimera ColoAd1, the VACV GL-ONC1, and the Seneca valley virus NTX-010—have been reported as being suitable for safe blood infusion in phase I human trials. Their therapeutic efficacy is currently under clinical investigation. The list is rapidly extending as additional candidates are translating into the clinic (e.g., measles virus MV-NIS, parvovirus H-1 ParvOryx, rhabdoviruses VSV-hIFN-β, and Maraba MG1-hMAGE-A3).

Efficacy of oncolytic virotherapy relies not only on viral oncolysis of infected cells but also on the induction of antitumor immunity. Immune mediators contributing to OV-induced antitumor immunity and their dynamics of action are subject to particular attention. Treatment with oncolytic reovirus typically induces the secretion of proinflammatory cytokines and the recruitment of antigen-presenting cells, natural killer (NK) cells, and T lymphocytes within the tumor environment.[3,4] Additionally, a recent study described concomitant inhibition of the infiltration and protumor effects of myeloid-derived suppressor cells and regulatory T cells.[5] In a murine melanoma model, reovirus-mediated priming of an antitumor cytotoxic response was independent of both virus replication and oncolysis.[6] A similar

[1]McMaster Immunology Research Center, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada; [2]Ottawa Hospital Research Institute, Centre for Innovative Cancer Research, Ottawa, Ontario, Canada

Correspondence: Brian D Lichty, Department of Pathology and Molecular Medicine, MDCL 5023, McMaster University, 1280 Main Street West, Hamilton, Ontario L8S 4K1, Canada. E-mail: lichtyb@mcmaster.ca

observation has recently been reported for the attenuated Maraba virus MG1 in the same model. Indeed, administration of MG1 resulted in a replication-independent, rapid, and intense activation of NK cells (Jiqing Zhang, Ottawa). For at least these two RNA viruses, the adjuvant role of virus-associated molecular patterns determined therapeutic efficacy. Such properties may be of particular interest in a perioperative setting. Indeed, the immunosuppression that follows surgery is associated with high risk of metastatic cancer recurrence.[7] It is speculated that activation of immune cells such as NK cells by replication-incompetent viruses has the potential to prevent tumor recurrence.[8]

The adaptive antitumor immune response is commonly generated within a few weeks after OV administration, but a clinical impact of immunotherapy may not become apparent in short-term follow-up. Intriguingly, an ongoing human clinical trial involving the engineered poliovirus PVS-RIPO for the treatment of high-grade glioma appears to fit this emerging paradigm (Matthias Gromeier, Duke University)—tumor shrinkage was delayed to several months after local infusion of PVS-RIPO. Biopsy samples illustrated massive cancer cell necrosis and infiltration of macrophages and lymphocytes. Involvement of OV oncolysis at that time point was excluded, as viral clearance was achieved within 2 weeks postinjection because of a strong humoral response. Mechanisms in understanding these results will probably require better characterization of the interactions among poliovirus, infected brain tumor cells, and the immune system.

OVs have been armed over the years with immunomodulatory factors to enhance their immunotherapeutic ability. Regardless of the protein expressed from the virus, such strategies allow for tumor-localized delivery at high local concentrations while avoiding toxicity that potentially arises from systemic administration. Transgenes encoding cytokines such as GM-CSF or interleukin-12 (IL-12) have been inserted into various OV backbones. One research group recently found that expressing IL-12 from an oncolytic HSV increased interferon-γ (IFN-γ) release, inhibited angiogenesis, and reduced the number of immunosuppressive regulatory T cells within the tumor.[9]

Expression of trastuzumab, a monoclonal antibody targeting HER2, from an oncolytic Ad resulted in improved efficacy in HER2-positive cancers (Paula Savola, Helsinki). New studies are investigating the ability of measles virus (MV) armed with CTLA4 or PD-L1 antibodies to prolong survival (Guy Ungerechts, Heidelberg). Other research groups have armed VACV with TRAIL (Rinat Maksyutov, Koltsovo) or CD40L (Karolina Autio and Suvi Parvainen, Helsinki) to induce apoptosis and promote T-cell expansion, respectively. The presence of the VACV-soluble type I IFN-neutralizing protein B18R within the tumor environment has previously been shown to enhance HSV and vesicular stomatitis virus (VSV) antitumor activity.[10–12] Recently, B18R has been expressed from a new immunomodulatory platform, the nonpathogenic commensal bacterium *Escherichia coli*. Tumor "preconditioning" with B18R expressing *E. coli* followed by treatment with VSV led to enhanced OV infection and greater therapeutic efficacy (Michelle Cronin, Cork).

Overexpression of tumor-associated antigens (TAAs) from OV genomes has also been exploited to generate potent tumor-specific T-cell responses. To extend the strategy to different types of tumors, corresponding immunogenic TAAs will need to be identified. The TAA dopachrome tautomerase has been targeted for OV-mediated murine melanoma immunotherapy.[13] Dopachrome tautomerase is currently being investigated for the treatment of glioma in murine models together with the glioma-associated antigens EphrinRA2 and ED-B. Oncolytic VSV[13] and Maraba virus both display potent vaccine booster ability. In macaques, a prime-boost strategy involving an oncolytic Maraba MG1 vaccine to boost Ad-primed response against the human tumor antigen MAGE-A3 generated remarkably strong MAGE-A3–specific responses (Jonathan Pol, McMaster University). This approach will be translated into patients with advanced MAGE-A3$^+$ tumors in the coming months. In addition to targeting one highly immunogenic TAA, it has been proposed that targeting multiple TAAs, even if individual responses are weak, could have therapeutic benefit by generating a significant overall antitumor response. As an illustration, mice with brain melanoma displayed prolonged survival following treatment with a VSV–complementary DNA (VSV-cDNA) library derived from human melanoma cells.[14] Such an approach applied early enough could prevent emergence of antigen-loss variants and thus recurrence. Indeed, the antigenic profile of recurring tumors differed from that of primary tumors. As a consequence, recurring tumors could only be cured by treating them with VSV-cDNA libraries derived from recurring tumors but not from parental tumors. Another interesting finding resided in the possibility of treating recurring melanoma tumors with cDNA libraries from recurring prostate cancer and vice versa. A comparative analysis led to the identification of shared TAAs specific to tumor recurrence (Richard Vile, Mayo Clinic).

Recent progress in the field of oncolytic virotherapy has been focused on circumventing the tumor microenvironment barriers that constitute the dense extracellular matrix (ECM), the tortuous tumor vasculature, and various immune-cell populations to improve delivery, dissemination, and efficacy. In a different light, it was revealed that the natural ability of oncolytic VACV to infect resident tumor endothelial cells and induce vascular shutdown is enhanced with the maturity of the developing vasculature.[15] The alternative strategy adopted by this OV, and potentially others, to "bite the hand that feeds" may be due to elevated levels of vascular endothelial growth factor, a key driver of vessel maturation recently found to have a direct immunosuppressive effect on the tumor vascular bed (Andrea McCart, Toronto). The tumor-associated stroma was recognized as another compartment conducive to OV infection due to an elegant interplay of growth factor signaling between activated, cancer-associated fibroblasts and tumor cells (Carolina Ilko, Ottawa).

These data combined, revealing the inherent immunosuppressive, OV-sensitizing effects of certain soluble growth factors within the tumor microenvironment, prompt us to consider whether this compartment can instead be exploited to reap the full benefits of OV therapy. Besides, it is known that components of the ECM can be natural targets for VACV and/or myxomavirus (MYXV) attachment.[16] On the other hand, in the context of oncolytic Ad, treatment of tumors with ECM-digesting enzymes, such as hyaluronidase, can improve spread and efficacy.[17] Researchers have extended these findings to show that in resistant models of pancreatic adenocarcinoma, an oncolytic Ad expressing hyaluronidase (VCN-01), had potent antitumor cytotoxicity when combined with gemcitabine, a first-line chemotherapy

for this cancer type (Miriam Bazam-Peregrino, Barcelona). Another group is also observing improved antitumor efficacy upon combining hyaluronidase with two additional proteolytic enzymes—chondroitinase and proteinase K—in conditionally replicating Ad (Alison Tedcastle, Oxford). Switching gears to brain cancers, microglial cells, which contribute up to one-third of the tumor mass, were shown to proliferate after administration of HSV and to interfere with HSV oncolysis. RAMBO virus, an oncolytic HSV expressing the antiangiogenic fragment vasculostatin (Vstat120), was able to reduce microglia activation and inflammation by suppressing its expression or secretion of tumor necrosis factor-α, as observed both *in vitro* and in mice with intracranial tumors (Balveen Kaur, Ohio State University).

Only a few OVs currently researched are naturally tumor-tropic in terms of their receptor specificity (e.g., Sindbis virus, coxsackievirus A21).[18–20] Research in the field is progressing toward identifying and exploiting inherent OV cell entry pathways and systematically modulating receptor specificity to increase tumor selectivity. VSV displays pantropic infectivity mediated by its coat protein (G), and its cellular port of entry was very recently identified as the low-density-lipoprotein receptor.[21] New studies revealed that the inherent neurotoxicity of VSV can be markedly reduced when G is replaced with MV H and F glycoproteins without affecting the rapid spread of the virus.[22] In a similar vein, others have replaced VSV-G with the lymphocytic choriomeningitis virus glycoprotein to render it nonneurotoxic.[23] With the additional use of mesenchymal stem cells as carrier cells, dampened antibody-mediated neutralization has been observed while achieving significant efficacy in orthotopic models of ovarian cancer (Catherine Dold, Innsbruck). Complement-mediated neutralization of VSV-G, which is far greater in human than mouse serum, can also be circumvented through pseudotyping with the less immunogenic Maraba virus G protein (Steven Russell, Mayo Clinic). In the case of Ads, chimeras have been generated by exchanging the Ad5 hexon with hexons from different serotypes to reduce Kupffer cell capture in the liver and lower antibody neutralization (e.g., Ad3, 6, 11, or 48). However, the field has started to move from a linear to a more systematic synthesis of novel OVs to accelerate identification of tumor-selective candidates. One group developed a platform of about 60 different Ads in which about 60% of the genome can be replaced (Clodagh O'Shea, Salk Institute). The aim will be to craft therapies specific for different tumor types by swapping coat proteins from Ads that naturally target the organ of interest.

Another strategy commonly applied to improve OV tumor selectivity consists of placing OV genome replication under tumor-restricted transcriptional regulators. This can be achieved by incorporating microRNA response elements (MREs) specific to microRNAs (miRs) overexpressed in normal tissues or downregulated in tumor cells. This approach is of particular interest when OVs are delivered systemically so as to limit off-target replication. MREs complementary to miRs specifically expressed in liver, muscle, and macrophages were recently introduced in the genome of Sindbis virus (Gray Kueberuwa, Oxford). Intravenous injection of the engineered virus appeared safe and demonstrated antitumor activity in a murine metastatic melanoma model.

A few studies are currently being conducted with OVs targeting cancer stem cells (CSCs). Whereas CSCs' origin and nature remain controversial, their dual role as tumor-initiating cells and as a source of treatment-resistant cells is recognized.[24] CD133 is commonly thought to be a CSC marker. An oncolytic MV has been retargeted to $CD133^+$ cells by modifying the hemagglutinin (H) protein so that it displays a CD133-specific single-chain antibody fragment.[25] Another team has restricted HSV replication to CSCs by placing ICP4 expression under control of the CD133 promoter. In mice treated with this engineered HSV, whole-tumor shrinkage was observed despite targeting only $CD133^+$ cells, representing as little as 30% of the tumor mass (Kaoru Terai). With regard to these promising preclinical results, this targeted approach deserves further investigation and testing.

A constant focus for the field is to look for ways to predict how a certain tumor is going to react to a given OV and whether therapeutic success will be achieved. Human pancreatic ductal adenocarcinoma cells showed high heterogeneity in their permissiveness to VSV. Resistant cells demonstrated retention of type I IFN responses, constitutive high-level expression of IFN-stimulated genes, and defects in apoptosis (Valery Grdzelishvili, North Carolina). Introduction of JAK/STAT inhibitors resulted in increased viral infection, replication, and oncolysis.[26] Measles vaccine strain binds to both CD46 and signaling lymphocytic activation molecule (SLAM), often coexpressed in hematological malignancies.[27] Using NIS-expressing MV vectors able to recognize either receptor and high-resolution imaging, investigators found that SLAM-dependent entry led to more efficient tumor regression and survival in a mouse model of mantle cell lymphoma.[28] SLAM may be further exploited as a positive predictive marker for therapy. In the case of HSV-1, short hairpin RNA knockdown of histone deacetylase 6 (HDAC6) reduced degradation of HSV virions in glioma cell lines. This resulted in an increased HSV-1 oncolysis on primary glioma samples (Hiroshi Nakashima, Brigham & Women's Hospital).

Although treatment with short hairpin RNA may not be realistic *in vivo*, drug-targeted inhibition of HDAC6 may improve HSV efficacy against GBM. Seneca valley virus (SVV) is an oncolytic picornavirus with inherent tumor selectivity. By screening a large number of small-cell lung cancer lines, a research group found that the messenger RNA ratio of late neurogenic transcription factor NEUROD1 to early neurogenic transcription factor ASCL1 correlated with the degree of cell permissivity to SVV infection.[29] This ratio could be used as a predictive marker in the clinic for oncolytic SVV efficacy. Recently it was shown that Mnk kinase activity potentiated the efficacy of PVS-RIPO treatment (Michael Brown, Duke University). Interestingly, Mnk kinases are active in a variety of cancers and have been implicated in the development of chemoresistance.[30,31] This finding is encouraging for patients whose cancer failed to respond to chemotherapy and positions OV therapy as a promising complement to standard care strategies.

OV therapy also appeared as a precious add-on to standard-of-care stem cell transplantation (ASCT) for patients with diseases such as multiple myeloma (MM). Associated common concerns with ASCT are the development of graft-vs.-host disease (GvHD) and reintroduction of MM cells from the bone marrow samples. Therefore, one group has pretreated stem cell samples *ex vivo* with MYXV and successfully eliminated malignant cells before transplantation.[32–35]

Moreover, they have shown that infecting bone marrow with MYXV resulted in the infection of activated but not naive human T cells, abrogating GvHD post-ASCT.[36]

When standard monotherapies fail because of tumor resistance, combination strategies offer great promise. Targeting multiple cellular or molecular checkpoints that are vital for malignant cells could be one key to success. In this regard, synergistic efficacy between oncolytic virotherapy and chemotherapy has been reported in preclinical studies. With proper dosing, as well as route and timing of administration, all classes of anticancer drugs tested demonstrated positive interactions with OVs. As an example, combining the adenovirus Ad5/3-D24-GMCSF with the alkylating agents ifosfamide and the anthracycline doxorubicin significantly prolonged survival of immunocompetent rodents with sarcoma, in comparison to single agents (Mikko Siurula, Helsinki). Therapeutic improvement was associated with enhanced cell-death immunogenicity. In a hamster model of pancreatic adenocarcinoma, intratumoral administration of the oncolytic Ad VCN-01 allowed chemosensitization to the nucleoside analogue gemcitabine. In addition, a delayed antiviral humoral response was observed (Miriam Bazan-Peregrino, Barcelona). Both phenomena contributed to increased tumor growth control. Chemical agents affecting the microtubule network (e.g., docetaxel) also benefited from combination with OVs such as VSVΔM51 (Jean-Simon Diallo, Ottawa) or the coxsackievirus A21 (Min Yuan Quah, Newcastle) in ovarian and lung murine tumor models. The topoisomerase inhibitors irinotecan and mitoxanthrone improved antitumor efficacy when administered in combination with an oncolytic VACV (Kathryn Ottoline-Perry, Toronto) or HSV-1 (Samuel Workenhe, McMaster University) in colorectal peritoneal carcinomatosis and a mammary murine tumor model, respectively. Finally, a number of tyrosine and serine/threonine kinase inhibitors have displayed synergy with OVs in rodent tumor models. These include erlotinib with HSV-1 in the context of pancreatic cancer (Kazuo Yamamura, Nagoya), sorafenib in combination with reovirus in hepatitis C virus–associated hepatomas (Adel Jabar, Leeds), and mammalian target of rapamycin inhibitors together with HSV-1 in breast cancer models (Tommy Alain, McGill University).

Mutual benefits have also been reported between radiation therapy and OV therapy. For example, whole-brain irradiation has been shown to disrupt the blood–brain barrier. Therefore, one group demonstrated that head irradiation before systemic administration of reovirus enhanced virus delivery to intracranial tumors in mice (Liz Ilett, Leeds). This strategy will be evaluated in patients with recurrent high-grade brain tumors. Another approach consists of combining administration of ^{131}I with OV-mediated expression of the sodium-iodide symporter NIS. Enhanced antitumor activity has been confirmed with several OV backbones, including MV, VSV, and VACV. Furthermore, NIS-driven uptake of the radionuclide inside the tumor bed not only increases cytotoxicity but also permits noninvasive tracking of OV infection sites.[37–39]

The ability to track OVs *in vivo* through reporter protein expression enables identification of permissive sites and visualization of the extent of viral replication. Such information may be helpful to design delivery optimization strategies or readministration schedules. With computer assistance, it has even been possible to make a three-dimensional model of the inoculum. However, several factors can alter the quality of the signal, such as rapid virus clearance, high background in the tissue hosting the tumor (e.g., liver), or nonspecific uptake in surrounding organs. For example, using positron emission tomography imaging, monitoring of VSV-NIS delivery to murine liver tumors was not feasible because of iodine uptake in the stomach. Depending on the tissue targeted, replacement of the reporter protein and/or the associated radionuclide may solve the issue. In the present case, VSV imaging was achieved by switching from [^{131}I]/NIS to the enhanced HSV-1 thymidine kinase sr39tk–[^{18}F]FHBG tracker system (Kim Bentrup, Munich).

For practical reasons (and despite their immunogenicity), green fluorescent protein and luciferase remain the most common reporter proteins used for visualizing OV-infected tissues. However, tissue components strongly interact with visible light, quickly annealing the signal emitted by such bioluminescent reporters as the number of cell layers increases. Cold substitutes are emerging, such as the fluorogen-activating proteins (FAPs). FAPs can be fused to proteins of interest and are poorly immunogenic. Coupled to fluorogens that emit in near infrared, they already demonstrated sensitive *in vivo* imaging in preclinical models (Steve Thorne, Pittsburgh). The use of reporter-expressing OVs has also been proposed as a cancer diagnostic tool. A demonstration of this application was performed on blood samples treated with Ad TRAD-F35–142–3pT. This modified Ad possesses: (i) an Ad35 fiber (F35) that binds to the leukocyte receptor CD46, (ii) an E1 gene expressed under the control of the telomerase promoter that is constitutively active in malignant cells (TRAD), and (iii) green fluorescent protein expression silenced in the presence of miR-142–3p—a miR expressed only in normal blood cells (142–3pT). This approach showed impressive sensitivity, being able to detect a single malignant cell out of millions of normal ones (Fuminori Sakurai, Osaka). By reducing the number of false-negative test results, this application of OVs would allow for earlier treatment, increasing the likelihood of success.

As numerous neoplasms fail to respond to standards of care, there is an urgent need for efficient alternatives. There is hope that some OVs will join the arsenal of approved anticancer therapies in the near future. As mentioned, there has been a remarkable expansion of our knowledge of OV biology inside and outside the tumor bed in the past few years. Subsequent development of new-generation OVs and combination strategies should contribute to expand the list of promising candidates significantly. However, definition of a "promising candidate" must be qualified, as this term has tended to be used to refer to an OV that displayed therapeutic efficacy in preclinical studies. Unfortunately, OV preclinical evaluation is mostly performed in the same imperfect rodent models that conducted several first-generation "good candidates" to clinical failure owing to a lack of therapeutic benefits.

Because evaluation in human patients is a long and costly process, a more reliable preclinical selection is required. In this regard, a method of *ex vivo* short-term culture of patient tumor biopsy samples with maintained high viability has been developed. This methodology, based on staining assays, allows evaluation of oncolytic activity, with characterization of the type of tumor cell death induced, as well as identification of the various immune components present inside the tumor section (e.g., cell subsets,

cytokines). Such a model is obviously still imperfect because it cannot address certain issues, such as OV delivery. However, it could be useful to quickly compare the oncolytic activity of distinct viruses on the same patient tumor sample. Preclinical comparison of multiple OVs in one particular tumor model should help to identify which OVs best treat a defined type of cancer and thus help prioritize its translation into the clinic for a particular neoplasm. Such comparative studies have recently been performed and are likely to multiply in the future. Because it is not always possible for a research group to achieve such comparisons, a good alternative could reside in defining consensus tumor models and relative parameters for each type of neoplasm that then would enable researchers to evaluate their own OV and compare it within the community.

REFERENCES

1. Sheridan, C (2013). Amgen announces oncolytic virus shrinks tumors. *Nat Biotechnol* **31**: 471–472.
2. Breitbach, CJ, Burke, J, Jonker, D, Stephenson, J, Haas, AR, Chow, LQ *et al.* (2011). Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. *Nature* **477**: 99–102.
3. Gujar, SA, Marcato, P, Pan, D and Lee, PW (2010). Reovirus virotherapy overrides tumor antigen presentation evasion and promotes protective antitumor immunity. *Mol Cancer Ther* **9**: 2924–2933.
4. Gujar, SA, Pan, D, Marcato, P, Garant, KA and Lee, PW (2011). Oncolytic virus-initiated protective immunity against prostate cancer. *Mol Ther* **19**: 797–804.
5. Gujar, S, Dielschneider, R, Clements, D, Helson, E, Shmulevitz, M, Marcato, P *et al.* (2013). Multifaceted therapeutic targeting of ovarian peritoneal carcinomatosis through virus-induced immunomodulation. *Mol Ther* **21**: 338–347.
6. Prestwich, RJ, Ilett, EJ, Errington, F, Diaz, RM, Steele, LP, Kottke, T *et al.* (2009). Immune-mediated antitumor activity of reovirus is required for therapy and is independent of direct viral oncolysis and replication. *Clin Cancer Res* **15**: 4374–4381.
7. Tai, LH, de Souza, CT, Bélanger, S, Ly, L, Alkayyal, AA, Zhang, J *et al.* (2013). Preventing postoperative metastatic disease by inhibiting surgery-induced dysfunction in natural killer cells. *Cancer Res* **73**: 97–107.
8. Tai, LH, Zhang, J, Scott, KJ, Tanese de Souza, C, Alkayyal, AA, Ananth, AA *et al.* (2013). Perioperative influenza vaccination reduces post-operative metastatic disease by reversing surgery-induced dysfunction in natural killer cells. *Clin Cancer Res*; e-pub ahead of print 30 August 2013.
9. Zhang, W, Fulci, G, Wakimoto, H, Cheema, TA, Buhrman, JS, Jeyaretna, DS *et al.* (2013). Combination of oncolytic herpes simplex viruses armed with angiostatin and IL-12 enhances antitumor efficacy in human glioblastoma models. *Neoplasia* **15**: 591–599.
10. Fu, X, Rivera, A, Tao, L and Zhang, X (2012). Incorporation of the B18R gene of vaccinia virus into an oncolytic herpes simplex virus improves antitumor activity. *Mol Ther* **20**: 1871–1881.
11. Paglino, JC and van den Pol, AN (2011). Vesicular stomatitis virus has extensive oncolytic activity against human sarcomas: rare resistance is overcome by blocking interferon pathways. *J Virol* **85**: 9346–9358.
12. Le Boeuf, F, Diallo, JS, McCart, JA, Thorne, S, Falls, T, Stanford, M *et al.* (2010). Synergistic interaction between oncolytic viruses augments tumor killing. *Mol Ther* **18**: 888–895.
13. Bridle, BW, Stephenson, KB, Boudreau, JE, Koshy, S, Kazdhan, N, Pullenayegum, E *et al.* (2010). Potentiating cancer immunotherapy using an oncolytic virus. *Mol Ther* **18**: 1430–1439.
14. Pulido, J, Kottke, T, Thompson, J, Galivo, F, Wongthida, P, Diaz, RM *et al.* (2012). Using virally expressed melanoma cDNA libraries to identify tumor-associated antigens that cure melanoma. *Nat Biotechnol* **30**: 337–343.
15. Ottolino-Perry, K, Tang N, Head R, Ng, C, Arulanandam, R, Angarita, FA *et al.* (2013). Tumor vascularization is critical for oncolytic vaccinia virus treatment of peritoneal carcinomatosis. *Int J Cancer*; e-pub ahead of print 29 August 2013.
16. Chan, WM, Bartee, EC, Moreb, JS, Dower, K, Connor, JH and McFadden, G (2013). Myxoma and vaccinia viruses bind differentially to human leukocytes. *J Virol* **87**: 4445–4460.
17. Ganesh, S, Gonzalez-Edick, M, Gibbons, D, Van Roey, M and Jooss, K (2008). Intratumoral coadministration of hyaluronidase enzyme and oncolytic adenoviruses enhances virus potency in metastatic tumor models. *Clin Cancer Res* **14**: 3933–3941.
18. Wang, KS, Kuhn, RJ, Strauss, EG, Ou, S and Strauss, JH (1992). High-affinity laminin receptor is a receptor for Sindbis virus in mammalian cells. *J Virol* **66**: 4992–5001.
19. Berno, V, Porrini, D, Castiglioni, F, Campiglio, M, Casalini, P, Pupa, SM *et al.* (2005). The 67 kDa laminin receptor increases tumor aggressiveness by remodeling laminin-1. *Endocr Relat Cancer* **12**: 393–406.
20. Shafren, DR, Au, GG, Nguyen, T, Newcombe, NG, Haley, ES, Beagley, L *et al.* (2004). Systemic therapy of malignant human melanoma tumors by a common cold-producing enterovirus, coxsackievirus a21. *Clin Cancer Res*;**10**(Pt 1): 53–60.
21. Finkelshtein, D, Werman, A, Novick, D, Barak, S and Rubinstein, M (2013). LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. *Proc Natl Acad Sci USA* **110**: 7306–7311.
22. Ayala-Breton, C, Barber, GN, Russell, SJ and Peng, KW (2012). Retargeting vesicular stomatitis virus using measles virus envelope glycoproteins. *Hum Gene Ther* **23**: 484–491.
23. Muik, A, Kneiske, I, Werbizki, M, Wilflingseder, D, Giroglou, T, Ebert, O *et al.* (2011). Pseudotyping vesicular stomatitis virus with lymphocytic choriomeningitis virus glycoproteins enhances infectivity for glioma cells and minimizes neurotropism. *J Virol* **85**: 5679–5684.
24. Frame, FM and Maitland, NJ (2011). Cancer stem cells, models of study and implications of therapy resistance mechanisms. *Adv Exp Med Biol* **720**: 105–118.
25. Bach, P, Abel, T, Hoffmann, C, Gal, Z, Braun, G, Voelker, I *et al.* (2013). Specific elimination of $CD133^+$ tumor cells with targeted oncolytic measles virus. *Cancer Res* **73**: 865–874.
26. Moerdyk-Schauwecker, M, Shah, NR, Murphy, AM, Hastie, E, Mukherjee, P and Grdzelishvili, VZ (2013). Resistance of pancreatic cancer cells to oncolytic vesicular stomatitis virus: role of type I interferon signaling. *Virology* **436**: 221–234.
27. Noyce, RS, Bondre, DG, Ha, MN, Lin, LT, Sisson, G, Tsao, MS *et al.* (2011). Tumor cell marker PVRL4 (nectin 4) is an epithelial cell receptor for measles virus. *PLoS Pathog* **7**: e1002240.
28. Miest, TS, Frenzke, M and Cattaneo, R (2013). Measles virus entry through the signaling lymphocyte activation molecule governs efficacy of mantle cell lymphoma radiovirotherapy. *Mol Ther*; e-pub ahead of print 5 August 2013.
29. Poirier, JT, Dobromilskaya, I, Moriarty, WF, Peacock, CD, Hann, CL and Rudin, CM (2013). Selective tropism of Seneca valley virus for variant subtype small cell lung cancer. *J Natl Cancer Inst* **105**: 1059–1065.
30. Altman, JK, Glaser, H, Sassano, A, Joshi, S, Ueda, T, Watanabe-Fukunaga, R *et al.* (2010). Negative regulatory effects of Mnk kinases in the generation of chemotherapy-induced antileukemic responses. *Mol Pharmacol* **78**: 778–784.
31. Zhao, Y, Shen, S, Guo, J, Chen, H, Greenblatt, DY, Kleeff, J *et al.* (2006). Mitogen-activated protein kinases and chemoresistance in pancreatic cancer cells. *J Surg Res* **136**: 325–335.
32. Bartee, E, Chan, WS, Moreb, JS, Cogle, CR and McFadden, G (2012). Selective purging of human multiple myeloma cells from autologous stem cell transplant grafts using oncolytic myxoma virus. *Biol Blood Marrow Transplant* **18**: 1540–1551.
33. Rahman, MM, Madlambayan, GJ, Cogle, CR and McFadden, G (2010). Oncolytic viral purging of leukemic hematopoietic stem and progenitor cells with myxoma virus. *Cytokine Growth Factor Rev* **21**: 169–175.
34. Kim, M, Madlambayan, GJ, Rahman, MM, Smallwood, SE, Meacham, AM, Hosaka, K *et al.* (2009). Myxoma virus targets primary human leukemic stem and progenitor cells while sparing normal hematopoietic stem and progenitor cells. *Leukemia* **23**: 2313–2317.
35. Madlambayan, GJ, Bartee, E, Kim, M, Rahman, MM, Meacham, A, Scott, EW *et al.* (2012). Acute myeloid leukemia targeting by myxoma virus *in vivo* depends on cell binding but not permissiveness to infection *in vitro*. *Leuk Res* **36**: 619–624.
36. Bartee, E, Meacham, A, Wise, E, Cogle, CR and McFadden, G (2012). Virotherapy using myxoma virus prevents lethal graft-versus-host disease following xeno-transplantation with primary human hematopoietic stem cells. *PLoS One* **7**: e43298.
37. Belin, LJ, Ady, JW, Lewis, C, Marano, D, Gholami, S, Mojica, K *et al.* (2013). An oncolytic vaccinia virus expressing the human sodium iodine symporter prolongs survival and facilitates SPECT/CT imaging in an orthotopic model of malignant pleural mesothelioma. *Surgery* **154**: 486–495.
38. Li, H, Nakashima, H, Decklever, TD, Nace, RA and Russell, SJ (2013). HSV-NIS, an oncolytic herpes simplex virus type 1 encoding human sodium iodide symporter for preclinical prostate cancer radiovirotherapy. *Cancer Gene Ther* **20**: 478–485.
39. Li, H, Peng, KW and Russell, SJ (2012). Oncolytic measles virus encoding thyroidal sodium iodide symporter for squamous cell cancer of the head and neck radiovirotherapy. *Hum Gene Ther* **23**: 295–301.