Immunovirotherapy Based on Recombinant Vesicular Stomatitis Virus: Where Are We?

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Vesicular stomatitis virus (VSV), a negative-strand RNA virus of the Vesiculovirus genus, has demonstrated encouraging anti-neoplastic activity across multiple human cancer types. VSV is particularly attractive as an oncolytic agent because of its broad tropism, fast replication kinetics, and amenability to genetic manipulations. Furthermore, VSV-induced oncolysis can elicit a potent antitumor cytotoxic T-cell response to viral proteins and tumor-associated antigens, resulting in a long-lasting antitumor effect. Because of this multifaceted immunomodulatory property, VSV was investigated extensively as an immunovirotherapy alone or combined with other anticancer modalities, such as immune checkpoint blockade. Despite these recent opportunities to delineate synergistic and additive antitumor effects with existing anticancer therapies, FDA approval for the use of oncolytic VSV in humans has not yet been granted. This mini-review discusses factors that have prompted the use of VSV as an immunovirotherapy in human cancers and provides insights into future perspectives and research areas to improve VSV-based oncotherapy.

Keywords: vesicular stomatitis virus, oncolytic virus, genetically modified virus, cancer therapy, immunotherapy

INTRODUCTION

Vesicular stomatitis virus (VSV) is non-pathogenic, enveloped, negative-strand RNA Rhabdovirus with potent vaccine and oncolytic potential (1–6). VSV can infect nearly all cell types but cannot initiate a productive infection in healthy cells due to an antiviral response mediated by type-I interferons (IFNs).¹¹ However, defects in IFN signaling often coincide with tumorigenesis (7, 8). Thus, VSV is capable of infecting and selectively destroying cancer cells with minimal damage to normal cells, making it an attractive therapeutic agent. Furthermore, VSV is particularly appealing as an oncolytic vector (OV) and vaccine agent due to low anti-VSV immunity in the general population (pre-existing immunity to OVs limits their intratumoral spread) and fast replication kinetics in cancer cells (9).

The nonsegmented VSV genome is typical of viruses in the Vesiculovirus genus. The approximately 11-kb genome encodes five structural proteins, including the nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and the large polymerase protein (L) (10–12). VSV genome encapsidation is facilitated by specific interactions between the
N and P proteins (12). The N protein is essential for suppressing the transcription-termination signal during viral replication (13). The P and L proteins function as co-factors of the RNA-dependent RNA polymerase (RdRp); they exert indispensable and versatile functions, including regulating the initiation, elongation, and encapsidation of viral RNAs (14, 15). Specifically, the RdRp binds the encapsidated viral genome at the leader region, then sequentially transcribes each gene (16).

The M protein is involved in virus assembly and budding (17). It has also been shown to inhibit innate antiviral responses and alter host transcriptional machinery, ultimately coercing tumor cells to undergo apoptosis (18). Therefore, viruses with mutant M proteins were developed to restrict viral replication to tumor cells with an altered type-I IFN signaling axis (19).

The G protein forms spike-like structures on the viral particle surface and plays an essential role in the initial stages of infection (20). In the Indiana strain, the G protein was shown to mediate viral attachment via interaction with the low-density lipoprotein receptor (LDL-R) and its family members (21). The VSV-G protein is capable of binding LDL-R via the cysteine-rich LDL-R domains CR2 and CR3, resulting in clathrin-dependent endocytosis and intracellular uptake of the VSV genome (22, 23). However, several other reports showed that isogenic pairs of wild type LDL-R and LDL-R–knockout (-/-) (24, 25) cell lines can be infected efficiently by VSV and other closely related family members, highlighting the potential role of other surface proteins or cell-intrinsic mechanisms in viral entry (22). The broad cellular tropism of VSV is attributable to its G protein; thus, it is often replaced with entry proteins from other viruses to improve the safety and selectivity of VSV-based oncolytic vectors (26).

Numerous studies have shed light on the fundamental mechanisms of VSV–host cell interactions, the dynamics of viral gene expression, and the pathogenesis of viral infection (26). These findings have greatly expanded our understanding of the biology and structure of VSV, informing the design of recombinant VSV (rVSV) vectors with improved safety and selectivity towards a broad range of cancer cells. Despite this progress, VSV-based immunovirotherapy has not lived up to its expectations, and FDA approval has not yet been granted. Thus, we eagerly awaited the published outcomes of various completed, recruiting, or active cancer treatment trials (clinicaltrials.gov) in the United States using rVSV as an immunovirotherapy platform. In the meantime, it is equally important to review the past and recent developments of VSV vectors in cancer therapy to derive insights into ways to refine and improve the antitumor efficacy of such vectors.

**DEVELOPMENT OF VSV AS A VACCINE PLATFORM**

The use of reverse genetics enabled researchers to rescue infectious negative-strand RNA viruses from viral genomic cDNAs, leading to significant improvement in our ability to manipulate and study RNA viruses for vaccine development and cancer therapy applications (27, 28). Owing to their ability to prime robust humoral and cellular immunity, VSV vectors have also been used as vaccine agents to generate protective immunity against infections with highly lethal human viruses, including Ebola, HIV, Marburg, Lassa, Zika, and SARS-COV-2 viruses (29–40). Other vaccine candidates using attenuated VSV vectors were evaluated in preclinical models to prevent illnesses due to influenza (41), hepatitis B virus (42), different strains of coronavirus causing respiratory diseases (43, 44), *Yersinia pestis* (bubonic plague) (45), respiratory syncytial virus (RSV) (46), herpes simplex virus 2 (HSV2) (47), Dengue virus (48), Chikungunya virus (49), Nipah virus (50), and human papillomavirus (HPV) (51).

However, despite abundant evidence of therapeutic efficacy, only one VSV-based vaccine is FDA approved (52). This is mainly due to concerns related to the promiscuous nature of the VSV entry glycoprotein (VSV-G), allowing the virus to infect neurons and induce encephalitis in mice (7, 53, 54). Thereby, questions were raised regarding the potential neurotoxicity of VSV in humans following systemic delivery, limiting its widespread clinical deployment as a vaccine vector in humans. To address this critical concern, several groups have engineered VSV vectors with mutated G proteins or harboring G proteins from other non-neurotropic viruses to ablate interactions with LDL-R, which is highly expressed in neurons (49, 55–57). Many VSV-derived vectors that have progressed to preclinical and clinical testing as vaccine agents also displayed lytic potency and elicited a strong, durable cytotoxic T-cell response in permissive tumors (58, 59). While most oncolytic viruses such as VSV induce robust tumor-cell killing *in vivo*, recent clinical reports strongly suggest that, *in vitro*, OVIs turn “cold” tumors into hot tumors (60), as discussed below.

**RATIONALE FOR DEVELOPING VSV AS AN ONCOLYTIC AGENT**

Wild type VSV causes mild disease in cattle, horses, and swine, causing vesicles (blisters) around the mouth (61). The few reported cases of human VSV infections were limited to agricultural and laboratory workers, characterized by an incubation period between 8 to 48 hours, with mild flu-like symptoms (26, 62, 63). VSV is a highly cytopathic virus that infects nearly all cell types, but its infection and replication are enhanced in tumor cells with a defective IFN signaling pathway (64). This feature makes it an ideal oncolytic virus therapy agent. In addition, VSV has a fast kinetic cycle, does not integrate into the host genome (65), and is a potent inducer of apoptosis in the infected cancer cells—a critical feature of viral therapeutics (66, 67). The VSV genome is also relatively small and can accommodate the insertion of one or more foreign, functional genes (68). Importantly, VSV has demonstrated anticancer activity in a vast array of cancer cells, including osteosarcoma (69), cervical cancer (70), breast cancer (71), melanoma (72), hepatocellular carcinoma (73), pancreatic cancer (57), and glioblastoma (74).
Although VSV-based oncolytic vectors have shown efficacy in mouse models and led to multiple human studies (Table 1), barriers to FDA approval and clinical application remain. These barriers include variability in the efficiency by which VSV kills cancer cells, even among cancers from the same tissue of origin, and reports of VSV-induced encephalitis in laboratory animals and humans. Furthermore, the heterogeneous therapeutic responses in solid cancers (e.g., pancreatic cancer) are attributed to factors such as a fibrotic and dense extracellular matrix, hypoxia, high interstitial tumor pressure, and low pH in the tumor microenvironment, limiting viral spread and immunogenic cell death in response to oncolytic therapy.

The fact that VSV is cleared rapidly by the immune system (e.g., via neutralizing antibodies and complement molecules) has further dampened enthusiasm for this vector (7, 53, 54). These obstacles have severely limited the anticancer efficacy of VSV—particularly the inability to administer multiple doses to achieve tumor shrinkage and, most importantly, the inability to bypass the immune system and infect neoplastic cells.

A decade ago, the first VSV trial in human cancers was posted to clinicaltrials.gov; however, no trial results have been disseminated (Table 1). This lack of information raises pertinent questions about whether we can achieve the desired therapeutic outcomes with current VSV vectors. Although it is not clear when these results will be available, the eagerly awaited outcomes of these studies will undoubtedly guide the future development of VSV-based oncotherapy for clinical translation. Nonetheless, several groups developing and testing oncolytic vesiculoviruses have proposed ingenious viral engineering strategies (65, 89, 90) to improve patient safety and vector potency.

### STRATEGIES DESIGNED TO ENHANCE VSV ONCOLYTIC ABILITY

Each genetic modification approach attempted to improve the antitumor activity of rVSV and its safety profile. Although rapid progress in nanotechnologies has enabled the improvement of delivery, pharmacokinetics, bioavailability in the tumor of rVSV vectors, many of these studies are in the early preclinical stages (91). Thus, this section will focus on vector engineering strategies to enhance safety, immunogenic apoptosis, and immune clearance.

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**Table 1 | Reported VSV-based vaccine and cancer treatment clinical trials (http://clinicaltrial.gov).**

| Vector         | Purpose                        | Clinical Trial                                                                 | Identification     | Phase  | Status            |
|----------------|--------------------------------|-------------------------------------------------------------------------------|--------------------|--------|-------------------|
| VSV-IFNβ-NIS   | Cancer treatment               | Systemic VSV-IFNβ-NIS and Pembrolizumab in Refractory NSCLC and NEC          | NCT03647163        | I/II   | Recruiting**      |
| VSV-IFNβ      | Cancer treatment               | Administration of VSV-IFNβ-NIS Monotherapy and in Combination With Avelumab in | NCT02923466        | I      | Active not recruiting** |
| VSV-IFNβTyrp1  | Cancer treatment               | Modified Virus VSV-IFNβTyrp1 in Treating Patients With Stage III-IV Melanoma | NCT03865212        | I      | Recruiting**      |
| VSV-GP         | Cancer treatment               | Phase 1b Study to Evaluate ATP128, VSV-GP128 and Bl 754091 in Patients With  | NCT04064445        | I      | Recruiting**      |
| VSV-IFNβ-NIS   | Cancer treatment               | Intratumoral Administration of Recombinant VSV in Patients With Refractory Solid | NCT02923466        | I      | Recruiting**      |
| VSV-IFNβ-NIS   | Cancer treatment               | VSV-IFNβ-NIS With or Without Ruxolitinib Phosphate in Treating Patients With | NCT03120624        | I      | Recruiting**      |
| VSV-IFNβ-NIS   | Cancer treatment               | VSV-IFNβ-NIS in Treating Relapsed or Refractory Multiple Myeloma, Acute | NCT03017820        | I      | Recruiting**      |
| rSVαβ-G-ZEBOV-GP| Vaccine                       | Placebo-Controlled, Dose Response, Safety and Immunogenicity Study of Vesicular | NCT02314923        | I      | Completed (75)    |
| VSVαβ-G-ZEBOV  | Vaccine                        | Safety and Immunogenicity of Prime-Boost Vesicular Stomatitis Virus (VSV) Ebola | NCT02280408        | I      | Completed (76)    |
| VSV-EOBOV      | Vaccine                        | Immune Durability After VSV-EOBOV Vaccination                                | NCT02933931        | I      | Completed (77)    |
| VSV-ZEBOV      | Vaccine                        | VSV-ZEBOV Geneva Vaccine Trial                                               | NCT02287480        | II     | Completed (78, 79) |
| VSV-Indiana (one type of VSV vector) | Vaccine | Evaluating the Safety of and Immune Response to the VSV-Indiana HIV Vaccine in Healthy, HIV-Uninfected Adults | NCT01438606        | I      | Completed (93)    |
| rSVαβ-G-ZEBOV-GP| Vaccine                       | Phase I Trial of an Ebola Virus Vaccine (rSVαβ-G-ZEBOV-GP)                    | NCT02283099        | I      | Completed (62, 78, 81) |
| rSVαβ-G-ZEBOV  | Vaccine                        | STRIVE (Sierra Leone Trial to Introduce a Vaccine Against Ebola)              | NCT02376753        | III    | Completed (82-84) |
| rSV-HIVgag     | Vaccine                        | Vaccine Treatment for Ebola Virus in Healthy Adults (V920-001)                | NCT02269423        | I      | Completed (76)    |
| rSVN4CT1-EBOVGP1| Vaccine                       | Therapeutic Vaccine for HIV                                                  | NCT01859325        | I      | Completed (85)    |
|                |                                |                                                                              | NCT02718469        | I      | Completed (86)    |

**No publications, (published).
Optimizing rVSV Design to Enhance Immunogenic Apoptosis and Reduce Neurotoxicity

Studies have shown that mechanistically, VSV-induced oncolysis results in the release of a series of molecules, including tumor-associated antigens (TAA$s$), pathogen-associated molecular patterns (PAMP$s$), and damage-associated molecular patterns (DAMP$s$) (92–94). The build-up of TAAs in the tumor microenvironment elicits the recruitment and activation of tumor-specific cytotoxic (CD8+) T cells (92–94). PAMP$s$ and DAMP$s$ promote infiltration of neutrophils, natural killer (NK) cells, and dendritic cells (DC) into tumor sites. This simultaneous activation of innate and adaptive immunity is essential for priming a robust and durable antitumor immune response. These earlier works on the ability of rVSV to induce immunogenic cell death or apoptosis have influenced the field in many ways. For example, Wu and colleagues demonstrated that rVSV expressing murine gammaherpesvirus M3 protein (rVSV[M-Δ51]-M3) induced enhanced tumor necrosis and prolonged survival substantially in an animal model compared to parental VSV (19). M3 is a secreted chemokine-binding protein that binds to a broad range of mammalian chemokines with high affinity (19). In addition to decreasing neurotoxicity, delivery of exogenous M3 enhanced rVSV (M-Δ51)-M3 oncolytic activity by curtailing the activation of host innate immunity against oncolytic VSV in the tumor. Similar to the mutant M protein vectors, VSVs harboring mutations in G (95), P, or L proteins (96) with improved oncoselectivity and potency were also developed and evaluated preclinically. Although rVSVs with mutated viral proteins have enabled some safety improvements, as evidenced by no apparent neurovirulence and no visible pathogenesis in animal models, these vectors are often highly attenuated (i.e., reduce viral replication capacity) and thus are not appropriate for clinical deployment. Therefore, Russell and colleagues have adopted a different approach by incorporating microRNA target sequences (e.g., for miR-125) into the viral genome to decrease the ability of the virus to replicate in neurons (97).

Generation of rVSV Vectors With Improved Immunostimulatory Activity

Multiple studies have also attempted to increase VSV safety and oncolytic properties by inserting into the viral genome genes encoding immunostimulatory proteins, chemoaottractant molecules, or effectors that induce apoptosis in tumor cells (26, 59). One example is a VSV vector carrying the full-length p53 gene. Tumor protein p53 is a potent activator of apoptosis, and it is the most frequently mutated gene in human tumors (98). Indeed, the reactivation of p53 has been shown to potentiate antitumor immune activity (99). In an animal model, the vector VSV-M(Δ51)-p53, expressing p53, improved antitumor activity and enhanced CD49b+ NK and tumor-specific CD8+ T-cell responses (99, 100).

Immunostimulatory cytokines function in a synergistic or cascade fashion to modulate immune responses. Consequently, combining cytokines (101) with oncolytic viruses was seen as worth investigating for possible additive or synergistic long-term responses in clinical settings. Granulocyte–macrophage colony-stimulating factor (GM-CSF) is a potent immunostimulatory cytokine involved in the maturation and migration of macrophages and dendritic cells, which activate cytotoxic T cells (102). Hence, GM-CSF–expressing VSV (VSV-GM-CSF) vectors were developed, in which the transgene was inserted upstream of the VSV N gene or between the M and G genes (103–105). These vectors were attenuated and well-tolerated in vivo, and they triggered strong cellular and humoral antitumor immune responses (103–105). This work with VSV vectors expressing immunomodulatory cytokines demonstrated that tumor stage and type; immune mechanisms; and timing, dosage, and route of administration are crucial for obtaining the desired therapeutic effect with oncolytic viruses (106).

While IL-15 preferentially stimulates the proliferation of NK and memory CD8+ T cells and increases their antitumor activity (107), IL-12 functions as a “bridging” cytokine, providing an essential regulatory link between innate and adaptive immunity (108). Additionally, IL-23 has been shown to establish stable gene expression for activation of Th17 cells, but it is also crucial to activate innate immune cells, which are scattered across non-lymphoid organs (109). Thus, rVSV expressing IL-15, IL-12, or IL-23 (rVSV-IL-15, rVSV-IL-12, rVSV-IL-23) were generated and considerably improved synergistic antitumor efficacy compared to parental rVSV (110–112). Along the same line, IL-4 (113), thymidine kinase (113), IL-28 (114), Fms-like tyrosine kinase 3 ligand (115), and IFN-β (116) were expressed in VSV vectors, and their oncolytic activities have been documented across various cancer types. Based on encouraging preclinical studies, rVSV expressing human type-I IFN-β and a reporter known as sodium iodide symporter (VSV-IFNβ-NIS) has advanced through early to late phases of clinical testing (Table 1). Although VSV-based cytokine expression promotes superior oncolytic activity, it is essential to note that it can also potentiate viral clearance and impact the overall antitumor efficacy of the vectors.

Addressing Issues With Rapid Immune Clearance, Dense Stroma While Promoting Strong Apoptotic Activity

A plethora of viral engineering strategies has been proposed to enhance the oncolytic ability of VSV. Chimeric VSV displaying fusion (F) and hemagglutinin (H) proteins from Newcastle disease virus or measles virus was shown to abolish VSV-associated neurotoxicity and the effect of virus-neutralization antibody (NA$s$)s on the bioavailability of viral vectors through the formation of syncytia-like structures (7, 57). The molecular mechanisms by which wild-type VSV and recombinant rVSV vectors induce intrinsic, extrinsic, or endoplasmic reticulum stress-mediated apoptosis have been elucidated in numerous studies (64, 65). This has prompted several authors to employ vectors such as VSV-vCKBPs (117), VSV-UL141 (118), and rVSV–FAST (119), capable of exerting robust oncolytic activity while resisting rapid viral clearance. It is reasonable to speculate that many viruses in tumor sites could infect tumor cells and induce enough oncolysis to eradicate the tumors on their own.
However, virus-mediated oncolysis also provides conditions for priming antitumor immunity by activating tumor-specific cytotoxic T cells (120–131). The most critical aspect of rVSVs in cancer vaccines’ context is their ability to efficiently modulate anti-tumor immune responses. Consequently, current oncolytic viruses, such as rVSV and rVSV-derived vectors, may be applicable to cancer patients with functional immune systems. In addition, the tumor microenvironment is complex, characterized by a sophisticated interplay between tumor cells and many components, including immune cells, extracellular matrix, fibroblasts, and various molecules, such as enzymes. This harsh environment is a known barrier to therapy, including immunotherapy and oncolytic virus therapy.15 It is now evident that rVSV vectors alone have limited long-term antitumor activity and may achieve only a partially curative effect. Combining rVSVs with other therapies, including radiotherapy, T-cell therapies, and immune checkpoint blockades, could serve to unleash their full oncolytic potential (132–138). We enthusiastically await the results of VSV clinical trials and expect novel combinations of VSV vectors with other cancer treatments to emerge in the coming years.

CHALLENGES AND FUTURE DIRECTIONS

Despite evidence of the therapeutic benefits of rVSV-based oncotherapy, most investigations remain in the preclinical stage due to numerous challenges. These limitations include neurotoxicity (e.g., due to the promiscuous nature of the VSV entry glycoprotein [VSV-G]), rapid clearance by the immune system (e.g., via pre-existing VSV antibodies), and hepatotoxicity (e.g., viral interaction with Kupffer cells) (139, 140). Several strategies were proposed to address these obstacles, including modifying the VSV-G protein to achieve optimal therapeutic benefits (7, 53, 54, 141). Moreover, the lack of biomarkers that could be used to select patients who would benefit from oncolytic virus therapy represents a significant hurdle that we must seriously consider in future designs and clinical testing. Despite these challenges, the therapeutic potential of rVSV in cancer treatments is indisputable; indeed, VSV-IFN-β has advanced into late-phase clinical testing, renewing enthusiasm for oncolytic VSV.

DISCUSSION

Early research into the biology of VSV, including genomic structure, immunogenic properties, and pan-tropism, paved the way for developing this promising oncolytic agent and vaccine vector. However, the seamless clinical translation of VSV oncotherapy still faces significant challenges, and VSV has not yet been utilized to its full potential as an oncolytic vector. As the mechanisms of tumor resistance to molecular therapy continue to be elucidated, we fully expect new VSV vectors with enhanced potency and selectivity to be evaluated soon.

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YZ and BN contributed to the study concept, drafting, and critical revision of the manuscript. Editorial support was provided by the Science Communication Group at the University of Arkansas for Medical Sciences. All authors approved the final, submitted version of the manuscript.

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14. Morin B, Rahme AH, Whelan SP. Mechanism of RNA Synthesis Initiation by the Vesicular Stomatitis Virus Polymerase. *EMBO J* (2012) 31(5):1320–9. doi: 10.1038/emboj.2011.483

15. Billel PA, Rowe JE, Fitch WM, Nichol ST. Phosphoprotein and Nucleocapsid Protein Evolution of Vesicular Stomatitis Virus New Jersey. *J Virol* (1990) 64(6):2498–504. doi: 10.1128/JVI.64.6.2498-2504.1990

16. Whelan SP, Wertz GW. The 5′ Terminal Trailer Region of Vesicular Stomatitis Virus Contains a Position-Dependent Cis-Acting Signal for Assembly of RNA Into Infectious Particles. *J Virol* (1999) 73(1):307–15. doi: 10.1128/JVI.73.1.307-315.1999

17. Raux H, Obiang L, Richard N, Harper F, Blondel D, Gaudin Y, Albertini AA. Crystal Structure of Mokola Virus Glycoprotein in its Post-Fusion Conformation. *Proc Natl Acad Sci U S A* (2015) 112(16):5221–6. doi: 10.1073/pnas.1415883112

18. Ahmed M, McKenzie MO, Puckett S, Hojnacki M, Poliquin L, Lyles DS. Assembly of RNA Into Infectious Particles. *J Virol* (2009) 83(1):440–53. doi: 10.1128/JVI.01864-08

19. Wu L, Huang TG, Meseck M, Altomonte J, Ebert O, Shinozaki K, et al. rVSV M Delta 51-M3 is an Effective and Safe Oncolytic Virus for Cancer Therapy. *Hum Gene Ther* (2012) 23(3):221–9. doi: 10.1089/hum.2011.0527

20. Belot L, Ouldali M, Roche S, Legrand P, Gaudin Y, Albertini AA. Crystal Structure of Mokola Virus Glycoprotein. *J Virol* (2012) 86(10):5253–61. doi: 10.1128/JVI.05346-11

21. Kim MN, Braden JS, Rentrop KH, Marcondes-Silva MM, Wang K, Zheng X, et al. Recombinant Vesicular Stomatitis Virus as Vaccine Vectors. *Vaccine* (2012) 30(18):2435–41. doi: 10.1016/j.vaccine.2012.02.013

22. Zgoba K, Mathew S, Babb JS, Lu X, Guo M, Moretto WJ, et al. Single-Dose Administration of an Attenuated Oral Vesicular Stomatitis Virus Vaccine Protects against Respiratory Challenge with a Highly Pathogenic Strain. *Infect Dis Ther* (2016) 5(2):337–50. doi: 10.1007/s13088-016-0121-4

23. Thompson EJ, McQuiston JH. The Current State of the Laboratory Diagnosis of Zoonotic and Other Notifiable Viral Respiratory Diseases. *J Virol* (1995) 69(11):7583–92. doi: 10.1128/JVI.69.11.7583-7592.1995

24. Leibowitz MS, Alspaugh JA, Longnecker KC, Hladik W, McDaniel B, Gaskill AE, et al. A Vesicular Stomatitis Virus-Based Vaccine Induces Humoral Response and Protects Mice Against Lethal Infection. *J Virol* (2005) 79(10):5853–61. doi: 10.1128/JVI.02125-04

25. Pearl TB, Lamb RA. Replication Competent or Attenuated, Nonpropagating Vesicular Stomatitis Viruses. *Vaccine* (2005) 23(13):1647–53. doi: 10.1016/j.vaccine.2004.08.022

26. Kanki PJ, Pongsuphapet W, Huber M, Vyas S, Butka J, Haddox J, et al. Optimization of Single Dose VSV-Based COVID-19 Vaccination in Nonhuman Primates. *PloS One* (2014) 9(4):e94355. doi: 10.1371/journal.pone.0094355

27. Robins K, Chakrabartty A, Kim JS, Desai S, Narendran S, Syed AS, et al. Intranasal Vaccination With a Dual Serotype of Recombinant Vesicular Stomatitis Virus Carrying the Genetically Modified Zika Virus E Protein Gene. *J Gen Virol* (2021) 102(4). doi: 10.1099/jgv.0.001588

28. Rose NF, Marx PA, Luckay N, Nixon DF, Moretto WJ, Donahoe SM, et al. An Effective AIDs Vaccine Based on Live Attenuated Vesicular Stomatitis Virus Reombinants. *Cell* (2001) 106(5):539–49. doi: 10.1016/S0092-8674(01)00482-2

29. Munis AM, Bentley EM, Takeuchi Y. A Tool With Many Applications: Factors and Functions Involved in Vesicular Stomatitis Virus Entry. *Virology* (2001) 282(2):207–16. doi: 10.1006/viro.2001.0534

30. Finkelshtein D, Werman A, Novick D, Barak S, Rubinstein M. LDL Receptor and its Family Members Serve as the Cellular Receptors for Vesicular Stomatitis Virus. *Proc Natl Acad Sci U S A* (2013) 110(18):7306–11. doi: 10.1073/pnas.1214411110

31. Nikolic J, Belot L, Raux H, Legrand P, Gaudin Y, Albertini AA. Structural Basis for the Recognition of LDL-Receptor Family Members by VSV Glycoprotein. *Nat Commun* (2018) 9(1):1029. doi: 10.1038/s41467-018-03432-4

32. Johanssodt HK, Mancini R, Kartenbeck J, Amato L, Helenius A. Host Cell Factors and Functions Involved in Vesicular Stomatitis Virus Entry. *J Virol* (2009) 83(1):440–53. doi: 10.1128/JVI.01864-08

33. Esletcher P, Konopka T, Santoro F, Chen D, Gapp BV, Kralovich R, et al. Megabase-Scale Deletion Using CRISPR/Cas9 to Generate a Fully Haploid Human Cell Line. *Genome Res* (2014) 24(12):2059–65. doi: 10.1101/ gr.177220.114

34. Kravtsova-Ivantysv Y, Shomer I, Cohen-Kaplan V, Snijder B, Superti-Furga G, Gonen H, et al. KPC1-Mediated Ubiquitination and Proteosomal Processing of NF-Kappab1 P105 to P50 Restricts Tumor Growth. *Cell* (2015) 161(2):333–47. doi: 10.1016/j.cell.2015.03.001

35. Marziani A, Feldmann F, Geisbich T, RWF, TF, Feldmann H, Safronetz D. Vesicular Stomatitis Viruses-Based Vaccines Against Lassa and Ebola Viruses. *Emerg Infect Dis* (2015) 21(2):305–7. doi: 10.3202/eid2102.141649

36. Jalabononen-Ronen Y, Tamir H, Melamed S, Politi B, Shifman O, Achdout H, et al. A Single Dose of Recombinant VSV-G-Spike Vaccine Provides Protection Against SARS-CoV-2 Challenge. *Nat Commun* (2020) 11(1):6402. doi: 10.1038/s41467-020-2238-7

37. Choi JA, Wu K, Kim GN, Saeedian N, Sohn SH, Park G, et al. Induction of Protective Immune Responses Against a Lethal Zika Virus Challenge Post-Vaccination With a Dual Serotype of Recombinant Vesicular Stomatitis Virus Carrying the Genetically Modified Zika Virus E Protein Gene. *J Gen Virol* (2021) 102(4). doi: 10.1099/jgv.0.001588
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With Vesicular Stomatitis Virus: Actions in the Brain. J Virol (2017) 91(6): e02154–16. doi: 10.1128/JVI.02154-16

Dahlke C, Zinser ME, Kasonta R, Lunemann S, Rechtein A, et al. Detectable Vesicular Stomatitis Virus (VSV)-Specific Humoral and Cellular Immune Responses Following VSV-Ebola Virus Vaccination in Humans. J Infect Dis (2017) 219(4):556–61. doi: 10.1093/infdis/jiy565

Bourgeois-Daigneault MC, Roy DG, Falls T, Twumasi-Boateng K, St-Germain LE, Marguierie M, et al. Oncolytic Vesicular Stomatitis Virus Expressing Interferon-Gamma has Enhanced Therapeutic Activity. Mol Ther Oncolytics (2016) 3:16001. doi: 10.1038/mtoto.2016.1

Dold C, Rodriguez Urbiola C, Wollmann G, Egerer L, Muik A, Bellmann L, et al. Application of Interferon Modulators to Overcome Partial Resistance of Human Ovarian Cancers to VSV-GP Oncolytic Viral Therapy. Mol Oncol (2021) 16:3021. doi: 10.1038/s41986-021-01259-z

Kim J, Castellano JM, Jiang H, Basak JM, Parsadanian M, Pham V, et al. Overexpression of Low-Density Lipoprotein Receptor in the Brain Markedly Inhibits Amyloid Deposition and Increases Extracellular A Beta Clearance. Neuron (2009) 64(5):632–44. doi: 10.1016/j.neuron.2009.11.013

Rozo-Lopez P, Drolet BS, Londono-Renteria B. Vesicular Stomatitis Virus Variants Selectively Infect and Kill Human Melanomas But Not Normal Melanocytes. J Virol (2013) 87(12):6644–59. doi: 10.1128/JVI.03311-12

Ebert O, Shinozaki K, Huang TG, Savontaus MJ, Garcia-Sastre A, Woo SL. Oncolytic Vesicular Stomatitis Virus Treatment for Treatment of Orthotopic Hepatocellular Carcinoma in Immune-Competent Rats. Cancer Res (2003) 63(13):3605–11.

Cary ZD, Willingham MC, Lyes DS. 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 (2011) 85(12):5708–17. doi: 10.1128/JVI.02393-10

Heppner DGf., Kemp TL, Martin BK, Ramsey WJ, Nichols R, Dasen EJ, et al. Safety and Immunogenicity of the rVSVG-ZEBOV-GP Ebola Virus Vaccine Candidate in Healthy Adults: A Phase 1b Randomised, Multicentre, Double-Blind, Placebo-Controlled, Dose-Response Study. Lancet Infect Dis (2017) 17(8):854–66. doi: 10.1016/S1473-3099(17)30313-4

Regules JA, Beigel JH, Paolino KM, Voell J, Castellano RA, Hu Z, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine. N Engl J Med (2017) 376(4):330–41. doi: 10.1056/NEJMoa1414216

Huttner A, Agnandji ST, Combescre C, Fernandes JF, Bache EB, Kabwende L, et al. Determinants of Antibody Persistence Across Doses and Continents After Single-dose rVSV-ZEBOV Vaccination for Ebola Virus Disease: An Observational Cohort Study. Lancet Infect Dis (2018) 18(7):738–48. doi: 10.1016/S1473-3099(18)30165-8

Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 Trials of rSVSV Ebola Vaccine in Africa and Europe. N Engl J Med (2016) 374(17):1647–60. doi: 10.1056/NEJMoa1502924

Huttner A, Dayer JA, Verly S, Combescre C, Auderset F, Desmeules J, et al. The Effect of Dose on the Safety and Immunogenicity of the VSV Ebola Candidate Vaccine: A Randomised Double-Blind, Placebo-Controlled Phase 1/2 Trial. Lancet Infect Dis (2015) 15(10):1156–66. doi: 10.1016/S1473-3099(15)00154-1

Fuchs JD, Frank I, Elizaga ML, Allen M, Frahm N, Kochar N, et al. First-In-Human Evaluation of the Safety and Immunogenicity of a Recombinant Vesicular Stomatitis Virus Human Immunodeficiency Virus-1 Gag Vaccine (HVTN 090). Open Forum Infect Dis (2015) 2(3):ofv082. doi: 10.1093/ofid/ofv082

Dahlke C, Zinser ME, Lunemann S, Krahl C, Zinser ME, Biedenkopf N, et al. Dose-Dependent T-Cell Dynamics and Cytokine Cascade Following rSVSV-ZEBOV Immunisation. EBioMedicine (2017) 19:167–18. doi: 10.1016/j.ebiom.2017.03.045

Jarrett OD, Seward JF, Fomah AE, Lindblad R, Jalahh MI, EI-Khorazzy J, et al. Monitoring Serious Adverse Events in the Sierra Leone Trial to Introduce a Vaccine Against Ebola. J Infect Dis (2018) 217(suppl_1):S24–32. doi: 10.1093/infdis/jiy042

Samai M, Seward JF, Goldstein ST, Mahon BE, Lisk DR, Widdowson MA, et al. The Sierra Leone Trial to Introduce a Vaccine Against Ebola: An Evaluation of rSVSV-ZEBOV-GP Vaccine Tolerability and Safety During the West Africa Ebola Outbreak. J Infect Dis (2018) 217(suppl_1):S6–S15. doi: 10.1093/infdis/jiy020

Legardy-Williams JK, Carter RJ, Goldstein ST, Jarrett OD, Sefzer E, Fomah AE, et al. Pregnancy Outcomes Among Women Receiving Reswdelta-ZEBOV-GP Ebola Vaccine During the Sierra Leone Trial to Introduce a Vaccine Against Ebola. Emerg Infect Dis (2020) 26(3):541–58. doi: 10.3201/eid2603.191018
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85. Gianella S, Chaillon A, Chun TW, Sneller MC, Ignacio C, Vargas-Meneses MV, et al. HIV RNA Rebound in Seminal Plasma After Antiretroviral Treatment Interruption. J Virol (2020) 94(15):e00415–20. doi: 10.1128/JVI.00415-20
86. Clarke DK, Xu R, Matassov D, Latham TE, Ota-Seltik A, Gerardi CS, et al. Safety and Immunogenicity of a Highly Attenuated Rvvsnv41t–EBOVGP1 Ebola Virus Vaccine: A Randomised, Double-Blind, Placebo-Controlled, Phase 1 Clinical Trial. Lancet Infect Dis (2020) 20(4):e455–60. doi: 10.1016/S1473-3099(19)30614-0
87. Quiroz E, Moreno N, Peralta PH, Tesh RB. A Human Case of Encephalitis Due to Semireplication-Competent Vesicular Stomatitis Virus as a Novel Platform for Oncolytic Virotherapy. J Virol (2020) 10(13):57–62. doi: 10.1128/JVI.00415-20
88. Patterson WC, Mott LO, Jenney EW. A Study of Vesicular Stomatitis in Tumors. Cancer Res (2011) 71(23):10440–8. doi: 10.1158/0008-5472.CAN-11-2552
89. Howard F, Muthana M. Designer Nanocarriers for Navigating the Systemic Suppressor P53 is a Highly Attenuated, Potent Oncolytic Agent. Nature Nanotechnol (2006) 1(2):126–30. doi: 10.1038/nnano.2006.28
90. Pol JG, Atherton MJ, Bridle BW, Stephenson KB, Le Boeuf F, Hummel JL, et al. Senescence and Tumour Clearance is Triggered by P53 Restoration in Murine Liver Carcinomas. Cancer Res (2007) 67(1):38–46. doi: 10.1158/0008-5472.CAN-06-3057
91. Hamada M, Yura Y. Effective Delivery and Replication of Oncolytic Virus for Successful Treatment of Head and Neck Cancer. Int J Mol Sci (2020) 21(19):7073. doi: 10.3390/ijms21197073
92. Bartlett DL, Liu Z, Sathaiwa M, Ravindranathan R, Guo Z, He Y, et al. Oncolytic Viruses as Therapeutic Cancer Vaccines. Mol Cancer (2013) 12(1):103. doi: 10.1186/1475-2048-12-103
93. Dunsch CD, Zhou Q, Jayakar HR, Weimar JD, Robertson JH, Pfeffer LM, et al. Recombinant Vesicular Stomatitis Virus Vectors as Oncolytic Agents in the Treatment of High-Glioma in an Organotypic Brain Tissue Slice-Glioma Coculture Model. J Neurosurg (2004) 100(6):1049–59. doi: 10.3171/2004.6.1049
94. Muik A, Dold C, Geiss Y, Volk A, Werbizki M, Dietrich U, et al. Semi-replication-Competent Vesicular Stomatitis Virus as a Novel Platform for Oncolytic Virotreatment. J Mol Med (Berl) (2012) 90(8):959–70. doi: 10.1007/s00109-012-0883-6
95. Kelly EF, Nace R, Barber GN, Russell SJ. Attenuation of Vesicular Stomatitis Virus Encephalitis Through microRNA Targeting. J Virol (2010) 84(3):1550–62. doi: 10.1128/JVI.01788-09
96. Ozaki T, Nakagawa A. Role of P53 in Cell Death and Human Cancers. Cancers (Basel) (2011) 3(1):994–1013. doi: 10.3390/cancers3010094
97. Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and Tumour Cessation is Triggered by P53 Restoration in Murine Liver Carcinomas. Nature (2007) 445(7128):656–60. doi: 10.1038/nature05529
98. Heiber JF, Barber GN. Vesicular Stomatitis Virus Expressing Tumor Suppressor P53 is a Highly Attenuated, Potent Oncolytic Agent. J Virol (2011) 85(20):10440–50. doi: 10.1128/JVI.01540-11
99. Pearl TM, Markert JM, Cassidy KA, Gholme MG. Oncolytic Virus-Based Cytokine Expression to Improve Immune Activity in Brain and Solid Tumors. Mol Ther Oncolytics (2019) 13:14–21. doi: 10.1016/j.jmo.2019.03.001
100. Shi Y, Liu CH, Roberts AI, Das J, Xu G, Ren G, et al. Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and T-Cell Responses: What We do and Don’t Know. Cell Res (2006) 16(2):126–33. doi: 10.1038/sj.cr.7301017
101. Ramsburg E, Pocker C, Bozic S, Luboleski R, Rubik M, Malin A, et al. A Vesicular Stomatitis Virus Recombinant Expressing Granulocyte-Macrophage Colony-Stimulating Factor Induces Enhanced T-Cell Responses and is Highly Attenuated for Replication in Animals. J Virol (2005) 79(24):15043–53. doi: 10.1128/JVI.79.24.15043-15053.2005
102. Bergman I, Griffin JA, Gao Y, Whitaker-Dowling P. Treatment of Implantated Mammary Tumors With Recombinant Vesicular Stomatitis Virus Targeted to Her2/neu. Int J Cancer (2007) 121(2):425–30. doi: 10.1002/ijc.22680
103. Jenner A, Cassidy T, Belaidi K. In Silico Trials Predict That Combination Strategies for Enhancing Vesicular Stomatitis Oncolytic Virus are Determined by Tumor Aggressivity (Vol 9, E001387, 2021). J Immunother Cancer (2021) 9(10):e001387. doi: 10.1136/jitc-2020-001387corr1
104. LeBoeuf F, Gebremeskel S, McMullen N, He H, Greenshields AL, Hoskin DW, et al. Reovirus FAST Protein Enhances Vesicular Stomatitis Virus Determined by Tumor Aggressivity (Vol 9, E001387, 2021). J Immunother Cancer (2021) 9(10):e001387. doi: 10.1136/jitc-2020-001387corr1
122. Patel MR JB, Ji Y, Drees J, Tang S, Xiong K, Wang H, et al. Vesicular Stomatitis Virus Expressing Interferon-β is Oncolytic and Promotes Antitumor Immune Responses in a Syngeneic Murine Model of non-Small Cell Lung Cancer. Oncotarget (2015) 6(32):(33165–77). doi: 10.18632/oncotarget.5320

123. Russell SJ, Peng KW, Bell JC. Oncolytic Virotherapy. Nat Biotechnol (2012) 30(7):658–70. doi: 10.1038/nbt.2287

124. Stojdl DF, Lichty B, Knowles S, Marius R, Atkins H, Sonenberg N, et al. Exploiting Tumor-Specific Defects in the Interferon Pathway With a Previously Unknown Oncolytic Virus. Nat Med (2000) 6(7):821–5. doi: 10.1038/77558

125. Zeyaullah M, Patro M, Ahmad I, Ibraheem K, Sultan P, Nehal M, et al. Constitutive Interferon Pathway Activation in Tumors as an Oncolytic Properties of a Mumps Virus Vaccine Strain in Human Melanoma Cell Lines. Mol Biol (Mosk) (2018) 52(4):659–66. doi: 10.1134/S0026893318040027

126. Kevin J Harrington IP J, Hechtb R, Stephen Hodic F, Szabod Z, Ammour YI, Ryabaya OO, Milovanova AV, Sidorov AV, Shohin IE, Zverev VV, et al. Radiation Attenuates Prostate Tumor Antiviral Responses to Vesicular Stomatitis Virus Containing IFNbeta, Resulting in Pronounced Antitumor Effects. Mol Cancer Res (2013) 24(7):644–51. doi: 10.1158/1521-6440.MCR-12-0441-

127. Ammour YI, Ryabaya OO, Milovanova AV, Sidorov AV, Shohin IE, Zverev VV, et al. Oncolytic Properties of a Mumps Virus Vaccine Strain in Human Melanoma Cell Lines. Mol Biol (Mosk) (2018) 52(4):659–66. doi: 10.1134/S0026893318040027

128. Kurokawa C, Iankov ID, Anderson SK, Aderca I, Leontovich AA, Maurer MJ, et al. Activating Systemic T-Cell Immunity Against Self Tumor Antigens to Support Oncolytic Virotherapy With Vesicular Stomatitis Virus. Hum Gene Ther (2011) 22(11):1343–53. doi: 10.1089/hum.2010.216

129. Son HA, Zhang L, Cuong BK, Van Tong H, Cuong LD, Hang NT, et al. Combination of Vaccine-Strain Measles and Mumps Viruses Enhances Oncolytic Activity Against Human Solid Malignancies. Hum Gene Ther Clin Dev (2017) 27(3):111–22. doi: 10.1089/humc.2016.061

130. Son HA, Zhang L, Cuong BK, Van Tong H, Cuong LD, Hang NT, et al. Combination of Vaccine-Strain Measles and Mumps Viruses Enhances Oncolytic Activity Against Human Solid Malignancies. Cancer Invest (2018) 36(7):1098–1106. doi: 10.1080/07357907.2018.1435439

131. Grossardt C, Engeland CE, Bossow S, Halama N, Zaouk I, Leber MF, et al. Granulocyte-Macrophage Colony-Stimulating Factor-Armed Oncolytic Measles Virus is an Effective Therapeutic Cancer Vaccine. Hum Gene Ther (2013) 24(7):644–54. doi: 10.1089/hum.2012.205

132. Suzuki M. Partners in Crime: Combining Oncolytic Viroimmunotherapy With Other Therapies. Mol Ther (2017) 25(4):836–8. doi: 10.1016/j.ymthe.2017.03.005

133. Udayakumar TS, Betancourt DM, Ahmad A, Tao W, Totiger TM, Patel M, et al. Radiation Attenuates Prostate Tumor Antiviral Responses to Vesicular Stomatitis Virus Containing IFN-beta, Resulting in Pronounced Antitumor Systemic Immune Responses. Mol Cancer Res (2020) 18(8):1232–43. doi: 10.1158/1541-7786.MCR-19-0836

134. Shen W, Patnaik MM, Ruiz A, Russell SJ, Peng KW. Immunovirotherapy With Vesicular Stomatitis Virus and PD-1 Blockade Enhances Therapeutic Outcome in Murine Acute Myeloid Leukemia. Blood (2016) 127(11):1449–58. doi: 10.1182/blood-2015-06-652503

135. Durham NM, Mulgrew K, McGlinchey K, Monks NR, Ji H, Herbst R, et al. Oncolytic VSV Primes Differential Responses to Immuno-Oncology Therapy. Mol Ther (2017) 25(8):1917–32. doi: 10.1016/j.ymthe.2017.05.006

136. Gebremeskel S, Nelson A, Walker B, Oliphant T, Lobert L, Mahoney D, et al. Natural Killer T Cell Immunotherapy Combined With Oncolytic Vesicular Stomatitis Virus or Reovirus Treatments Differentially Increases Survival in Mouse Models of Ovarian and Breast Cancer Metastasis. J Immunother Cancer (2021) 9(3):e002096. doi: 10.1136/jitc-2020-002096

137. Wonghida P, Diaz RM, Pullido C, Rommelfanger D, Galivo F, Kaluza K, et al. Exploiting Tumor-Specific Defects in the Interferon Pathway With a Previously Unknown Oncolytic Virus. Nat Med (2015) 6(32):(33165–77). doi: 10.1038/nm.3788

138. Melzer MK, Lopez-Martinez A, Altomonte J. Oncolytic Vesicular Stomatitis Virus as a Viro-Immunotherapy: Defeating Cancer With a “Hammer” and “Anvil”. Biomedicines (2017) 5(1):8. doi: 10.3390/biomedicines5010008

139. Altomonte J, Ebert O. Sorting Out Pandora’s Box: Discerning the Dynamic Roles of Liver Microenvironment in Oncolytic Virus Therapy for Hepatocellular Carcinoma. Front Oncol (2014) 4:85. doi: 10.3389/fonc.2014.00885

140. Zhang L, Steele MB, Jenks N, Grell J, Sukasampaisan L, Naik S, et al. Safety Studies in Tumor and Non-Tumor-Bearing Mice in Support of Clinical Trials Using Oncolytic VSV-IFNbeta-NIS. Hum Gene Ther Clin Dev (2016) 27(3):111–22. doi: 10.1089/humc.2016.061

141. Yokoda R, Nagalo BM, Vernon B, Oklu R, Albadawi H, DeLeon TT, et al. Oncolytic Virus Delivery: From Nano-Pharmacodynamics to Enhanced Oncolytic Effect. Oncolytic Virother (2017) 6:39–49. doi: 10.2147/OV.S145262

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