Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology) *2025 26(6):546-556*
www.jzus.zju.edu.cn; www.springer.com/journal/11585
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Review

https://doi.org/10.1631/jzus.B2400381

New characteristics of cancer immunotherapy: trends in viral tumor immunotherapy with influenza virus-based approaches

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Abstract: Immunomodulatory cancer therapy is witnessing the rise of viral immunotherapy. The oncolytic influenza A virus, although promising in preclinical investigations, remains to be implemented in clinical practice. Recent progress in genetic engineering, coupled with experiential insights, offers opportunities to enhance the therapeutic efficacy of the influenza A virus. This review explores the use of the influenza virus, its attenuated forms, and associated vaccines in cancer immunotherapy, highlighting their respective advantages and challenges. We further elucidate methods for engineering influenza viruses and innovative approaches to augment them with cytokines or immune checkpoint inhibitors, aiming to maximize their clinical impact. Our goal is to provide insights essential for refining influenza A virus-based viral tumor immunotherapies.

Key words: Oncolytic virus; Influenza A virus; Antitumor; Reverse genetic technology; Vaccine; Viral immunotherapy

1 Introduction

The potential use of viruses in cancer therapies has been continuously explored for more than a hundred years. Since the 19th century, certain naturally occurring or engineered oncolytic viruses (OVs) have been identified to specifically target and annihilate cancer cells without causing harm to healthy cells (Kelly and Russell, 2007; Chaurasiya et al., 2021). This selective operation not only induces the direct lysis of tumor cells but also sparks strong and potentially enduring immune responses against tumor antigens (Bommareddy et al., 2018). In the 1950s, significant breakthroughs in oncolytic virotherapy (OVT) were made, thanks to the modernization of cellular and tissue cultural technologies, accompanied by the establishment of cancer mouse models via xenografting (Davola and Mossman, 2019). In recent decades, substantial progress in the genetic engineering of viruses and the study of microorganisms at their molecular levels has accelerated the use of various OVs in medical research (Kabiljo et al., 2020). Talimogene laherparepvec (T-VEC) has gained recognition in this field. In 2015, T-VEC received approval from the US Food and Drug Administration (FDA) for its use in treating advanced melanoma (Johnson et al., 2015). As of now, there are at least five viral treatment products available on the market, including Rigvir, Oncorine (H101), T-VEC, Delytact, and nadofaragene firadenovec, as outlined in Table 1 (Shalhout et al., 2023). The majority are replication-competent OVs, with the exception of nadofaragene firadenovec, which serves as a replication-incompetent vector to administer interferon α-2b (IFNα2b). An increasing number of OVs are entering clinical trials, and these advances herald a new era in which OVs can join other immunotherapy agents to provide a versatile strategy for the targeting and efficient elimination of cancer cells (Kaufman et al., 2015; Raja et al., 2018).

To date, over ten DNA and RNA viruses have been utilized as OVs, including adenovirus and herpes simplex virus. Detailed reviews are available in

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Received July 23, 2024; Revision accepted Nov. 11, 2024;
Crosschecked Apr. 28, 2025; Published online May 23, 2025

Table 1 Currently approved virotherapeutic products

Name	Location (approval year)	Prototypal species	Gene modification	Types used for cancer
Rigvir (discontinued)	Armenia (2016), Georgia (2015), Latvia (2004)	Picornaviridae family, *Enterovirus* genus, enteric cytopathic human orphan (ECHO), type 7	None	Stages I and II melanoma
H101	China (2005)	Adenovirus	Delete E1B-55 KDS, and partly delete E3 (Liang, 2018)	Nasopharyngeal carcinoma
T-VEC	Israel (2017), Australia (2016), Europe (2015), USA (2015)	Herpes simplex virus type 1 (HSV-1)	Encode for GM-CSF and delete the genes *ICP34.5* and *ICP47* (Rasa and Alberts, 2023)	Unresectable stages IIIB–IV melanoma
Delytact	Japan (2021)	HSV-1	Add an additional deletion mutation to the *α47* gene of the second-generation oncolytic HSV-1 G207 (Rasa and Alberts, 2023)	Glioblastoma
Nadofaragene firadenovec	USA (2022)	Adenovirus	Deliver cDNAs encoding the human *IFNα2b* gene and restrict replication (Shalhout et al., 2023)	Non-muscle invasive bladder cancer

cDNA: complementary DNA; GM-CSF: granulocytemacrophage colony-stimulating factor; *IFNα2b*: interferon α-2b; T-VEC: talimogene laherparepvec.

recent publications (Tian et al., 2022; Rasa and Alberts, 2023; Shalhout et al., 2023; Alhaskawi et al., 2024). DNA viruses are favored for their high genomic stability and have consequently been a focal point of clinical research. Conversely, RNA viruses mitigate the insertional mutation risk, offering a safety advantage by generally avoiding the initiation of chronic diseases (Haseley et al., 2009).

This review delves into the exploration of one of the RNA viruses—the influenza virus, predominantly known as the pathogen causing the flu disease—in the burgeoning field of oncolytic viral therapy. Recent studies have revealed its promising applicability not only in targeting and eliminating cancer cells but also as a dual-purpose intervention that can additionally serve as a protective vaccine against influenza for patients undergoing cancer treatment (Pollyea et al., 2010; Pedrazzoli et al., 2014; Newman et al., 2020). A Phase 1 clinical trial (ClinicalTrials.gov identifier: NCT05600582) with CodaLytic, an intratumoral OV derived from the influenza virus used for metastatic or untreatable breast cancer patients, has been withdrawn due to a funding decision by the sponsor. This review will further elaborate on the advances in influenza virus immunotherapy and mechanisms of influenza A virus (IAV) oncolysis, their advantages and disadvantages, approaches to influenza viral engineering, ways of genetically engineering influenza viruses, and how these ideas can be used to optimize the design of OVs based on the IAV.

2 Influenza A virus for tumor immunotherapy

2.1 Influenza virus and the reverse genetic system

With respect to its bio-structure, the influenza virus is a wrapped negative-stranded RNA virus devoid of reverse transcriptase or any DNA-incorporating functionality. The IAV is one of the virus's four subtypes (A, B, C, and D) and is predominantly considered in OV development for tumor immunotherapy. This viral subtype contains eight separate RNA fragments (Bouvier and Palese, 2008). These fragments encode structural proteins, including polymerase basic protein 1 (PB1), PB2, polymerase acidic protein (PA), hemagglutinin (HA), nucleoprotein (NP), neuraminidase (NA), matrix protein 1 (M1), and M2, as well as nonstructural proteins, such as NS1 and NS2 (Fig. 1) (Egorov et al., 1998), which leverage a complex interplay to facilitate the virus's life cycle. Membrane proteins on the viral envelope include both HA and NA. The former attaches itself to sialic acid receptors present on the

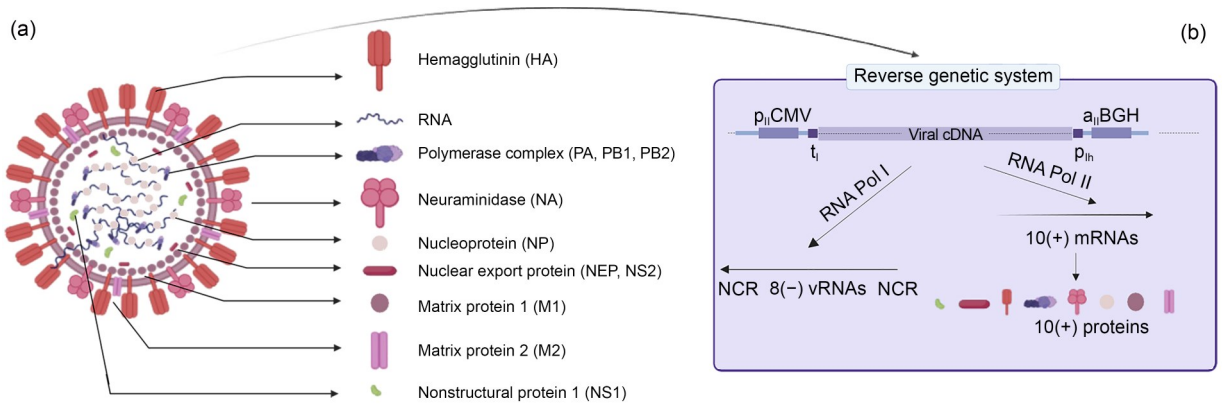

Fig. 1 Model diagram of the influenza virus (a) and reverse genetic system of influenza virus (b). PA: polymerase acidic protein; PB: polymerase basic protein; NCR: non-coding region; cDNA: complementary DNA; mRNA: messenger RNA; vRNA: viral RNA; p_{II}CMV: Pol II promoter of the human cytomegalovirus; a_{II}BGH: polyadenylation signal of the gene encoding bovine growth hormone; p_{Ih}: Pol I promoter; t_I: Pol I terminator. Created with BioRender.com.

host cell surface and facilitates virus ingress into cells via receptor-mediated endocytosis. Conversely, the latter assists with discharging newly formed viral particles by detaching sialic acid from the surfaces of host cells (Colman et al., 1983; Stegmann, 2000). Inside the virus and infected cells, a viral ribonucleoprotein (vRNP) complex is formed with the combination of polymerase basic proteins (PB1 and PB2), PA, and NP, orchestrating viral RNA (vRNA) synthesis (Eisfeld et al., 2015; Miyake et al., 2019; Morris et al., 2020). Matrix proteins (M1 and M2) govern the structure and proton channel functionality of the virus, contributing to viral particle assembly and pH stabilization within the cytoplasm (Martin and Heleniust, 1991; Su et al., 2018). Nuclear export protein (NEP) or NS2 transports vRNP from the nucleus to the cytoplasm, a critical step in determining viral replication levels (Robb et al., 2009). NS1 stands as a vital antagonist to hosts' natural immune responses, modulating vRNA synthesis and limiting host cell messenger RNA (mRNA) polyadenylation (Li et al., 2021).

The packaging of the IAV genome is organized by a specific interaction network of intersegment RNA–RNA interactivity. Non-coding regions (NCRs) flanking a wide central coding region are integral in packaging signals and genome replication promotion. This region envelops 12 nucleotides at the 3' termination, 13 nucleotides at the 5' termination, and fragment-distinctive sections. This structural organization, coupled with other elements like segment-unique packaging signals, plays a pivotal role in fostering intricate RNA–RNA interactions that facilitate the assembly of octameric supramolecular genomic complexes. This sophisticated viral replication and assembly process presents challenges to the genetic engineering of recombinant IAV because of the essential functions of each gene and the constrained viral genome size (Ferhadian et al., 2018; Li et al., 2021; Jakob et al., 2022).

Since its inception in 1999 by Neumann and colleagues in Kawaoka's group, the plasmid-centric reverse engineering method has revolutionized the field of flu virus studies, evolving progressively from utilizing 12 plasmids to a single plasmid setup (Fodor et al., 1999; Neumann et al., 1999, 2005; Hoffmann et al., 2000; Zhang et al., 2009). Predominantly relying on the 8-plasmid system (Hoffmann et al., 2000), the technology utilizes RNA polymerase I (Pol I) and RNA polymerase II (Pol II) sites to facilitate the simultaneous transcription of complementary RNA (cRNA) and vRNA (Fig. 1), thereby enhancing the ability to manipulate influenza viruses through feasible mutations and gene modifications.

2.2 Live competent influenza virus for viral immunotherapy

OVs frequently selectively multiply within and eradicate cancer cells, causing little or no damage to normal cells (Chaurasiya et al., 2021). As a respiratory virus, inhaled IAV could potentially be effective in treating lung cancer or metastasized lung cancer (Kumlin et al., 2008). Kasloff et al. (2014) reported that IAV exhibits tropism against human pancreatic ductal adenocarcinoma cells. Consequently, it triggers the

replication of vRNA and induces cell self-destruction (apoptosis) in vitro. In addition, it has produced substantial antitumor responses in in vivo studies.

IAV infection can induce diverse tumor cell death pathways, encompassing apoptosis, necrosis, and autophagy, and engage different viral proteins, such as PB1-F2 protein (PB1-F2), NP, and NA, in the process (Atkin-Smith et al., 2018; Kabiljo et al., 2020). Particularly, NA facilitates apoptosis via interactions with host proteins and the activation of the transforming growth factor-β (TGF-β) pathway (Schultz-Cherry and Hinshaw, 1996; Chen et al., 2001; Tripathi et al., 2013). M2 and NS1 induce autophagy, promoting immunogenic reactions. This process involves the mobilization of adenosine triphosphate (ATP) and danger-associated molecular pattern (DAMP) from inside the cell (García-Sastre et al., 1998; Zhirnov and Klenk, 2013). This infection with the virus and the rupture of tumor cells catalyze antigen release, fostering the initiation of both native and adaptive immune reactions, the foundation of immunological cell death (ICD) pathways, which is crucial in OV immunotherapy (Mardi et al., 2022; Palanivelu et al., 2023). Additionally, IAVs foster sustained antitumor immunity post-recovery, with resident alveolar macrophages exhibiting enhanced phagocytic and tumor cytotoxic functionalities, a manifestation of trained immunity (Masemann et al., 2021; Wang et al., 2023). Therefore, leveraging live competent influenza viruses as OVs alongside ICD enhancers could be a formidable strategy in immunotherapy, presenting a technique for sensitizing tumor cells to treatment.

2.3 Live attenuated and inactivated influenza vaccines for immunotherapy

While natural IAV can trigger potent immune responses, it carries the risk of causing influenza (Francisci et al., 2010). Thus, researchers have also attempted to use influenza viral vaccines as safer alternatives in potential immunotherapy applications to mitigate this risk while still harnessing the immune response benefits.

NS1 is a gene that can inhibit the host's antiviral response by interfering with ubiquitination in different ways (Lamotte and Tafforeau, 2021). IAV with *NS1* deletion cannot proliferate in regular cells; however, it generates infectious elements in cells that lack protein kinase R (PKR), making the virus an attractive candidate with the ability to activate Ras signaling pathways to treat tumors (Bergmann et al., 2001). Live attenuated influenza virus (LAIV) vaccine generated by the deletion of *NS1* (DelNS1-LAIV) induces cross-protective neutralizing antibodies, as well as $CD8^+$ and $CD4^+$ T cell immunities, inducing tumor cell death.

Additionally, methods for high-throughput library screening that simultaneously enhance the effectiveness and safety of LAIVs are available. We have comprehensively analyzed the type I interferon (IFN) sensitivity of all single-nucleotide alterations across the complete viral genome, utilizing a quantitative genomics system for the IAV. By incorporating eight IFN-provoking mutations, we managed to create a highly IFN-sensitive (HIS) virus. The HIS virus shows significant attenuation in hosts competent for IFN but can induce a fleeting IFN reaction that triggers a potent response from both the cellular and humoral immune systems (Du et al., 2018). Our initial findings indicate that the HIS virus can inhibit the growth of multiple murine tumors through intra-tumoral injection.

Besides LAIV, Newman et al. (2020) have documented that FDA-approved non-adjuvanted inactivated vaccines, when administered intratumorally, also have the capacity to attenuate tumor growth. This is achieved through the augmentation of anti-tumor $CD8^+$ T cells and a reduction in regulatory B cells present within tumors. Notably, this approach transforms immunologically dormant "cold" tumors into reactive "hot" tumors, fostering a systemic response that renders previously resistant tumors susceptible to checkpoint blockade therapies. Inactivated oncolytic viruses activate antitumor defenses through the stimulator of interferon genes (STING) pathway, enabling them to induce tumor immunity that surpasses the effects of their active viral counterparts (Dai et al., 2017; Zeng et al., 2024). Toll-like receptor (TLR) is activated through the interaction with virus-originated pathogen-associated molecular patterns (PAMPs). This mechanism may activate an innate immune response, potentially transforming the tumor microenvironment (Newman et al., 2020).

2.4 Combination therapy using IAV-based viral immunotherapy

Currently, the standalone clinical efficacy of OV therapy remains somewhat limited. Various preclinical

trials have exhibited augmented results when pairing OVs with treatments such as radiotherapy, immune checkpoint blockades, and cytotoxic drugs (Blake et al., 2018; Zhu et al., 2022).

For instance, the combination of oncolytic IAV and immune-checkpoint inhibitors (ICIs) has showcased potential clinical usage against ICI-resistant non-small cell lung cancer and metastatic pulmonary melanoma, among other cancers (Sitnik et al., 2020; Masemann et al., 2021). The advantage of combining viral immunotherapy with other therapies is that IAV infection abolishes tumor-mediated immunosuppression. IAV infection can have a long-lasting oncolytic effect, and the immune capacity of tumor-infiltrating immune cells in the uninfected area is restored. The resulting abundance of tumor-associated immune cells provides an opportunity to modulate their tumor-supporting and immunosuppressive phenotypes through viral infections or ICIs. Previous studies have shown that more than 50% tumor shrinkage can be achieved when IAV is combined with ICIs for melanoma lung metastasis (Sitnik et al., 2020). Similarly, IAV can synergistically amplify local immune responses when combined with cytokines. In addition, the oncolytic efficacy of IAV may be amplified when combined with cytotoxic medications. This occurs because these drugs boost the replication of DelNS1 and its ability to induce cell apoptosis through the caspase pathway, which is triggered by the IAV infection as mentioned above (van Rikxoort et al., 2012).

2.5 Limitations of IAV-based viral immunotherapy and the rationale for modifying IAV to improve its efficacy

The application of the influenza virus to viral immunotherapy in clinical practice also has certain limitations. Because of factors such as physical barriers and antiviral immunity, OVT has yielded positive results in only one Phase 1/2 clinical trial. The feasibility of administering a neoadjuvant influenza vaccine within a tumor has been verified in this cohort. The results offering definitive conclusions regarding safety and efficacy have not yet been obtained (Gögenur et al., 2023). Because of the widespread presence of neutralizing antibodies and safety concerns, intravenous administration of influenza viruses has been challenging, even when the virus is protected by various methods to reach target cells (Sui et al., 2011; Ji et al., 2024). For these reasons, influenza viruses are more suitable for solid tumors and for intra-tumoral injections, which is currently the most widely studied form of administration for influenza vaccines.

Even with intra-tumoral injections, there are significant challenges to address. In the tumor microenvironment, elements such as a dense extracellular structure, coupled with anomalous vasculature and lymphatic networks, can lead to interstitial hypertension, thereby limiting the dissemination of OVs (Atkin-Smith et al., 2018). Furthermore, the effectiveness of mono-viral therapy is typically restricted to the site of injection, necessitating a combined approach with cytokines to achieve a holistic anti-tumor response.

With advances in viral genetic engineering methodologies, recombinant variants of IAV offer a more effective method for showcasing the immunomodulatory properties of the virus. We describe the modification strategy for IAV in the following sections.

3 Modifying influenza virus to enhance its efficacy

3.1 Ways to engineer influenza virus

A variety of strategies have been deployed in the engineering of IAV. While the compact genome and intricate packaging signals of viruses pose challenges for viral engineering, researchers have successfully performed insertions and edits on numerous viral genes. The genes frequently targeted in these engineering and editing efforts are delineated in Fig. 2. In general, *NS1*, *HA*, *PB1*, and *PA*, as well as *NA*, are usually edited, as we detail below.

3.1.1 NS1

Beyond the fundamental role of the primary thirty nucleotides within the 3' coding section in viral packaging within the NS segment (segment 8) (Fujii et al., 2005), NS1 is not a critical component for the duplication of viruses, thus establishing it as a prime target for gene modification. As mentioned above, alterations to the NS1 protein induce a heightened IFN response, which is a hallmark of efficacious OV strategies.

Bergmann et al. (2001) discovered that influenza *NS1* knockout virus DelNS1 has specific oncolytic properties and replicates selectively in cells expressing carcinogenic Ras. Subsequent studies leveraged

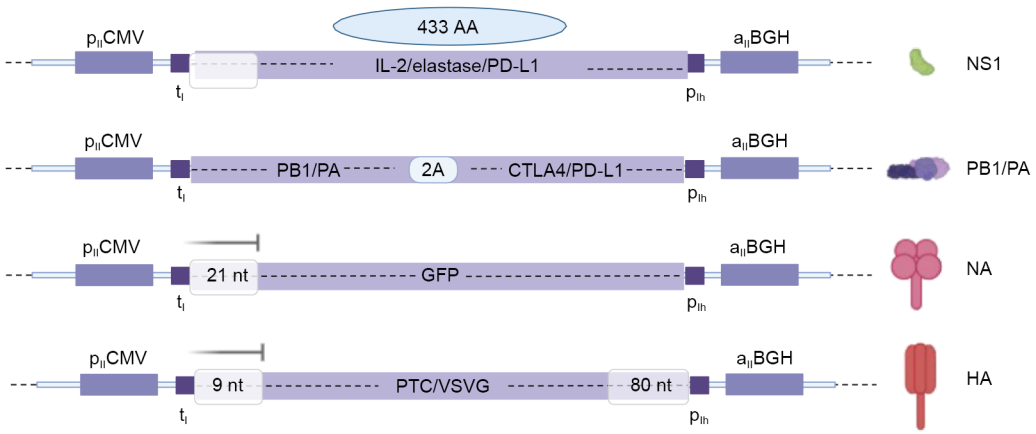

Fig. 2 Genes inserted and edited by influenza viruses. p_{II}CMV: Pol II promoter of the human cytomegalovirus; a_{II}BGH: polyadenylation signal of the gene encoding bovine growth hormone; p_{Ih}: Pol I promoter; t_I: Pol I terminator; AA: amino acids; PB1/PA: polymerase basic protein 1/polymerase acidic protein; NS1: nonstructural protein 1; NA: neuraminidase; HA: hemagglutinin; PD-L1: programmed death-ligand 1; CTLA4: cytotoxic T lymphocyte-associated antigen-4; GFP: green fluorescent protein; PTC/VSVG: premature termination codon/vesicular stomatitis virus glycoprotein.

the NS1 protein open reading frame (ORF) as a stable vector for the expression of long transgenes, with the capability to consistently express foreign genes up to 433 amino acids in length when initiated from the NS fragment (Kuznetsova et al., 2014). These *NS1*-engineered influenza viruses have not only proven safe in clinical trials but have also showcased promising characteristics for utilization as oncolytic agents (Kabiljo et al., 2020).

3.1.2 HA

As the most plentiful glycoprotein on the surface of the influenza virus, HA promotes viral entry by engaging the receptor and mediating the amalgamation of the virus–host membrane. Watanabe et al. (2003) revealed that efficiently integrating the *HA* vRNA into virions necessitates the presence of 9 nucleotides at the 3' terminus coding section and 80 nucleotides at the 5' terminus; thus they successfully engineered a virus to express foreign genes consistently, replacing the coding regions of two membrane proteins with different viral sequences while maintaining the necessary virion incorporation signals. Even without these replaced viral sequences, the final virus, which only carried the vesicular stomatitis virus glycoprotein (VSVG) on its surface, was still able to form green fluorescent protein (GFP)-marked clusters across multiple cycles of replication. This successfully exhibited the stable incorporation and maintenance of a pair of exogenous genetic sequences in the IAV (Watanabe et al., 2003). In a more recent study, Ji et al. (2024) introduced the fourth premature termination codon (PTC) in the HA segment and conjugated the new virus with a dibenzocyclooctyne-modified antigenic peptide (Ag-DBCO) incubated with cholesterol-modified cytosine-phosphate-guanine (CpG), resulting in the creation of a peptide-armed PTC virus, which can lead to robust absorption of the antigen, a specific response of immune cells, and a significant increase in the number of tumor-infiltrating lymphocytes.

3.1.3 PB1 and PA

Through the use of reverse genetics, the modification of *PA* and *PB1* genes to enable the expression of foreign genes has now become possible. For example, Lei et al. (2022) successfully cloned the heavy and light chains of antibodies downstream of the *PB1* and *PA* genes, respectively. Yang et al. (2022) engineered a chimeric virus, named rFlu-huCTLA4, which contains the heavy and light chains of human cytotoxic T lymphocyte-associated antigen-4 (CTLA4) antibodies in the PB1 and PA of the PR8 virus and selectively destroys liver cancer cells. Furthermore, Sun et al. (2023) engineered the recombinant oncolytic influenza virus armed with programmed death-ligand 1 (PD-L1) antibody (rgFlu/PD-L1), which activated the cyclic GMP-AMP synthase (cGas)-STING pathway in $CD8^+$ T cells and caused them to kill hepatocellular carcinoma (HCC) cells. PB1 and PA of rgFlu/PD-L1 express the PD-L1 heavy and light chains, respectively, while PR8 is used as the backbone. A common strategy involves inserting the porcine teschovirus 1 (PTV-1)

2A sequence along with the signal peptide sequence between PB1 or PA and the heavy or light chain genes to make it possible for proteins to be simultaneously translated.

3.1.4 NA

While not as extensively explored as *HA*, the *NA* gene has also been leveraged for foreign gene expression. Initial studies by Liu and Air (1993) facilitated the creation of a mutation pertaining to the *NA* vRNA segment of IAV. This substantial modification resulted from the utilization of bacterial sialidase, along with viral NA antibodies. Castrucci et al. (1992) substituted an unrelated amino acid sequence (FLAG) for a portion of NA in influenza virus A/WSN/33 (H1N1), which resulted in a 100-fold increase in the median lethal concentration (LC_{50}) of the engineered virus. The virulence of the engineered virus with FLAG replacement decreased. Fujii et al. (2023) have proven that the separate components of the vRNA sequence are vital for the effective formation of virions. Both ends of the *NA* vRNA coding region, the 3' and the 5', play a part in the incorporation into virions, with the 3' end having a more substantial role.

3.2 Armed influenza viruses

There are several editing strategies for boosting the influenza virus immunogenicity and spreading, attracting more immune cells to target tumor cells, and presenting tumor-associated antigens (TAAs), which are delineated below.

3.2.1 Expression of proteases and increased spread in the tumor

The extracellular matrix (ECM) can act as a physical obstruction, impeding the propagation of OVs (Vähä-Koskela et al., 2007). The influenza virus must be able to penetrate the ECM through the unstable tumor vascular system. Therefore, its ability to spread through tumors is limited, and it can only infect a subset of the tumor cells (de Silva et al., 2010). To increase the spread of the influenza virus in the tumor and improve targeting, Kuznetsova et al. (2017) exchanged the trypsin cleavage site within the influenza virus with partial *NS1* deletion for elastase. These elastase-reliant viral entities displayed powerful effects when tested on murine models of specific cancers, such as B16 (a type of melanoma) and PANC-1 (a human pancreatic ductal adenocarcinoma cell line).

3.2.2 Expression of cytokines

Drawing parallels with the FDA-endorsed T-VEC, which encodes the granulocyte-macrophage colony-stimulating factor (GM-CSF), influenza viruses have been engineered to harbor various immune-stimulating transgenes. These primarily encompass cytokines integral to T cell and dendritic cell (DC) activation, including interleukin-2 (IL-2), IL-15, and respective receptor ligands like CC-chemokine ligand 20 (CCL20) or GM-CSF (Kuznetsova et al., 2014; de Graaf et al., 2018). Significantly, IL-15-modified influenza virus with partial *NS1* deletion exhibited enhanced therapeutic efficacy, amplifying natural killer (NK) cell and T cell responses in murine models (Hock et al., 2017). Similarly, it has greater anticancer activity in human hepatoma cell line Hep-G2 xenograft models when leveraging *NS1*-deleted IAV integrated with GM-CSF (Yang et al., 2019).

3.2.3 Expression of TAAs and immune response initiation

TAAs are molecules that exhibit abnormal expression in cancer cells and can be used as targets of antitumor immune responses. The introduction of the transient expression of TAA via the influenza virus can trigger the body's production of particular antibodies and cellular defenses, as well as durable immune memory, while providing resistance to virus-related antigens. Zheng et al. (2000) found that recombinant influenza viruses can be used as therapeutic agents to prevent and treat cancers where the TAA is known. To induce specific immune memory for cancer epitopes, Efferson et al. (2003) used the IAV to establish a vaccine peptide targeting human epidermal growth factor receptor 2 (HER2). This approach resulted in the effective activation of effector T cells along with memory T cells through in vitro DC-centered experiments. In addition to genetic engineering, there are other methods by which viruses carry TAAs. Ji et al. (2024) conjugated HA with Ag-DBCO via click chemistry, leading to the formation of a new IAV.

3.2.4 Expression of ICI drugs

The method of encoding macromolecular proteins by influenza virus vectors has been used in many

applications, among which ICIs are the most common antibodies. Hamilton et al. (2018) utilized the influenza virus genome to express the CTLA4 ICI antibody, enhancing anti-cancer effects in a B16 melanoma model. Building upon this, Ji et al. (2024) introduced anti-PD-L1 nanoantibodies into the *PB2* gene sequence, optimizing the localized expression of anti-PD-L1 nanoantibodies at infection sites through nasal inhalation and showing superior therapeutic efficacy in mouse models of melanoma lung metastasis.

4 Conclusions

With the advancement of reverse genetic techniques, the utilization of the influenza virus in cancer therapy has grown significantly. However, no influenza virus has been officially approved for clinical use as an OV. To improve safety and efficacy, attenuated viruses can be constructed and carry foreign genes simultaneously. For example, DelNS1 and HIS were used as vectors to insert cytokine, TAA, or ICI sequences. In addition, it can be administered by inhalation or combined with other drugs to achieve a better oncolytic effect. Modified influenza viruses have been proven safe and to have beneficial properties as oncolytic agents. Existing studies have described various factors controlling their immunogenicity and established strategies to optimize their oncolytic effects. As for the future, the challenges ahead will be to identify the appropriate cancer, injection method and dose, transportation and storage issues, and the most appropriate combination of therapies for this promising new immunotherapeutic agent to allow its incorporation into routine clinical practice. The impact of influenza virus immunotherapy and combination therapy with other kinds of anticancer drugs also needs to be investigated.

Acknowledgments

This work was supported by the Leading Innovative and Entrepreneur Team Introduction Program of Zhejiang (No. 2022R01002), the National Natural Science Foundation of China (Nos. 82272300 and 82102893), and the Fundamental Research Funds for the Central Universities (No. 226-2024-00062), China.

Author contributions

Shiyao HU, Yong SHEN, and Yushen DU conceived the research project. Shiyao HU, Yiqi CAI, and Yushen DU drafted the manuscript and produced original figures. Shiyao HU, Yushen DU, and Yiding CHEN performed critical revisions of the text and figures. Yushen DU, Yingkuan SHAO, and Yiding CHEN provided supervision for the research and guided the discussions. Every author contributed to the final article and agreed upon the version published.

Compliance with ethics guidelines

Yushen DU is a Young Scientist Committee Member for *Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)* and was not involved in the editorial review or the decision to publish this article. Shiyao HU, Yiqi CAI, Yong SHEN, Yingkuan SHAO, Yushen DU, and Yiding CHEN declare that they have no conflicts of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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