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Review

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

Shiyao HU[1,3], Yiqi CAI[2], Yong SHEN[1,3], Yingkuan SHAO[1,3], Yushen DU[3]✉, Yiding CHEN[1,3]✉

[1]*Department of Breast Surgery and Oncology, The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China*
[2]*College of Life Sciences, Zhejiang University, Hangzhou 310012, China*
[3]*Cancer Institute (Key Laboratory of Cancer Prevention and Intervention, China National Ministry of Education), The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310009, China*

Abstract: Immunomodulatory cancer therapy is witnessing the rise of viral immunotherapy. The oncolytic influenza A virus, although promising in preclinical investigations, still 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. Starting in 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 induces not only the direct lysis of tumor cells but also sparks strong and potentially enduring immune responses against tumor antigens (Bommareddy et al., 2018). The 1950s saw significant breakthroughs in oncolytic virotherapy (OVT), 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 level 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 U.S. 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

✉ Yiding CHEN, ydchen@zju.edu.cn
Yushen DU, lilyduyushen@zju.edu.cn
Shiyao HU, https://orcid.org/0000-0001-8043-9061
Yiding CHEN, https://orcid.org/0000-0002-2263-6275
Yushen DU, https://orcid.org/0000-0002-8015-4207

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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 oncolytic viruses, with the exception of Nadofaragene firadenovec, which serves as a replication-incompetent vector to administer interferon alfa-2b (IFNα2b). An increasing number of oncolytic viruses are entering clinical trials, and these advances herald a new era in which OVs can join other immunotherapy agents in providing a versatile strategy for the targeting and efficient elimination of cancer cells (Kaufman et al., 2015; Raja et al., 2018).

Table 1 Currently approved virotherapeutic products

Name	Location	Prototypal species	Gene modification	Types used for cancer
Rigvir (Discontinued)	Armenia (2016), Georgia (2015), Latvia (2004)	Picornaviridae family, enterovirus genus, ECHO, type 7	None	Stage I–II melanoma
H101	China (2005)	Adenovirus	Delete E1B-55 KDS, part deleted E3 (Liang, 2018)	Nasopharyngeal carcinoma
T-VEC	Israel (2017), Australia (2016), Europe (2015), USA (2015)	Herpes Simplex Virus type 1 (HSV-1)	Encode for granulocyte-macrophage colony-stimulating factor (GM-CSF) and delete the genes ICP34.5 and ICP47 (Rasa and Alberts, 2022)	Unresectable stage 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, 2022)	Glioblastoma
Nadofaragene firadenovec	USA (2022)	Adenovirus	Deliver cDNAs encoding the human IFNA2B gene and restricts replication (Shalhout, et al., 2023)	Non-muscle invasive bladder cancer

To date, over ten DNA and RNA viruses have been utilized as OVs. Examples include adenovirus and herpes simplex virus, among others. Detailed reviews are available in recent publications (Rasa and Alberts, 2022; Tian et al., 2022; Shalhout, et al., 2023). 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., 2020a). 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 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 influenza A virus.

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 influenza A virus 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 PB1, PB2, PA, HA, NP, NA, 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 hemagglutinin (HA) and neuraminidase (NA). The former attaches itself to sialic acid receptors present on the host cell's 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, PB2), polymerase acidic protein (PA), and nucleoprotein (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 virus's structure and proton channel functionality, contributing to viral particle assembly and pH stabilization within the cytoplasm (Martin and Helenius, 1991; Su et al., 2018). Nuclear export protein (NEP) or nonstructural protein 2 (NS2) transport vRNP from the nucleus to the cytoplasm, a critical step in determining viral replication levels (Robb et al., 2009). Nonstructural protein 1 (NS1) stands as a vital antagonist to hosts' natural immune responses, modulating viral RNA synthesis and limiting host cell mRNA polyadenylation (Li et al., 2021).

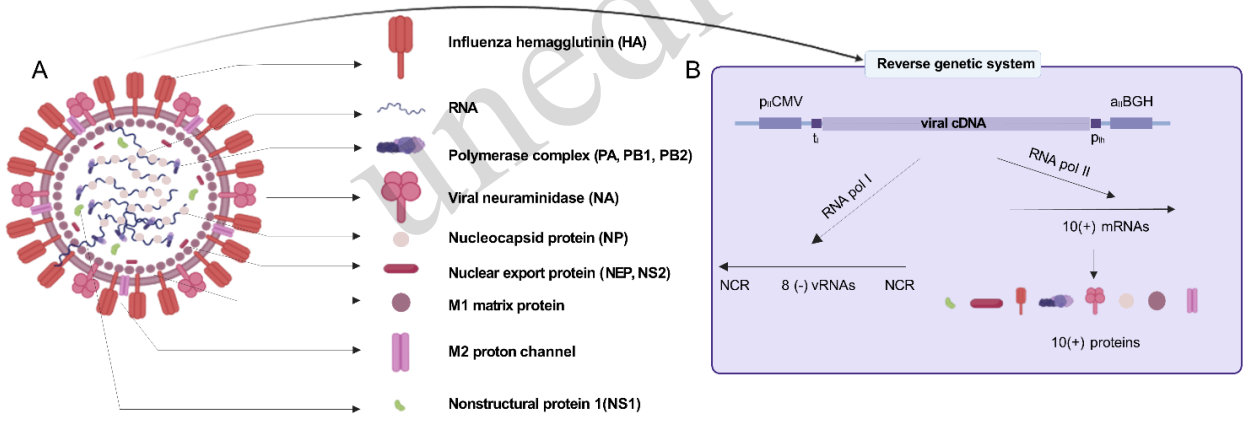

Fig. 1 Model diagram of the influenza virus (a) and reverse genetic system of influenza virus (b). p_{II}CMV: pol II promoter of the human cytomegalovirus; a_{II}BGH: polyadenylation signal of the gene encoding bovine growth hormone; $p_{I}h$: pol I promoter; t_I: pol I terminator. Created with BioRender.com

The packaging of the influenza A virus (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 and 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; Hoffmann et al., 2000; Neumann et al., 2005; 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, shown in 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

Oncolytic viruses frequently have the selectivity to 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). Samantha B. Kasloff found that IAV exhibits tropism against human pancreatic ductal adenocarcinoma cells. Consequently, it triggers the replication of viral RNA and induces cell self-destruction (apoptosis) in vitro. In addition, it produced substantial antitumor responses in in vivo studies (Kasloff et al., 2014).

IAV infection can induce diverse tumor cell death pathways, encompassing apoptosis, necrosis, and autophagy, engaging 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 transforming growth factor-β (TGF-β) pathways (Schultzcherry 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 patterns (DAMP) from inside the cell (Garcia-Sastre et al., 1998; Zhirnov and Klenk, 2013). This infection with the virus and the rupture of tumor cells catalyzes antigen release, fostering the initiation of both native and adaptive immune reactions, a foundation of immunological cell death (ICD) pathways, which are 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 a safer alternative in potential immunotherapy applications to mitigate this risk while still harnessing the immune response benefits.

As 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 are available for high-throughput library screening that simultaneously enhance the effectiveness and safety of LAIV. 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 yet 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. 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. The initiation of defenses against tumors by inactivated OVs, afforded via the stimulator of interferon genes (STING) pathway, allows it to provide tumor immunity beyond that provided by its active viral counterpart (Dai et al., 2017). Toll-like receptor (TLR) is activated through 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., 2020b).

2.4 Combinational therapy of IAV 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 influenza A virus 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 produce 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 infection 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 mentioned above (Van Rikxoort et al., 2012).

2.5 Limitations of IAV viral immunotherapy and rational for modifying IAV to improve 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 had 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. Results offering definitive conclusions regarding safety and efficacy have not yet been obtained (Gogenur 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 in order to reach the target cells (Sui et al., 2011; Ji et al., 2023). For these reasons, influenza viruses are more suitable for solid tumors and with 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 (TME), 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 the advances in viral genetic engineering methodologies, recombinant variants of IAV offer a more effective method for showcasing the virus's immunomodulatory properties. We describe the modification strategy for IAV in the following sections.

3 Modifying influenza virus to enhance efficacy

3.1 Ways to engineer influenza virus

A variety of strategies have been deployed in the engineering of IAV. While the virus's compact genome and intricate packaging signals 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.

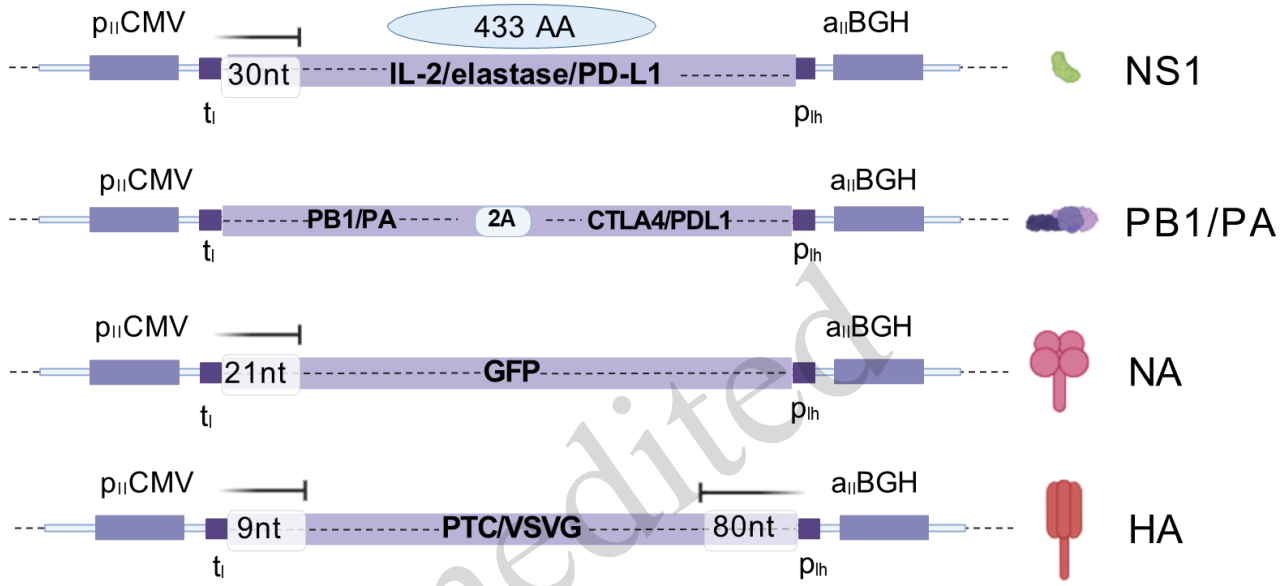

Fig. 2 Genes that have been inserted and edited by influenza viruses.

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 interferon (IFN) response, which is a hallmark of efficacious OV strategies.

In 2001, Bergmann et al. discovered that influenza NS1 knock-out virus DelNS1 has specific oncolytic properties and replicates selectively in cells expressing carcinogenic Ras (Bergmann, et al., 2001). Subsequent studies leveraged 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. Tokiko Watanabe (2003) revealed that efficiently integrating the HA vRNA into virions necessitates the presence of nine nucleotides at the 3' terminus coding section and 80 nucleotides at the 5' terminus. Utilizing that insight, Tokiko Watanabe

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, De zhong Ji et al. introduced the fourth premature termination codons in the HA segment and conjugated the new virus with a dibenzocyclooctyne-modified antigenic peptide incubated with cholesterol-modified CpG, resulting in the creation of a peptide-armed premature termination codons virus, which can lead to a robust absorption of the antigen, a specific response of immune cells, and a significant increase in the number of tumor-infiltrating lymphocytes (Ji, et al., 2023).

3.1.3 PB1 and PA

Utilizing 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 (Lei et al., 2022). Yang et al. 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 PR8 virus's PB1 and PA and selectively destroys liver cancer cells (Yang et al., 2022). Furthermore, Sun et al. engineered the rgFlu/PD-L1, which activated the cGas-STING pathway in CD8 T cells and caused them to kill HCC cells (Sun et al., 2023). PB1 and PA of rgFlu/PD-L1 express the programmed cell death of 1 ligand 1 (PD-L1) heavy chain and light chain, 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 the PB1 or PA and the heavy or light chain genes to make it possible for proteins to be simultaneously cotranslated.

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 (Liu and Air, 1993). 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 LC50 of the engineered virus (Castrucci et al., 1992). The virulence of the engineered virus with flag replacement decreased. Fujii has 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 (Fujii et al., 2003).

3.2 Armed influenza viruses

There are several editing strategies for boosting the influenza virus’s immunogenicity and spreading, attracting more immune cells to target tumor cells, and presenting tumor-associated antigens, 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 spread through the tumor 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 the targeting, Kuznetsova et al. 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 form of pancreatic ductal adenocarcinoma) (Kuznetsova et al., 2017).

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), interleukin-15 (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 cell (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 (Penghui et al., 2019).

3.2.3 Expression of tumor-associated antigens (TAA) and immune responses initiation

Tumor-associated antigens (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. In 2000, H Zheng et al. found that recombinant influenza viruses can be used as a therapeutic agent to prevent and treat cancers where the TAA is known (Zheng et al., 2000). To induce specific immune memory for cancer epitopes, Efferson et al. used the influenza A virus 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 (Efferson et al., 2003). In addition to genetic engineering, there are other ways to make viruses carry TAAs. De zhong Ji et al. conjugated HA with a DBCO-modified antigenic peptide (Ag-DBCO) via click chemistry, leading to the formation of a new IAV (Ji et al., 2023).

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 immune checkpoint inhibitors are the most common antibodies. Hamilton et al. utilized the influenza virus genome to express the CTLA4 immune checkpoint inhibitor antibody, enhancing anti-cancer effects in a B16 melanoma model (Hamilton et al., 2018). Building upon this, Ji et al. 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, showing superior therapeutic efficacy in mouse models of melanoma lung metastasis (Ji, et al., 2023).

4 Conclusion

With the advancement of reverse genetics 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 oncolytic virus. 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 effect. 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.

Data availability statement

Not applicable

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Author contributions

The research project was conceived by Shiyao HU, Yong SHEN, and Yushen DU. The manuscript was drafted and original figures were produced by Shiyao HU, Yiqi CAI, and Yushen DU. The text and figures underwent critical revisions by Shiyao HU, Yushen DU, and Yiding CHEN. 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 submitted. Yushen DU and Yiding CHEN made equal contributions to the study and shared the responsibility for correspondence.

Compliance with ethics guidelines

Shiyao HU, Yiqi CAI, Yong SHEN, Yingkuan SHAO, Yushen DU, and Yiding CHEN declare that they have no conflict of interest.

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

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