



## Views &amp; Comments

## From Flu to Therapy: Development of Influenza Viruses as Platforms for Combating Infections and Cancer



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The influenza virus [1], traditionally viewed as a major human pathogen, has recently gained attention as a versatile platform for therapeutic development. Progress in reverse genetics and viral vector engineering has made it possible to precisely manipulate the influenza genome, enabling the insertion of foreign genes and attenuation of virulence [2]. Not only are these engineered influenza viruses being explored as pathways to next-generation vaccines against influenza itself, but they are also under investigation as delivery vehicles for heterologous antigens targeting other viral infections and cancers [3]. Owing to their ability to generate strong mucosal and systemic immune responses, they are particularly attractive for immunotherapy and vaccine design. However, conventional influenza vaccine platforms, such as egg-based inactivated and live-attenuated formulations, are constrained by long production cycles, insufficient immunogenicity in vulnerable populations, and strain mismatch-driven reductions in protective efficacy. These limitations underline the need for vaccine platforms that offer improved genetic stability, rapid programmability, and enhanced immunogenicity.

To address these challenges, novel approaches are being developed that precisely modulate viral fitness and biosafety. The incorporation of non-canonical amino acids (ncAAs) into influenza viral proteins represents a promising and transformative strategy, as it enables site-specific attenuation of replication without compromising antigen presentation. This is due to the introduction of premature termination codons (PTCs) at selected loci within essential viral genes and the resultant generation of what we term “PTC viruses” [4]. The system is based on engineered synthetic biology tools—in particular, an orthogonal transfer RNA (tRNA)/aminoacyl-tRNA synthetase pair that selectively identifies a designated ncAA. At the PTC site, the orthogonal pair inserts the ncAA, with no cross-reaction with the host’s endogenous translational machinery, leading to the formation of a tightly regulated “genetic firewall” that restricts viral replication strictly to the orthogonal translation system.

This platform was implemented using the mammalian cell line XH 293, which we engineered to stably express these orthogonal components, thereby enabling efficient ncAA incorporation during

viral replication. Consequently, replication of the engineered PTC virus was confined to XH 293 cells and could proceed only in the presence of the appropriate ncAA. Notably, even with ncAA supplementation, the virus was unable to replicate in unmodified mammalian cells, resulting in a robust, multilayered biosafety mechanism. In mice, ferrets, and guinea pigs, the PTC virus elicited significantly stronger immune responses than a commercially available inactivated influenza vaccine. Subsequently, all of the immunized mice survived challenge with a wild-type influenza virus, whereas unvaccinated controls succumbed.

Owing to its controllable nature, this PTC virus can be repurposed as a cancer vaccine platform, in addition to being used in infectious disease prevention [5]. To effect antitumor immunity, a tumor-associated antigen was first tethered to the viral hemagglutinin (HA) protein via bioorthogonal click chemistry. Dendritic cell activation was achieved via synthesis of a CpG-rich Toll-like receptor 9 (TLR9) agonist and conjugation of the oligonucleotide to an N-terminal cholesterol moiety. Immune activation was further potentiated by inserting an anti-programmed cell death ligand 1 (PD-L1) nanobody gene into the viral genome. Collectively, these elements—checkpoint blockade, antigen, and PTC flu—constitute the chimeric antigen peptide (CAP) Flu system. In a lung metastasis model, intranasal administration of the CAP Flu system improved dendritic cell recruitment and activation in tumors and draining lymph nodes, resulting in the robust induction of humoral and cellular immunity and significant suppression of tumor growth [6].

Compared with conventional viral vectors such as adenovirus and vesicular stomatitis virus (VSV), the PTC influenza system offers several advantages: ① a uniquely orthogonal and genetically stable attenuation mechanism; ② strong mucosal immunity, which most viral vectors lack; and ③ consistent and stoichiometric antigen display, as antigens are physically tethered to viral proteins in contrast to relying on intracellular transcription/translation as in messenger RNA (mRNA) vaccines, which can vary with delivery efficiency and innate immune activation. The intrinsic instability of codon-deoptimized or temperature-sensitive influenza strains is avoided as well.

Translating the ptc platform to the clinic entails major challenges. preexisting immunity to influenza may limit vector spread and antigen expression, and potential solutions include the use of rare influenza subtypes, rotating HA/neuraminidase (NA) backbones, or

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partially shielding immunodominant epitopes. The clinical safety of ncAAs must also be evaluated, including their biodistribution and metabolic fate. Furthermore, the tumor-targeting specificity of the CAP Flu system may need to be optimized for non-pulmonary tumors, potentially via ligand-directed viral entry or improved delivery routes. Recent studies have revealed that influenza vectors can express neoantigens or cytokines in cancer immunotherapy, emphasizing the broader relevance of influenza-based platforms [7,8].

In brief, the PTC influenza system enables the rational design of replication-defective yet immunogenic influenza viruses with uniquely controllable safety profiles. Its modular architecture facilitates programmable antigen payloads, incorporation of immunomodulators, and orthogonal replication control. By integrating conserved epitopes or multi-subtype antigens, this platform addresses major limitations of conventional influenza vaccines and viral vectors while providing a foundation for developing universal influenza vaccines. Moreover, the plug-and-play nature of the platform ensures synergy with chemotherapy, radiotherapy, checkpoint blockade, and other immunotherapies. With ongoing advances in synthetic biology, the PTC influenza platform represents a compelling and versatile strategy for next-generation vaccines and viral immunotherapies.

#### CRediT authorship contribution statement

**Demin Zhou:** Writing – review & editing, Writing – original draft. **Dezhong Ji:** Writing – original draft, Writing – review & editing. **Jiandong Jiang:** Writing – review & editing.

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