VACCINES

A new route to vaccines using PROTACs

An influenza vaccine is created by attenuating the live virus through targeted proteolysis.

Brad Gilbertson and Kanta Subbarao

Influenza has been overshadowed by the COVID-19 pandemic, but it remains a major threat to global health. Improved vaccines that deploy innovative technologies will be welcome additions to our options for the control of influenza. In a novel application of targeted degradation published in *Nature Biotechnology*, Si et al[1]. describe the development of proteolysis-targeting chimeric (PROTAC) influenza vaccines. The PROTAC viruses target a selected viral protein to the ubiquitin–proteasome system, dramatically attenuating their replication while retaining their capacity to elicit robust and broad humoral, mucosal and cellular immunity. The authors demonstrate protection against homologous and heterologous viral challenge with influenza A virus in mice and ferrets. The concept of using PROTACs to attenuate live viruses for use as vaccines — not only against influenza but also against other viral diseases for which effective vaccines are lacking — is promising and warrants further investigation.

Despite their clinical efficacy and widespread uptake, influenza vaccines have well-known limitations. Influenza viruses undergo antigenic drift — a gradual accumulation of point mutations due to error-prone replication; mutations in the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) can generate variants that evade immunity induced by prior infection or vaccination. Although influenza vaccines are re-formulated annually to account for antigenic drift, further antigenic drift during the 6- to 8-month period of vaccine manufacture can diminish their efficacy. Influenza viruses can also undergo antigenic shift — the introduction of a virus with a novel HA into the human population. Antigenic shift usually occurs through the exchange of gene segments between animal and/or human influenza viruses and can generate variants that cause pandemics.

Current seasonal influenza vaccines induce strain-specific immunity and generally lack efficacy against variants that have undergone antigenic drift or shift. Improved vaccines that can elicit broader and more durable immunity are a public health priority, and many next-generation influenza vaccines are in development. These approaches use an array of innovative techniques to shorten production time, avoid deleterious mutations resulting from manufacture in hen's eggs, or increase the breadth of protection.

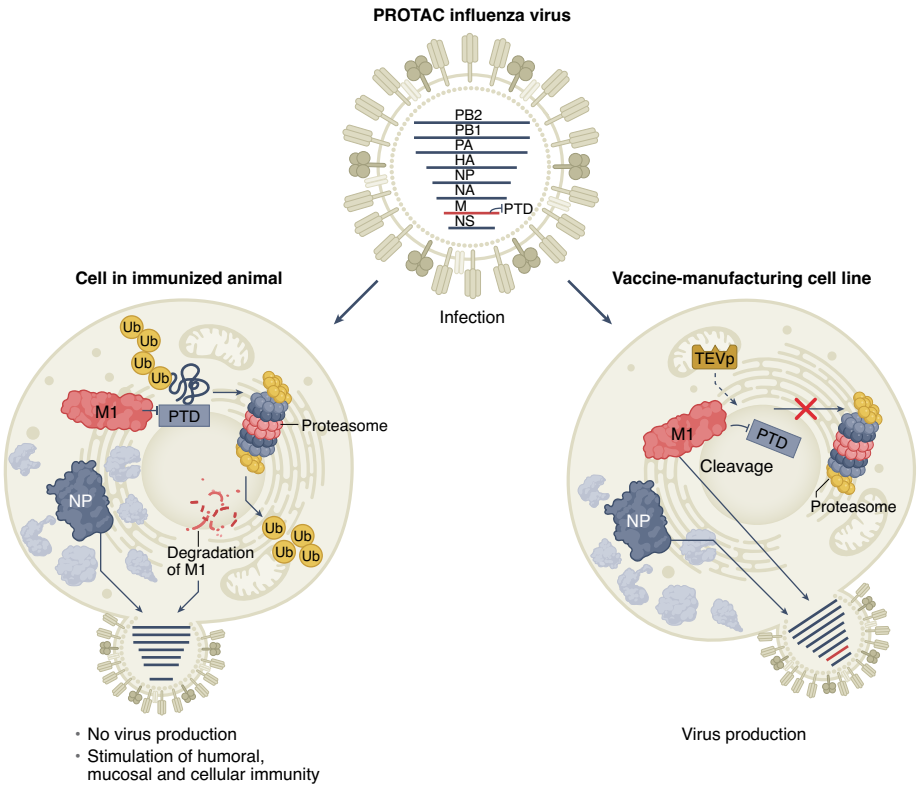

Fig. 1 | Overview of the PROTAC virus production system. PROTAC viruses are attenuated by proteasome-mediated targeted degradation of the ubiquitinated viral protein (M1–PTD) in conventional cells, resulting in deficient protein synthesis and attenuation of replication (left). The influenza nucleoprotein (NP), which is not fused to PTD, is not degraded. For vaccine manufacture, the PROTAC viruses replicate efficiently in cells engineered to express the TEV protease (TEVp), which cleaves the PTD (right). M1, matrix protein; Ub, ubiquitin; PTD, proteasome-targeting domain.

PROTACs are bifunctional molecules that induce the degradation of a protein of interest by targeting it to the ubiquitin–proteasome system[2]. They typically contain two covalently linked moieties, one specific to the protein of interest and the other to E3 ligase. Targeted protein degradation technology has been investigated primarily in the context of cancer[3]. It has also been applied as a promising antiviral approach against pathogenic viruses, including SARS-CoV-2, to degrade viral or host-related proteins[4–7]. The targeting of a viral protein for degradation was first reported for the hepatitis B virus (HBV)-encoded X-protein, which is essential for HBV replication[4]. Later, development of a PROTAC targeting the hepatitis C virus NS3/4A protease[5] was notable because this approach also overcame

the problem of viral drug resistance that often emerges during conventional antiviral therapy targeting enzymatic activity. More recently, angiotensin converting enzyme-2 (ACE-2)-derived peptides were designed to promote proteasomal degradation of the receptor-binding domain of the SARS-CoV-2 spike protein, thereby inhibiting infection[7].

Si et al[1]. engineered a conditionally removable proteasome-targeting domain (PTD) onto the matrix gene segment of an influenza A virus (Fig. 1). The PTD contains the peptide ALAPYIP, recognized by the von Hippel–Lindau tumor suppressor protein (VHL), the substrate-recognition component of $CRL2^{VHL}$ E3 ubiquitin ligase. For vaccine manufacture, virus replication should not be attenuated. Therefore, the PTD includes a tobacco etch virus cleavage site linker, which is selectively cleaved by TEV protease, sparing the protein from degradation in cells that stably express TEV protease (Fig. 1).

The authors first show that the PTD directs the viral M1 protein to the host proteasome in conventional Madin Darby canine kidney (MDCK) cells (Fig. 1). They used a proteasome inhibitor and a VHL E3 ubiquitin ligase inhibitor to confirm that viral protein degradation and PROTAC virus attenuation were dependent on the proteasome and ubiquitin ligase. To quantify the attenuation, they measured the replication kinetics of the M1–PTD and wild-type viruses in conventional MDCK cells and in MDCK cells transduced to stably express TEV protease. As expected, the M1–PTD virus replicated efficiently only in cells expressing TEV protease (Fig. 1). In MDCK cells, replication competence decreased >20,000-fold relative to wild-type virus. Similarly impressive attenuation was observed in vivo in both BALB/c mice (10,000- to 50,000-fold versus wild-type) and ferrets (100- to 800-fold). Notably, M1-PTD was also genetically stable over more than 20 passages in the TEV-protease-expressing cells.

The ubiquitous expression of VHL in most human tissues and cell types underpins the safety of potential PROTAC vaccines. Their ability to replicate stably and efficiently in MDCK cells, which are approved by the FDA for human vaccine production, is a bonus for this vaccine platform.

PROTAC technology may offer unique advantages for the generation of broad and robust immune responses. Cellular immunity promotes viral clearance through cytotoxic T cell recognition of foreign peptides presented by major histocompatibility complex (MHC) proteins on antigen-presenting cells. Viral epitopes presented on MHC class I are derived from proteasomal degradation of viral proteins. The level of ubiquitination of viral proteins correlates strongly with presentation of viral peptides on MHC[8]. Thus, the PROTAC approach may augment the presentation of viral epitopes on MHC and may promote enhanced T cell responses against the virus.

Furthermore, similarly to the synthetic attenuated virus engineering (SAVE) approach based on codon-pair deoptimization[9], PROTAC vaccines may induce superior protection against heterologous viruses because they incorporate a larger complement of gene segments from circulating wild-type viruses compared with the FluMist or M2-deleted single-replication influenza vaccine (M2SR)[10], which incorporates several gene segments from a master donor strain.

Further research is needed into any potential safety concerns of PROTAC vaccines. Mutations in human VHL result in several abnormalities, collectively called VHL disease[11]. The fact that VHL is a tumor-suppressor protein and that VHL loss is found in clear cell renal carcinomas[12] has raised concerns about the use of VHL as a target for PROTACs. As the authors note, there may be individuals for whom the PROTAC approach or specific PROTAC viruses may not be suitable — for example, people receiving proteasome inhibitor therapy or those with defective expression of a specific E3 ligase. However, there are many different PTD–E3 ligase pairs and PTD–viral protein linkers that could be investigated: more than 600 E3 ligases have been identified in the human ubiquitin–proteasome system. Although personalized selection from a suite of E3 ligases would not be feasible for population-based vaccine production, there is scope to develop improved PROTAC vaccine candidates that are safe, efficacious and cost-effective.

Implementation of a seasonal influenza vaccine strategy using PROTAC viruses would require the design and co-administration of four vaccines to protect against both circulating influenza A subtypes (H1N1 and H3N2) and B lineage viruses (Victoria and Yamagata). It may be prudent to target the same viral protein in the four viruses to reduce the risk that replicative competence would be restored through genetic complementation or reassortment. PROTAC vaccines would not face the problems of egg-adaptive mutations and introduction of glycosylation motifs that compromise the effectiveness of egg-based vaccines, and they could be manufactured more rapidly.

Although Si et al. demonstrated their approach with influenza virus, it should be applicable to many other pathogens. A potential challenge for wider application relates to the structure of PTD–viral protein fusions. The authors evaluated PTDs linked to eight different influenza proteins (M1, PB2, PB1, PA, NP, M2, NEP and NS1) and found differences in the efficiency of cleavage that were related to protein structure. Although for all proteins the PTD allowed efficient proteolysis, the cleavage site linker was buried in seven out of the eight proteins, leading to low efficiency of cleavage required to prevent degradation during vaccine production. Choosing the best viral protein(s) to target and ensuring efficient cleavage are among the details to be investigated as the authors' approach is extended to additional viral pathogens. ❐

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Competing interests
The authors declare no competing interests.