## Development of singly replicating virus vaccines against seasonal influenza

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The seasonal influenza vaccines used in Japan are primarily egg-based inactivated vaccines; however, egg-propagated viruses frequently acquire egg-adaptive mutations in the hemagglutinin antigen, reducing antigenic fidelity and contributing to mismatches that lower vaccine effectiveness. The requirement for frequent strain updates also increases manufacturing and regulatory burdens. Moreover, inactivated vaccines induce limited immune responses at the nasal mucosa, which is the primary site of influenza infection, resulting in insufficient mucosal immunity, with limited secretory IgA and tissue-resident cellular responses. These limitations highlight the need for improved vaccine modalities that elicit broader and more durable protection.

In the 2024/25 season, the intranasal live attenuated influenza vaccine FluMist was approved for use in Japan only for individuals aged 2–18 years, excluding older adults at high risk for severe diseases. Although intranasal administration of FluMist does stimulate mucosal immunity, the limited effectiveness of the vaccine in adults further underscores the need for next-generation vaccines that combine antigenic stability, strong mucosal and systemic immunity, and enhanced safety across all age groups.

This research focuses on developing a novel intranasal single-cycle replication influenza vaccine based on M2-knockout (M2KO) viruses. The objective is to establish a trivalent formulation targeting H1N1, H3N2, and influenza B viruses and to advance the candidate through a Phase I clinical trial. The M2KO platform is engineered by deleting the gene encoding the M2 ion channel protein, which is essential for virus replication. While the recombinant virus can be propagated in complementing cell lines expressing M2, the resulting virions lack the M2 gene and therefore cannot replicate in normal human or animal cells. Upon intranasal administration, the virus infects epithelial cells of the nasal mucosa and expresses all viral proteins except M2, enabling robust antigen presentation without productive replication.

This transient antigen expression is expected to induce strong mucosal immunity, systemic antibody responses, and cellular immune activation, offering broader and more durable protection than conventional inactivated vaccines. Compared with FluMist, which retains limited replicative capacity, the replication-incompetent M2KO platform provides a substantially improved safety profile, particularly for vulnerable populations. Overall, the M2KO semi-live vaccine represents a promising next-generation modality with the potential to overcome key limitations of current seasonal influenza vaccines.