HUMAN VACCINES & IMMUNOTHERAPEUTICS
2018, VOL. 14, NO. 3, 637–646
https://doi.org/10.1080/21645515.2017.1338547

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REVIEW



# Factors affecting immune responses to the influenza vaccine

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**ABSTRACT**

Annual administration of the seasonal influenza vaccine is strongly recommended to reduce the burden of disease, particularly for persons at the highest risk for the viral infection. Even during years when there is a good match between the vaccine and circulating strains, host-related factors such as age, preexisting immunity, genetic polymorphisms, and the presence of chronic underlying conditions may compromise influenza vaccine responsiveness. The application of new methodologies and large-scale profiling technologies are improving the ability to measure vaccine immunogenicity and our understanding of the immune mechanisms by which vaccines induce protective immunity. This review attempts to summarize the general concepts of how host factors can contribute to the heterogeneity of immune responses induced by influenza vaccines.

**ARTICLE HISTORY**
Received 1 March 2017
Revised 10 May 2017
Accepted 31 May 2017

**KEYWORDS**
aging; genetic polymorphism; immune response; immunocompromised; influenza vaccine

## Introduction

Influenza is a contagious acute respiratory disease that causes seasonal epidemics with 3–5 million hospitalizations and 250,000–500,000 deaths annually worldwide (WHO influenza center website). Although influenza viruses can cause disease in any age group, complications leading to serious illness and death following infection are predominantly observed among those aged ≥ 65 y and immunocompromised individuals. Thus, annual influenza vaccination has long been recommended for these vulnerable and most-at-risk groups.

Currently, licensed influenza vaccines contain components of 3/4 virus strains responsible for seasonal epidemics that are changed annually on the basis of global surveillance data.[1] Vaccine-induced neutralizing antibodies against the hemagglutinin (HA) represent the most effective correlate of protection against homologous influenza virus strains.[2] Even during years when there is a good match between the vaccine and the circulating strains, influenza vaccine effectiveness (VE) in preventing influenza illness may vary among vaccine recipients depending on age and health status of the individual.[3,4] In addition, preexisting immunity and genetic and hormonal factors may account for the inter-individual variability with respect to the immunological parameters used for assessing vaccine responsiveness. Over the last few years, new methodologies have increased our knowledge on the cellular and molecular mechanisms of vaccine immunity and help explain how host factors contribute to mechanisms that mediate the efficacy of vaccines against influenza.

### *Preexisting immunity*

The impact of influenza exposure history from prior infections and vaccinations on current-season strains remains controversial.[5-9] However, there are few data related to the effect of vaccinations received in previous seasons, and these data may be affected by bias and uncontrolled confounding. Thus, annual VE studies in large population groups of varying ages and that consider virus type/subtype and the vaccine type would help determine the appropriate response to annual vaccination. Furthermore, the specificity of the end-point or outcome measure used in the study should be considered. Currently, hemagglutination inhibition (HI) titers are used to evaluate immunogenicity of influenza vaccines, and values ≥ 40 are used as the surrogate correlate of 50% protection in adults.[10,11] Low rates of seroconversion after a trivalent inactivated influenza vaccine (TIV) administration and low ratios of post- to pre-vaccination HI titers are usually observed in subjects who have been repeatedly vaccinated,[12] although some studies have shown that the influenza vaccine was able to raise and maintain protective antibody titers.[13,14] In addition, cell-mediated immune responses may correlate better with protection in these individuals than HI titers.[15-18]

The application of new methodologies has improved our understanding of antibody-mediated responses to vaccination or infection. Andrews et al.[19] recently determined how the specificity of B cell responses are biased by recent past exposures. The authors conducted a longitudinal analysis of the plasmablasts, memory and serological responses upon vaccination with TIV in multiple individuals between 2006 and 2013. They found that repeated vaccination with the same vaccine strains from year to year reduced the overall vaccine-induced B cell response. In particular, high preexisting serological antibody levels to an influenza virus strain correlate with a low production of antibody secreting cells and memory B cells following repeated vaccination. Conversely, a robust B cell response is generated from antigenically novel vaccine strains. Preexisting serum antibodies, acquired through multiple

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immunizations, are hypothesized to bind and mask viral epitopes in the vaccine, thus reducing activation of memory B cells by recognizing these particular epitopes. Thus the level of preexisting serum antibodies modulates the magnitude of the response to repeated vaccinations, ensuring that a broad and diverse memory pool is ready to respond to a wide range of virus variants.

Fonville et al.[20] recently introduced the antibody landscape, which is a novel method for determining and representing serum antibody titers toward different antigenic variants in response to influenza infection or vaccination. The authors use antigenic cartography[21] to map the antigenic relationship between influenza strains; then, HI titers of a given serum are added with elevations corresponding to antigenic variants with higher antibody levels. A substantial heterogeneity among the antibody landscapes of 69 individuals monitored for infection over 6 y was observed, although each individual antibody landscape was stable from one year to the next. In another cohort, the in-depth serological phenotyping of 225 individuals who received a previously encountered vaccine strain or a more novel strain showed that the use of an antigenically updated virus variant stimulated antibodies to the novel variant and provided "back boosting" of titers to previous antigenic clusters.[20] By using this approach, the authors could quantify this "back boosting" and correlate the antigenic differences among the influenza viruses. Importantly, their findings indicate that vaccine updates may be beneficial and improve vaccine efficacy in previously exposed individuals.

Molecular-based technologies have strongly contributed to provide data describing immune cells and their functions. Lee et al.[22] described a molecular-level analysis of the serum antibody repertoire in a relatively small cohort of young adults before and after seasonal influenza vaccination. A high-resolution proteomics analysis of immunoglobulins coupled with high-throughput sequencing of transcripts encoding B cell receptors was used to characterize the antibody repertoire at the individual clonotype level in the sera of these subjects. By these means, the authors could demonstrate that high serum antibody titers before vaccination strongly correlate with the boosting of preexisting antibodies and the emergence of fewer vaccine-elicited antibodies. In particular, they found an unexpectedly high fraction of serum antibodies recognized by both the H1 and H3 monovalent vaccines, thus providing new insights for the development of a universal influenza vaccine.

Even though strain-specific neutralizing antibodies confer protection against infection with matching influenza virus strains, non-neutralizing antibodies and cellular immune responses to the virus that are cross-reactive to other viral strains also contribute to reduce disease severity and infectivity. A meta-analysis of 5 clinical studies performed between 2008 and 2013 in individuals receiving influenza live viral challenges provides further evidence on the ability of preexisting heterosubtypic cellular immunity to reduce both viral shedding and symptom scores in humans.[23] Notably, Trieu et al.[24] showed that both strain-specific and cross-reactive CD4+ and CD8+ T cells were maintained in healthcare workers who received a single AS03-adjuvanted H1N1pdm09 vaccine in 2009 and subsequently repeated annual vaccinations, whereas these cells declined significantly in those who received a single vaccine and no further vaccination. In addition, recent evidence that inactivated influenza vaccines could induce ADCC-antibodies against both homologous and heterologous influenza viruses in children and adults and that stalk-reactive antibodies appear to be preexistent within the memory compartment further highlights the role of preexisting cross-reactive immune responses against influenza.[25,26]

Recently, Gostic et al.[27] hypothesized that childhood HA imprinting may provide lifelong protection against severe infection and death from HA subtypes in the same phylogenetic group. In particular, their analysis of all known human cases of H5N1 and H7N9 with reported patient age revealed strong evidence that individuals born before the emergence of H3N2 (group 2) in 1968 showed protection against severe cases of H5N1 (group 1) but not H7N9 (group 2), and those born after 1968 showed the opposite pattern. As suggested by the authors, HA group imprinting also may contribute to the greater impact of seasonal H3N2 compared with H1N1 in older age groups. These findings provide new insights on herd immunity against zoonotic influenza virus strains in the human population and raise questions about the impact of influenza vaccination both on imprinting and boosting cross-reactive anti-HA responses that might be relevant for future research.

Overall, influenza exposure history based on prior infections and vaccinations heavily contributes to the complexity of the polyclonal antibody response to influenza viral strains in humans. For this reason, multiple approaches are necessary and may help explain the heterogeneity found among several serological studies. In addition, a better understanding of the impact of cross-reactive immune responses to influenza protection would help determine possible additional benefits provided by repeat vaccines.

## Immunosenescence

Over the past years, several studies have been conducted with large numbers of individuals and across multiple influenza seasons to assess vaccine efficacy and effectiveness in older adults. Although sometimes controversial when comparing older adults to unvaccinated individuals, the results clearly show that vaccine responses in older adults are reduced compared with young adults.[28,29] Indeed, influenza vaccines are estimated to be $< 50\%$ effective against influenza in older individuals even with a high rate of influenza vaccination and a well-matched vaccine.[3,30-32]

Physiological changes and a decline in immune function termed "immunosenescence" are responsible for a higher susceptibility to influenza, higher mortality rates and a poor influenza vaccine response. The influence of this age-related dysfunction on the innate and adaptive immune systems has been largely documented.[33,34] In particular, inappropriate activation of Toll-like receptors (TLRs) and costimulatory molecules in immune cells from older adults directly affects both cellular and humoral immunity and thus influenza-vaccine-induced antibody production.[35] The function and number of NK cells are altered in individuals with a diminished health status and correlate with low HI titers in vaccinated older individuals.[36,37] In addition, defects in the generation and function of B and T cells, reduced production of high affinity antibodies,

decreased expression of the costimulatory molecule CD28 on T cells, reduced telomerase activity, and Th1/Th2 cytokine disbalance were observed with aging, and parameters related to these functions are considered immunosenescence markers.[38-43] Furthermore, aging is characterized by a low-grade chronic inflammatory status, called "inflammaging," which is measured by circulating levels of IL-6, TNF-$\alpha$ and C-reactive protein, and latent infections with viruses such as cytomegalovirus (CMV).[44,45] In particular, latent CMV infection has been associated with an accelerated aging of the immune system in the elderly, and the virus has a negative effect on the in vivo and in vitro B cell responses to the seasonal influenza vaccine.[46,47] Recently, Frasca et al.[48] demonstrated for the first time a negative association between CMV seropositivity and the B cell predictive biomarkers of effective vaccine responses such as switched memory B cells and activation-induced cytidine deaminase in CpG-stimulated B cell cultures. In addition, CMV may also down-regulate the influenza vaccine-specific antibody response through the induction of late-differentiated T cells and the accumulation of senescent T cells, which lead to reduced influenza-specific memory T cells.[46,49] Thus, many components of the immune system contribute to the ability of an older individual to mount an effective response following vaccination against influenza. Because of this, a systems biology generated immune profile pre- and post-vaccination is essential to provide insight into the multiple signaling pathways and immunological processes.

Systems biology approaches have been used to identify molecular signatures of vaccine-induced immune responses in humans.[50-52] Among these, an increased expression of TNFRS17, which encodes the BCMA receptor for the B cell growth factor BAFF implicated in B cell differentiation, was a predictor of the antibody response in healthy adults vaccinated with TIV.[53] By integrating transcriptomic and microRNA (miRNA) expression data from a longitudinal study across 5 influenza seasons in diverse populations, Nakaya et al.[54] were able to identify several potential miRNA regulators of the interferon response to TIV vaccination. Importantly, their results revealed a negative association between enhanced NK cell and monocyte numbers in the elderly compared with young subjects and the magnitude of antibody response to vaccination. Furthermore, interferon-regulatory transcription factors (IRF1/IRF2/IRF6/IRF7/IRF9), chemokine/chemokine receptors (CCR5/CCR9/CCL5), cytokine/cytokine receptors (IFNG/IL10RA/TNFRSF1A), protein kinases (MAP2K4/MAPK3), growth factor receptor (TGFBR1) and several uncharacterized genes, such as ZNF300, NUP1333 and KLK1, were associated with the antibody response following influenza A/H1N1 vaccination in older adults.[55] Poland and collaborators, who first coined the term vaccinomics, in which information across transcriptomic, genomic, and proteomic data can be correlated with humoral and cellular immune measures to gain a greater understanding on vaccine immunogenicity, have also associated epigenetic markers with the influenza vaccine response.[56,57] In a recent work,[58] they show that sites of methylation regulation associated with the humoral response to vaccination in a cohort of 158 50-to 74-year-old individuals affect known cellular differentiation signaling and antigen presentation pathways. Specifically, they identified a group of CpGs that, when coordinately methylated, are associated with a higher antibody response, and when hypo-methylated are associated with a lower response. The availability of data sets (DNA methylation, mRNA expression, miRNA expression, and proteomics) could also provide evidence that, in addition to dysregulated immune function, immunosenescence may have effects on underlying biological processes and a variety of metabolic activities.[59]

Age-dependent comorbidities, including metabolic disorders, may also have an effect on the quality of the immune response to the influenza vaccine. Dementia and undernutrition, which are very common in frail elderly patients in geriatric medical long-term care, have been frequently observed among poor responders in vaccination studies.[60-63] The availability of high-dose influenza vaccines, intradermal influenza vaccines and MF59-adjuvanted vaccines has increased immunogenicity for influenza antigens in older adults.[64-66] Nonetheless, the immune responses in these individuals never achieve the levels observed in young adults receiving a standard vaccination.[67] Advances in scientific methodology and supporting technology over the years have contributed to increasing our knowledge in the field and may help improve current vaccination for the older adult population. Increasing evidence suggests that the modulation of signal transduction pathways such as the mammalian target of rapamycin (mTOR) pathway may have beneficial effects on aging and age-related conditions in humans. In particular, Mannick et al.[68] found that a 6-week treatment with the mTOR inhibitor RAD001, an analog of rapamycin, improved the response to influenza vaccination in elderly volunteers $\geq$ 65 y of age. A decrease in the percentage of PD-1-positive T cells observed in RAD001-treated cohorts compared with the placebo cohort may likely contribute to enhanced immune function in the elderly. Furthermore, RAD001 treatment increased the serologic cross-reactive response to heterologous strains of influenza not contained in the seasonal influenza vaccine. Keating et al.[69] also reported that treatment with rapamycin during immunization could provide cross-protective immunity against lethal infections with the influenza virus in mice. Rapamycin reduced the formation of germinal centers and inhibited class switching in B cells, facilitating the production of antibodies of lower affinity with a greater potential to be cross-reactive. In summary, these studies show how insights into the pathways may help guide the development of novel vaccines and strategies that provide better protection of at-risk populations in the future.

### Genetic polymorphism, sex-based differences and obesity

Several studies document the influence of the host genetic background on the immune response to influenza vaccination.[70-72] Polymorphisms of any of the involved genes in the molecular interactions within the immune system reflect the high variability between individuals to respond efficiently to vaccines. Even in healthy young individuals, there are subjects who do not develop a protective immune response to the influenza vaccine.

Associations between HLA class II alleles and nonresponsiveness to influenza vaccination were clearly observed in

individuals with HLA-DRB1*07, who produced lower antibody titers following a single administration of the influenza vaccine.[73] Conversely, a lower frequency of HLA-DBQ1*0603–9/14 (and DRB1*13) was found in nonresponders, compared with matched responders receiving the same vaccine.[73] A further study performed in an elderly cohort of recipients showed that HLA-DRB1*04:01 and HLA-DPB1*04:01 occurred at higher frequencies in individuals with seroprotective levels of the anti-HA antibodies compared with those non-seroprotected following influenza vaccination.[74]

Single-genetic polymorphisms (SNPs) in other genes have been associated with the influenza-specific antibody response after vaccination. Poland et al.[75] found associations of several SNPs in coding and noncoding regions of cytokine (IL6, IL18, IL12A IL12B, IFNg) and cytokine receptor (IL1R, IL2RG, IL4R, IL10RB, IL12RB, IFNAR2, TNFRS F1A) genes and H1N1 antibody titers in 184 18-to 40- year-old healthy recipients of the seasonal influenza vaccine. A recent work by Egli et al.[76] shows that a SNP in IL28B, a member of the IFN-$\lambda$ family, could be associated with an increased seroconversion rate after influenza vaccination. Notably, by using antagonist peptides to block the IL28 receptor subunit (IL28RA) in vitro, the authors could identify IL28B as a key regulator of the Th1/Th2 balance in influenza vaccination. Furthermore, Franco et al.[77] identified 20 genes involved in generalized biological functions, such as intracellular transport and membrane trafficking, that contributed to differential immune responses of healthy adults to influenza vaccination. Overall, the large number of SNPs that may be involved in immune responses highlights the complex interactions between different cell types and the need for a multidisciplinary approach with evidence-based principles that may be adopted for predicting influenza vaccine responsiveness.

Although limited data exist, sex-differences have also been reported in response to diverse vaccines across different age groups.[78,79] Both younger and older females have greater antibody responses than males following seasonal TIV. Engler et al.[80] also reported that healthy women who were vaccinated with a half dose of TIV had antibody titers similar to those in men who were given a full dose. Among older adults who received the high-dose TIV, Falsey et al.[64] documented higher antibody responses against each of the 3 influenza antigens in females compared with males. Sex steroid hormones contribute to differences in both innate and immune responses to infection and vaccination in men and women by directly binding to intracellular receptors in immune cells such as monocytes, B cells and T cells and the subsequent activation of hormone-responsive genes.[79,81] However, the age-related reduction of estrogen levels in women partially alters the sex-bias in vaccination. Conversely, there is evidence for an immunosuppressive role of testosterone in the response to influenza vaccination. Furman et al.[82] used a systems approach for the analysis of sex differences in the immune system and suggested that the natural variation in circulating free testosterone could explain the differences observed in the response to vaccines. They found that men with elevated serum testosterone levels and a high expression of genes involved in lipid metabolism had the lowest antibody responses to TIV. By repressing transcription factors such as FOS and JUN, which are involved in immune activation, testosterone would repress their further function to modulate genes implicated in the metabolism of lipids. In addition to sex hormones, other genes located on the X chromosome and encoding proteins such as TLR7, TLR8, GATA1, IRAK1, CD40L and FOXP3 may affect the immune response to vaccination.[83] Furthermore, the prevalence of miRNAs, which are crucial regulators of gene expression, on the X chromosome compared with the low number contained in the Y chromosome strongly implicate a role in sex-based differences in immunity.[84]

Obesity results in ineffective immune responses to influenza virus infection and represents a relevant risk factor for increased severity of influenza symptoms.[85-87] Paich et al.[88] first indicated that CD4+ and CD8+ T cells from obese individuals have substantial defects in activation and function that likely contribute to the increased morbidity and mortality from the H1N1pdm09 virus observed in these individuals compared with healthy weight adults. Obesity has also been clearly associated with a decreased immune response to influenza vaccination. Previous studies performed in mice subjected to diet-induced obesity showed a marked suppression of antibody production and neutralizing activity in response to a H1N1pdm09 vaccine, and thus a dramatically reduced protective efficacy against H1N1 virus challenge, compared with lean control mice.[89,90] A recent work[91] showed that despite the increased seroconversion conferred by the AS03-adjuvanted H1N1pdm09 vaccine, obese mice still succumbed to the influenza virus challenge and had higher morbidity and mortality compared with normal-weight mice. Human studies have had various results. Sheridan et al.[92] reported, for the first time, that a higher initial fold increase in antibodies to TIV in healthy obese individuals was followed, 12 months post-vaccination, by a greater decline in antibody titers and defective CD8+ T cell responses compared with healthy weight individuals. Recently, Frasca et al.[93] found reduced antibody responses to influenza vaccination in both young and elderly obese individuals that was associated with a decreased B cell function and higher levels of intracellular TNF-$\alpha$ and TLR4 compared with those detected in lean individuals. By contrast, other studies reported that antibody responses to influenza vaccination were similar in obese and lean older adults and among children and adolescents of various body mass index.[94,95] Recently, Esposito et al.[96] also found that in overweight and obese children aged 3–14 years, antibody responses to TIV were similar or in some cases slightly higher than in lean subjects of a similar age, even 4 months after vaccination. In addition to these conflicting results, obesity is a complex clinical condition that impairs various functions. Even if seroprotective antibodies are elicited, vaccination efficacy in obese individuals may be affected by obesity-associated decreases in key immunological and biological functions critical to counteract an influenza virus infection. However, this increasing population worldwide at a high risk of infection would have benefits from vaccination and thus should regularly receive a seasonal influenza vaccine.

### *Presence of chronic underlying medical conditions*

Individuals with autoimmune inflammatory diseases, such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE), or cancer are at an increased risk of influenza virus

infection because of their disease-associated impaired imbalances and the use of immunosuppressive therapies. Reduced immunogenicity of the influenza vaccine appears to reflect the impaired immunity in these patients, and the variability in the results of some studies are likely related to several factors, including disease heterogeneity and the varying degree of the patients' lymphopenia.[97-100] Nevertheless, there is evidence of influenza vaccine efficacy and effectiveness in these immunocompromised individuals.[101-104] In particular, Liao et al.[105] conducted a meta-analysis of influenza vaccine seroprotection, seroconversion and adverse effects in SLE patients. In this context, 18 studies with 1966 subjects, of whom at least 565 were patients with low-to-moderate SLE Disease Activity Index scores or SLE disease, showed that drugs such as glucocorticoids, azathioprine, methotrexate and mycophenolate mofetil decreased humoral responses to influenza vaccination compared with healthy controls. However, the immunogenicity of the influenza vaccine in SLE patients almost met that of the "Committee for Proprietary Medicinal Products" (CPMP) guidelines.[106] The mild and manageable side effects, detected in some of the SLE patients, further support the beneficial effects of influenza vaccination with TIV.

The current recommendation for cancer patients is to receive the influenza vaccine before the start of cytotoxic chemotherapy.[107-109] However, Keam et al.[110] conducted a large randomized clinical trial to determine the optimal timing of influenza vaccination during 3-week cytotoxic chemotherapy cycles. In particular, 83 adult patients with breast or lung cancer were randomly divided into 2 subgroups to receive the 2014–2015 seasonal TIV on day 1 or 11 during the chemotherapy cycle, and then stratified by age (< 60 and >60 years) and prior influenza vaccination status. Although generally lower than in healthy adults, antibody responses against influenza vaccine strains were comparable for patients who received the vaccine concurrently with chemotherapy (day 1) or within the cytopenic period (day 11).

In HIV-infected patients, depletion of CD4+ T cells due to HIV infection causes immunodeficiency and thus a higher risk of developing influenza-related complications. Despite a reduced immune response, the influenza vaccine appears to be safe and effective in reducing influenza disease in these patients.[111,112]In particular, early control of viremia by antiretroviral treatment (ART) can partially reverse B cell dysregulation and preserve influenza vaccine responsiveness.[113-115]Recently, Rinaldi et al.[116]measured both antibody and memory B-cell responses to the trivalent 2012–13 seasonal influenza vaccine in a cohort of vertically HIV-1 infected children and young adults receiving an influenza vaccination annually. Despite lower seroconversion rates after vaccination compared with healthy controls, similar frequencies of influenza-specific memory B cells for the 3 vaccine strains were detected by B-cell ELISPOT in both groups, suggesting that qualitative measures are more informative than standard serological markers in this population. Somewhat different results were reported by Wheatley et al.[117] who found lower frequencies and thus impaired induction of memory B cells in HIV+ individuals to influenza vaccination by using direct staining of HA-specific B cells with recombinant HA probes. Differences between the 2 studies may be due to the different cohorts analyzed or alternative methodologies used for memory B-cell measurements.

Transplant recipients, individuals with chronic obstructive pulmonary disease, and individuals with Down syndrome also have impaired immune responses to vaccination, including influenza.[118-120] Collectively, there is a need for more in-depth studies aimed at identifying the appropriate immunologic parameters to evaluate immunogenicity and assess vaccine effectiveness in immunocompromised individuals.

## Conclusions

Several host-related factors such as age, genetic polymorphisms, and the presence of chronic underlying conditions may contribute to a decline in immune responses and consequently compromise influenza vaccine responsiveness. Although complex and still challenging in many aspects, high-throughput technologies and the analysis of multi-omics experiments hold great promise to characterize the interactions between individual components of the immune system and thus better understand and predict vaccine-induced immunity.[121,122] Because of these studies, it is reasonable to believe that new insights about the efficacy of TIV in special populations such as the elderly or immunocompromised may enable rational design of specific vaccine formulations that target these most-at-risk groups.

In the meantime, further studies of influenza vaccination using alternative criteria rather than HI seroconversion to categorize subjects into 'responders" and 'nonresponders' will certainly help to establish the influenza vaccine efficacy. Among these, the use of human challenge models would be extremely informative for correlating the protection and development of new drugs and vaccines.[123,124]In addition, there are intense ongoing efforts to develop more immunogenic and broadly protective influenza vaccines that are based on highly conserved viral antigens, such as the HA stalk region and internal viral proteins, and early results seem to be promising.[125,126]

Lastly, and more importantly, the efficacy of current licensed vaccines may be lower in high-risk groups than in normal subjects, but the vaccines are still capable of reducing disease severity and mortality. Although countries have recommendations in place, vaccination coverage rates remain too low, and there is a need to improve vaccine awareness and education among patients, their families and healthcare workers to prevent the hospitalizations and deaths attributed to influenza in these groups each year.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Funding

This work was supported by the Ministry of Health, Italy (grant number RF-2010–2318269).

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