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REVIEW



# Cell culture-derived flu vaccine: Present and future

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**ABSTRACT**

The benefit of influenza vaccines is difficult to estimate due to the complexity of accurately assessing the burden of influenza. To improve the efficacy of influenza vaccines, vaccine manufacturers have developed quadrivalent influenza vaccine (QIV) formulations for seasonal vaccination by including both influenza B lineages. Three parallel approaches for producing influenza vaccines are attracting the interest of many vaccine manufacturing companies. The first and oldest is the conventional egg-derived influenza vaccine, which is used by the current licensed influenza vaccines. The second approach is a cell culture-derived influenza vaccine, and the third and most recent is synthetic vaccines. Here, we analyze the difficulties with vaccines production in eggs and compare this to cell culture-derived influenza vaccines and discuss the future of cell culture-derived QIVs.Keywords: Influenza vaccine, cell culture-derived, quadrivalent.

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## Introduction

Influenza A virus (IAV) is an Orthomyxovirus carrying a segmented, single-stranded RNA genome. Based on host tropism and severity of disease, influenza viruses are classified into types A, B, and C. Of these, type A, and to a lesser extent type B, are clinically important to humans. They are further classified based on the subtype of one of the 2 viral surface glycoproteins, the hemagglutinin (H) and the neuraminidase (N). IAV infections remain a serious burden for human health during seasonal outbreaks. At the same time, there is a constant threat of newly emerging highly virulent pandemic strains.[1,2]

"Vaccines have saved hundreds of millions of lives over the last century, and influenza has an important place in preventive health care programs. One estimate looking at excess deaths attributed to influenza found that, in milder influenza seasons, there were around 8 deaths per 100,000 population, while in more severe but non-pandemic years, the figure would be 44 per 100,000 3. Vaccination is the most effective single public health intervention able to dramatically reduce the impact of seasonal influenza.[4] Influenza has a high incidence of infection and transmission, and particularly debilitating to children and the elderly. Additionally, it incurs a high cost of healthcare- the total impact of an influenza epidemic (total estimated direct and indirect costs) in industrialised countries may reach 56.7 million € per million people3-. The virus strains change every year, cross-protection engendered by infection or vaccination is low, making it difficult to prepare and stockpile the vaccine in advance. It is nearly impossible to predict a widespread outbreak."

Global rates range from 5 to 10% in adults, 20 to 30% in children and the elderly, and up to 50% in specific populations and settings, with varying severity of disease. The annual global burden of severe influenza is estimated to be 3,000,000 to 5,000,000, causing 250,000 to 500,000 deaths, with 95% of these predicted to occur in developing countries.[5,6]

To improve the efficacy of their vaccines, a number of vaccine manufacturers have developed quadrivalent influenza vaccine formulations for seasonal vaccination by including both influenza B lineages, expected to substitute the trivalent formulation over time.[8] Recently, the Food and Drug Administration (FDA) and European Medicines Agency (EMA) has approved a quadrivalent formulation in which an additional strain of influenza B is added.[7-8]

The production of an optimal influenza vaccine requires constant global influenza monitoring for the emergence and circulation of new viruses other than those circulating in the previous season.[9] Seasonal influenza vaccine content is based on surveillance data on influenza virus circulation (http://who.int/influenza/gisrs_laboratory/en/).[10]

Seasonal influenza vaccine production is an enormous challenge for manufacturers because from the moment the WHO announces the seasonal strains in February for the Northern Hemisphere and in September for the Southern Hemisphere only a 6-month window is available for manufacturers to develop and supply the vaccines in July-August for the beginning of the vaccination campaign in September in the Northern Hemisphere and in March to start in April in the Southern Hemisphere.[11]

The majority of the currently licensed influenza vaccines are made using embryonated hens' eggs (Table 1) and a production system established in the 1940s. Fertilized hens' eggs are used as minifactories operated in parallel for influenza virus replication. The egg-based production system is still the most extensively used method to generate the 500 million vaccine doses.[12] Today, three parallel approaches for producing influenza vaccines are being considered by many vaccine manufacturing

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**Table 1.** Available seasonal influenza vaccines in the EU/EEA (2015–16 season).

| Manufacturer | Name of product* | Vaccine type | Adjuvant | Administration route | Produced in | Age recommended |
|---|---|---|---|---|---|---|
| Mylan | Trivalent: Influvac Imuvac | Inactivated | None | Intramuscular | Egg | From 6 months |
| AstraZeneca | Quadrivalent: Fluenz tetra (Flumist quadrivalent) | Live attenuated | None | Intranasal | Egg | From 24 months to 17 years |
| GlaxoSmithKline | Trivalent: Fluarix** Alpharix Influsplit Quadrivalent: Fluarix Tetra Alpharix Tetra Influsplit Tetra | Inactivated/ split | None | Trivalent: Intramuscular or subcutaneous Quadrivalent: Intramuscular | Egg | Trivalent: From 6 months Quadrivalent: From 3 years |
| Seqirus | Trivalent: Agrippal Fluvirin Optaflu Fluad | Inactivated /subunit | None None None Squalene (MF59) | Intramuscular or subcutaneous Intramuscular | Egg Egg Cell Egg | From 6 months From 4 years From 18 years From 65 years |
| Omninvest | Trivalent: Fluval AB | Inactivated | Aluminium phosphate gel | Intramuscular | Cell | From 6 months |
| Pfizer/ CSL Australia | Trivalent: Afluria*** Enzira | Inactivated/Split | None | Intramuscular | Egg | From 5 years |
| Sanofi Pasteur | Trivalent: Vaxigrip** Intanza 9$\mu$g Intanza 15$\mu$g | Inactivated Inactivated Inactivated | None None None | Intramuscular Intradermal Intradermal | Egg Egg Egg | From 6 months From 18-59 years From 60 yrs |

*The same product may be sold under different names **Split virion by Triton X-100 and formaldehyde inactivated ***Beta-propriolactone-inactivated and taurodeoxycholate split virion vaccine.
Overview of available seasonal influenza vaccines in the EU/EEA (2015–16 season).[87] (modified)

companies; the first and oldest is the conventional egg-derived influenza vaccine, the second is a cell culture-derived influenza vaccine, and the third and most recent technology is the production of synthetic vaccines. The following table displays the most important milestones in the development of influenza vaccines (Table 2).

## Difficulties with vaccines produced in eggs

Current influenza virus vaccines are most commonly grown in the allantoic cavity of embryonated hen eggs (ECE); the virus is then harvested, inactivated, purified and processed. High titers can be obtained by growing influenza virus in ECE, and extensive experience in large-scale production has led to the streamlining and automation of a highly standardized process. With current manufacturing capacity, 413 million doses of a trivalent influenza vaccine are produced every year for the world population.[13]

There is also extensive safety data available as billions of doses of ECE-produced influenza vaccine have been administered to humans. However, besides being labor-intensive, ECE-derived influenza vaccines have several drawbacks[3]:

**Table 2.** Historical path of the development of influenza vaccine.[8]

| | |
|---|---|
| 1930 | First experimental influenza vaccines (egg) |
| 1940 | Inactivated influenza vaccines (egg) |
| 1960 | Split influenza vaccines (egg) |
| 1980 | Subunit influenza vaccines (egg) |
| 2001 | Influenza vaccines cell-culture derived (MDCK) |
| 2013 | Modern DNA technology (Flublok) |

- First and foremost, the dependency uppon eggs.
  - Because 1 to 2 ECE are required for the production of each human dose of influenza vaccine, the method requires the availability of a large number of eggs simultaneously or within a short window of time, necessitating considerable planning (up to a year in advance) to produce sufficient numbers of ECE.
  - In addition, the eggs need to be set synchronously since inoculations need to be carried out 10–12 days after initiation of incubation.
  - More importantly, the eggs need to be from specific pathogen-free flocks or at least certified as 'clean' in order to avoid adventitious agents. This could be an issue especially in developing countries since the vaccine manufacturer will have to rely on the quality control of the supplier of the ECE.[14]
  - In the case of a pandemic strain, sufficient quantities of hen eggs may not be readily available to produce specific vaccines because the timeline from strain identification to vaccine is about four-six months.[13]
- Secondly, some virus strains, especially the recent H3N2 strains, do not grow well in ECE, and others such as the highly pathogenic avian influenza strains, H5N1, could be lethal to embryos, resulting in low titres.[14]
- Third, occasional breakdown in sterility during downstream processing could lead to rejection of large volumes of vaccine bulk, leading to a need to revisit the long

process of planning and execution, or to the undersupply of vaccine when needed.[3]

- Fourth, influenza viruses appear to mutate more frequently around the receptor-binding site and be selected when passaged in ECE, compared to passage in cells cultured in vitro,[15] which could potentially affect vaccine efficacy.
- Fifth, despite an extensive purification process, residual allergenicity of egg protein is a serious concern.[16]

The increased demand and the sustained threat of a pandemic outbreak have accelerated the introduction of new manufacturing strategies for influenza vaccine production.

## Cell culture-derived vaccines

In 1995, the WHO recommended developing an alternative influenza virus cultivation system.[17] One favored option is cell culture. In contrast to egg-based production processes, cell-based production technology allows manufacturers to respond to market needs faster and in shorter production cycles and also allows a greater surge capacity, greater process control, and a more reliable and well-characterized product.

Bulk production begins with the cultivation of the virus in a fermenter equipped with numerous process parameters to control temperature, pH, dissolved oxygen, and other factors. Two methods of mass cultivation of cells are recognized in the industry today, microcarrier cultures and free-cell suspension cultures. Both systems begin cultivation of the cell line in a fermenter, which can be scaled up to thousands of liters.[18]

The cell line used to cultivate the virus must be able to propagate the virus in large quantities, must be rapid and efficient in expressing the desired virus, and must be suitable for a wide variety of flu strains. It is desirable that the cell line be able to grow in a chemically defined synthetic medium that does not contain animal-derived components. It should also be scalable for industrial processes.[18]

Regardless of the cell-cultivation method, the cell line must be grown in a nutrient medium. A medium is a solution of either synthetic (serum-free) nutrient components or a complex substance of animal-derived protein or serum. There is less risk associated with synthetic media, provided they promote the growth of the cell line. The use of serum-free synthetic media has increased significantly, particularly when using serum presents a safety hazard and a potential source of unwanted contamination.[18]

Preparation of a cell line for propagation begins with the thawing of the cell line "seed" lot (In contrast, it can take up to six months to organize the egg supply for initial inoculation.) "First-pass" cell line propagation begins with the small-scale pre-culture propagation of seed cells after thawing. The cells are then introduced to the fermenter vessel with the selected nutrient medium. When the cell line reaches a predetermined cell density, the virus is introduced and begins to propagate in the cell line; after approximately three days the virus is harvested. After treatment of the infected cell line, the virus is released into the supernatant, and the cellular debris is centrifuged away. This occurs in a clean, closed environment, whereas harvesting of an egg-based virus is largely a manual process that requires extracting infected cells, breaking down cell walls, and then collecting the virus.[18]

Three cell lines were commercially proposed for cell culture-derived influenza vaccines (CCIV): *Madin Darby canine kidney cells* (MDCK), *Vero cells* (Kidney epithelial cells from an African green monkey) used for more than twenty years for polio vaccine production, and *PER.C6*, a human retina-derived cell line.[8] MDCK cells and Vero cells were especially promising cell-line candidates.

By 1998, Baxter Vaccines had already developed a Vero cell-based process to produce a new vaccine derived from cell culture. Solvay Biologicals licensed Influvac, a split virus vaccine produced in adherent MDCK cells, in the Netherlands in 2001. Baxter licensed in Europe Celvapan, a Vero cell-derived pandemic vaccine based on a wild-type "A/California/04/2009 (H1N1pdm09)"strain, in October 2009.[12,19] Preflucel, also manufactured by Baxter, is a seasonal influenza vaccine based on a Vero cell-line platform to produce three inactivated influenza viruses, including the A/H1N1 pandemic strain. During the 2008–2009 influenza season, a Phase III clinical study of Preflucel was conducted in the US. The study demonstrated that a Vero-derived vaccine was safe and well tolerated in both youths and adults.[20,21] However, on 20 October 2011, the EMA was informed by the Austrian Medicines Regulatory Agency that Baxter had recalled large batches from the EU market owing to increasing suspicion of side effects; as a precautionary measure, the EMA then recalled all batches from European markets.[22] PER.C6 cell lines have been shown to meet both EU and US regulatory requirements for the production of influenza vaccines. Having obtained a license to use PER.C6 for influenza vaccine production, Sanofi Pasteur started a Phase I clinical trial of their H7N1 vaccine in 2009. This was the first study conducted on a cell-based H7 pandemic virus vaccine candidate, and, although the vaccine was well tolerated, the results showed poor immunogenicity and humoral immune responses; thus, the vaccine did not meet the criteria for vaccine approval of the Committee for Medicinal Products for Human Use (CHMP).[23] To date, no flu vaccines derived from PER.C6 have been approved for use in humans.

Currently the cell culture-derived vaccines in the market are:

- Optaflu/ This vaccine was first approved in the EU.It is a trivalent subunit vaccine composed of two influenza A (H1N1, H3N2) strains and one type B strain, produced in MDCK cells from egg-adapted influenza viral seeds. The MDCK 33016 cell line grows in suspension in a serum-free and protein-free medium12. On 1 June 2007, Optaflu was manufactured by Novartis Vaccines and approved by the European Medicines Agency (EMA) for intramuscular use in the EU.[24] Currently this vaccine belongs to Seqirus.
- Flucelvax. In November 2012, the Food and Drug Administration (FDA) approved the first cell culture-derived influenza vaccine, Flucelvax. As the original brand name of this product, Optaflu, was deemed unacceptable by the Center for Biologics Evaluation and Research (CBER) in the US, the name "Flucelvax" was proposed and accepted.[25] Currently this vaccine belongs to Seqirus.
- *Celtura*, German regulatory authorities approved Celtura (Novartis), an MF59-adjuvanted, MDCK-CCIV A/H1N1

cell culture-derived pandemic vaccine, in November 2009.[26] A post-licensure vaccine surveillance study conducted in 2012 confirmed the good safety profile of Celtura.[27]

- *Preflucel and Celvapan.* Preflucel is Baxter's seasonal influenza vaccine formulated with inactivated H1N1, H3N2, and influenza B produced in Vero cells licensed in the EU in 2010. Baxter developed the monovalent Celvapan for H5N1 or H1N1, which was approved for commercialization in Europe in 2009.[12]
- *Flucelvax Quadrivalent*: This vaccine (Seqirus, Inc) was approved by the FDA in 2016. It is a tetravalent subunit vaccine composed of two influenza A (H1N1, H3N2) strains and two influenza B strains (Victoria, Yamagata), produced in MDCK cells from egg-adapted influenza viral seeds.

## Advantages of cell culture-derived influenza vaccines

- ✓ First, cell lines can be extensively characterized and stored for future use without the need for repeated full range testing, and cell culture avoids dependency on supply and quality control of a raw material such as ECE. In the event of outbreaks of avian influenza in poultry, the readily available supply of fertilized eggs may be insufficient, which frequently occurs in various continents.[28]
- ✓ Second, certain viruses grow better in cells, avoiding the down-time required for the generation of high-growth reassortants. Alternatively, high-growth reassortants can be directly generated in cells.[29,30] Recent data have suggested that most (over 90%) human isolates belonging to H3N2 are not recoverable in eggs.[15,31]
- ✓ Third, greater control during the standardized manufacturing process and sterility of the cell culture, medium and raw material reduce the risk of microbial contamination of the final product.[32,33]
- ✓ Fourth, allergies to egg proteins can be avoided. Indeed, conventional egg-based vaccines contain detectable amounts of some egg proteins; a risk of severe adverse allergic events following influenza immunization among egg-allergic vaccines is well-documented.[34] Egg allergy is the most common food allergy, especially in young children; a recent meta-analysis reported an overall lifetime prevalence of self-reported egg allergy of 2.5% (95% CI: 2.3–2.7%).[35]
- ✓ Fith, the process of serial passages in eggs may introduce important adaptive mutations, thereby altering matching and vaccine effectiveness.[36] By contrast, propagating the virus in cell lines does not lead to major changes in the amino acid sequence of hemagglutinin (HA).[15]

Furthermore, immune responses elicited by mammalian cell-derived vaccines have been shown to be more cross-reactive than responses produced by ECE-derived vaccines although protective efficacy may not be affected.[37] Finally, the same facilities can be used for the production of other vaccines when not being used for the production of influenza vaccine for extended periods.

## Mdck cell-derived influenza vaccines

Compared to other cell lines, the MDCK cells present several advantages for influenza vaccine production.

First, MDCK cells are the most suitable substrates among cultured cells to obtain primary isolates of influenza viruses.[34,38-41] So, comparisons of various cell lines for supporting the replication of live attenuated influenza viruses showed that MDCK cells are better than Vero: Medical Research Council-5 (MRC-5) human fetal lung fibroblast, Wistar Institute-38 (WI-38) human fetal diploid lung, fetal rhesus lung (FRhL), A549 human lung carcinoma and National Cancer Institute (NCI) H292 human mucoepidermoid bronchiolar carcinoma cells.[42-44]

Second, MDCK cells are the most suitable for large-scale production of influenza virus.[42, 45,46] Head-to-head comparison in laboratory scale bioreactors showed that MDCK cells yielded more virus than did Vero cells.[46]

Third, influenza virus replicates more rapidly in MDCK cells compared to other cell lines.[47] and can be adapted to produce high titers in MDCK cells in as few as 3 to 10 passages, i.e., in 10–30 days, depending on the strain. This may reduce the lead time for vaccine production.[48] Fewer passages during adaptation would also reduce the chances of accumulation of mutations around the receptor binding site of the HA protein. In addition, trypsin does not need to be added frequently for viral propagation in MDCK cells, avoiding potential chances of contamination, although trypsin inhibitors have also been reported to be secreted by MDCK cells.[49]

Fourth, the use of MDCK cells may be significantly more advantageous for the production of some influenza B virus vaccines.[45]

Finally, MDCK cells are refractory to human and mouse prions.[50] and in vitro data suggest that MDCK cell-derived components are not allergenic.[51,52] Extensive literature exists on the adaptation of MDCK cells for scaling up influenza vaccine production. The cells can be easily adapted to and grown in serum-free media, and in suspension, as well as on various microcarriers maintained under various bioreactor conditions.[45,53,54] Subclones of MDCK cells adopted to grow in suspension and to support robust virus production have also been described,[44,53,54] although adherent MDCK cells appear to support more robust virus production-replication of virus-, than suspension MDCK cells.[55]

## Safe and inmunogenic influenza vaccines derived from mdck

Influenza vaccines derived from MDCK cells are also safe and immunogenic. Initial studies, which compared ECE- and MDCK cell-derived vaccines in Phase I clinical trials, demonstrated the comparable safety and immunogenicity of both vaccines in children, healthy adults and the elderly.[56-59] Other studies found that MDCK cell-derived vaccines were, at least equivalent, and sometimes better and more efficacious as compared to ECE-derived antigens.[56,57,60-63]

In one instance, at-risk adult and elderly subjects who did not respond serologically to a previous ECE-derived vaccine

responded better when boosted with MDCK cell-derived vaccine as compared to an ECE-based vaccine.[64]

Since the early 1990s, reports of more than 20 clinical studies involving greater than 20,000 subjects in over a dozen countries, as well as large-scale immunization programs have further confirmed the safety and immunogenicity of MDCK cell-derived influenza vaccines.[57,59,60,65-67]

Recent studies that compare the safety and tolerability of cell culture-derived and egg-derived seasonal influenza vaccines in children at risk or healthy 4- to 17-year-olds show a similar profile in percentages of participans reporting solicited reactions, systemic reactions and reporting of unsolicited adverse events.[68,69] Immunogenicity results indicate that Flucelvax QIVc is effective against influenza similar to that provided by Flucelvax TIV and The risks of vaccination with Flucelvax Quadrivalent appear to be minor, and similar to that associated with trivalent Flucelvax. This results have served to recent approval of Flucelvax Quadrivalent by the FDA with a favorable overall benefit-risk profile for persons aged 4 years and older.[70]

## Present and future of cell culture-derived flu vaccine: Quadrivalent influenza vaccines

To reduce seasonal influenza epidemics, most industrialized countries have implemented influenza immunization strategies. However, two antigenically distinct lineages of influenza B viruses have circulated globally since 1985 and have co-circulated since 2001[71] – the Yamagata and Victoria lineages. The influenza B-lineage vaccine strains induce little or no cross-reactive protection against the alternate B-lineage,[72] such as in trivalent influenza vaccines (TIVs). The type B lineage selected for inclusion in the annual vaccine differs from the predominant circulating lineage in around 25% -50% of seasons.[73,74] In a mismatched season, influenza vaccine effectiveness may be suboptimal against influenza B epidemics, potentially leading to an increased public health burden during those seasons.[75,76] Influenza B accounts on average, for approximately 20–30% of influenza isolates from respiratory samples across seasons.[73,77] although the reported frequencies vary from year to year and from region to region. A large body of evidence from numerous countries demonstrates that influenza B accounts for a significant proportion of the overall burden of influenza that inundates healthcare services annually. Although the risk of breakthrough influenza A from vaccine strain mismatch remains, the risk of breakthrough influenza B from vaccine lineage mismatch can be eliminated by quadrivalent influenza vaccines (QIVs).[78]

This observation led the development of QIVs that included both of the circulating influenza B lineages.[72,79] Currently, some countries already include QIV next to TIV in their vaccination recommendations, like the US, Canada, UK and Australia.[80] However, in many other countries, including most European countries, TIVs are still used because either QIVs are not yet available, QIV procurement agreements with healthcare providers might still be being implemented.[81] or potential added benefits of QIVs are not or not yet recognized by national immunization technical advisory groups (NITAGs). A decision about switching from TIV to QIV is based on various criteria, of which a beneficial cost-effectiveness profile is often one of the principal aspects being considered by NITAGs in Europe.[82] Evaluations from different countries show very large variability in the seasonal impact of QIVs.[78] It is essential to make existing on disease outcomes and costs related to influenza B lineage viruses more available. More research will be required on the immunogenicity of natural influenza infection and vaccination with an emphasis on cross-reactivity between different influenza B viruses and duration of protection. The published dynamic models showed substantially greater improvement in health outcomes based on the use of QIVs as compared with the more conservative static models. However, although dynamic models better reflect the real-world impact of vaccination, dynamic transmission models are inherently more complex and require a greater degree of assumptions in terms of model inputs.[78]

To address the co-circulation of B-lineage viruses or B-lineage mismatch, QIVs have been developed and are likely to lead to more stable vaccine effectiveness across seasons, providing broader protection than TIVs and contributing to influenza prevention worldwide.[78]

For the future of flu vaccine, it will be necessary to do for coming improvements in flu vaccine such as more product differentiation (high dose, nasal via, use of adjuvants), even the potential ultimate ‘universal/ broadly protective’ flu vaccine. Until then, the extensive use of QIVs seems an excellent available option coupled with all advantages presented by the use of cell culture techniques for producing influenza vaccines.

## Conclusions

Although several companies continue to produce subunit egg-derived vaccines, new manufacturing platforms are being developed for new influenza vaccines. The development of faster and more innovative technologies will shorten manufacturing in comparison with egg-based vaccine production.

The production of vaccines by means of cell culture technologies has several advantages: Cell culture manufacturing is cleaner and faster, which is especially important in the case of a pandemic; the phenomenon of virus non-adaptation is avoided, and, lastly, the growing cell is controlled in defined culture media and validated cell banks in accordance with Good Manufacturing Practice (GMP), contrasting with the more lenient requirements applied to egg-based vaccine production. Immunogenicity has been found in different age groups, including children and adolescents similar to egg-derived vaccines. All developed estudies of safety and tolerability have shown a good profiles to CCIV The few available data on post-marketing surveillance confirm the robust safety of CCIV.

Creating a flexible and scalable system to supply influenza vaccine for the world’s population while considering safety and cost-effectiveness remains one of the major challenges of the influenza vaccine industry and the national and international public health agencies12. Despite advances, a pressing need for the traditional egg-based production remains and, for this reason it is necesary further development of cell culture-based vaccines, making their vaccines more effective and eficiency as possible and also deliver them as quickly as possible. These advances will be necessary to respond to a pandemic outbreak

of influenza virus, which is predicted as a potential threat in the upcoming years.

The availability of an alternative substrate, such as MDCK cells, may potentially prevent vaccine shortages and provide increased access to immunization. Moreover, cell culture-based technology has advantages over egg-based technology in terms of time-saving and flexibility.[8] Although CCIV is being increasingly used in routine immunization practice, its current market share is relatively small. This could be due to the fact that the vaccine is recommended only for those above 18 years of age[83,84.] However, given that egg allergy is much more prevalent among young children, this age group could gain substantial benefits from expanding the current age indication; indeed, a large pediatric trial.[63] found CCIV to be safe, well-tolerated and immunogenic in this population. Another explanation of CCIV's limited market share is that stakeholders in general may be less familiar with CCIV than with traditional vaccines.[85] Providing information on alternative options may be profitable, since this would increase the choices available to healthcare consumers. For example, the absence of egg allergens, antibiotics and preservatives in CCIV could be a constructive argument for increasing influenza immunization. The concomitant administration of influenza and pneumococcal vaccines is a common practice. Although CCIV is safe and well-tolerated in this age group when administered alone, co-administration of CCIV with pneumococcal vaccines may be associated with a higher rate of mild-to-moderate local and systemic reactions.[86]

Although varying from year-to-year, influenza B causes on average up to one-third of influenza infections each season. In parallel, the two influenza B lineages frequently co-circulate, and due to the complexity involved in accurately forecasting which B viruses will circulate, mismatches between the B strain selected for TIVs and circulating strains have occurred in up to half of the seasons. Evidence from clinical trials and observational studies suggest that B mismatched seasons are accompanied by a higher public health burden than well-matched seasons.[76] The risk of breakthrough influenza B from vaccine lineage mismatch can be decreased by QIV.

Based on the available evidence from clinical trials, epidemiological studies and modeling, several countries have progressively issued recommendations preferentially recommending QIVs over TIVs. Once budgetary constraints have been overcome, it seems plausible that the advantages offered by quadrivalent influenza vaccines drive future research toward quadrivalent cell culture-derived influenza vaccines.

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