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SPECIAL REPORT

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Strategy for approving a universal flu vaccine

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An urgent need exists for a universal flu vaccine to be more effective than current vaccines, yet there is no such vaccine in the market and no regulatory guideline for its approval. This report proposes a stepwise process for regulatory and marketing approval for such a vaccine. As a first step, the universal vaccine will be tested as a primer to existing HA-based vaccines. Regulatory approval and conditional marketing authorization will be granted based on safety assessments and improved hemagglutination inhibition antibodies (as a marker for efficacy) for the vaccines used in combination. Permanent market authorization will be granted next, based on clinical efficacy data, accumulated during several years, and ultimately the new vaccine will be approved as a standalone vaccine.

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KEYWORDS
- influenza virus
- M-001
- preventative treatment
- universal vaccine

Influenza disease

Influenza is an infectious disease caused by different strains of the influenza virus. The disease is endemic around the world, and manifests as seasonal or pandemic outbreaks. The influenza strains that are known to cause illness in humans are classified into types A and B based on the proteins in the virus. According to information published in March 2014 by the WHO [1], the global annual attack rate of seasonal influenza is estimated at 5–10% in adults and 20–30% in children, and between 250,000 and 500,000 of those infected die as a result of influenza-related complications. In addition, during seasonal influenza epidemics from 1979/1980 through 2000/2001, the estimated overall number of influenza-associated hospitalizations in USA ranged from approximately 54,000–430,000 per epidemic with an average of about 200,000 hospitalizations annually. On average 23,000 Americans die of the flu annually, 90% of whom are elderly persons (65+ years). Infants, adults over the age of 50 years and chronic disease patients are considered to be at-risk groups in that they suffer from influenza and its complications more than other groups.

Furthermore, according to the scientific journal, *Vaccine* [2], the financial cost attributed to influenza disease in USA was estimated at $87.1 billion annually, including $55.7 billion costs associated with the elderly (65+ years).

Current vaccines & their limitations

To date, the most common therapeutic treatments for influenza focus on pain and symptom relief. While antiviral treatments such as neuraminidase inhibitors may shorten the duration and severity of the disease, such treatments must be applied in the early stages of the course of the disease to

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be effective. Many countries around the world, including most European countries and USA, provide preventative treatment in the form of annual or seasonal influenza vaccines, which are especially recommended to persons in at-risk groups.

Seasonal vaccines target only some specific influenza strains that were circulating in previous seasons. The vaccines' targets are based on the WHO's educated guess regarding what particular strains will circulate in the upcoming season. Consequently, vaccine strains frequently do not match the circulating strains of that particular season. This is referred to as the 'vaccine-virus mismatch phenomenon'. CDC data on vaccine effectiveness [3] showed that during the seasons 2004/5 till 2014/15, the average vaccine effectiveness was only 40% in the general population; in the 2014/2015 influenza season, it was only 19% mainly due to a mismatch between the H3N2 strain contained in the vaccine and the circulating one. Furthermore, seasonal vaccine effectiveness is as low as 9% among the elderly [4], highlighting the need for a better vaccine especially for populations with weakened immune systems.

An additional drawback of currently available vaccines is associated with their long and complex production cycle resulting in restricted supply and lack of flexibility in responding to market needs. Upon selection of vaccine strains by the WHO, high-growth reassortant seed viruses are prepared and the vaccine manufacturers then work to produce the vaccine before the start of the influenza season. The vaccine strains are grown in hen eggs followed by purification steps and release testing. The whole process takes approximately 6 months in an ideal setting [5]. It should be noted that vaccines that are produced in eggs can pose a risk of anaphylactic responses in egg-allergic individuals; accordingly, the Advisory Committee on Immunization Practices recommends that such vaccines be used under medical supervision for allergic individuals [6]. There are several universal candidates under development [7]; the current report discussed the epitope-based vaccine candidate Multimeric-001 (M-001).

M-001 universal flu vaccine

M-001 vaccine is a multiepitope, peptide-based, single-chain recombinant protein that is expressed in *Escherichia coli* and further purified by chromatography [8]. The M-001 contains nine B- and T-cell epitopes derived from HA, nucleoprotein and matrix protein 1 sequences that are conservatively expressed among multiple influenza A and B viruses and it is inducing protective immunity against them [9,10], it is administered without an adjuvant. These proteins were intensively studied throughout the years and conserved epitopes within them were identified and examined in animal models. The epitopes selection was based also on their ability to bind multiple prevalent HLA molecules including both class-1 and class-2.

By using a vaccine that is based on conserved viral epitopes, the M-001 vaccine stimulates pre-existing memory T cells established by previous seasonal human influenza A infection. These T cells cross-react with future strains by targeting highly conserved internal proteins. In a manuscript by Lee *et al.* [11] that defined the immunity to H5N1 among healthy individuals, it was shown that memory $CD4^+$ and $CD8^+$ T cells isolated from the majority of these healthy individuals recognized multiple influenza peptides, including peptides from the H5N1 strain. Matrix protein 1 and nucleoprotein were the immunodominant targets of cross-recognition. They conclude that vaccine formulas inducing heterosubtypic T-cell mediated immunity may confer broad protection against avian and human influenza A viruses. Indeed, in preclinical trials, the M-001 vaccine induced protective immunity in mice against different influenza A and B strains, it also protected against infection with the highly pathogenic H5N1 strain [12].

The WHO's Preferred Product Characteristics (PPC) for next-generation influenza vaccines [13], including those vaccines that induce broadly protective and long-lasting immune responses was presented in August 2016 at the *8th WHO Meeting on Development of Influenza Vaccines That Induce Broadly Protective and Long-Lasting Immune Responses* [14]. BiondVax's M-001 meets or exceeds the current PPC recommendations both in terms of functionality and timeline. The WHO recommendations include:

- Statement of unmet public health need: "Safe and well-tolerated influenza vaccines that are effective at preventing severe influenza illness, that provide protection beyond a single year and that are programmatically suitable for use are needed for low- and middle-income countries."
- PPC Vaccine performance strategic goal 1: By 2022, influenza vaccines in advanced clinical

development that can feasibly provide greater protection against vaccine-matched influenza strains or against drifted influenza A strains than currently available unadjuvanted, inactivated influenza vaccines that protect against severe influenza A virus illness through at least 1 year after a primary series and that are suitable for high-risk groups in low- and middle-income countries.

These recommendations indicate it is recognized that there is an urgent need for BiondVax's concept and that there is a massive global market in developed and low- and middle-income countries for M-001.

Regulatory challenge & solution (prime-boost)

The basic concept for achieving regulatory approval of a novel universal influenza vaccine can be defined as 'evolution rather than revolution'. That is, a stepwise approach which will first rely on the existing surrogate marker (hemagglutination inhibition [HAI]) and then use newly defined markers. A different clinical pathway and end points will be employed for each of these two steps.

The current regulatory marker for protection against influenza is the HAI antibodies that target the outer variable regions of the viral hemagglutinin. Since M-001 [8] by design targets the conserved regions of the virus, it does not elicit such HAI antibody response by itself. Rather, it induces a cellular immune response. Seeking a conservative and cautious approach, BiondVax introduced the concept of 'evolution rather than revolution' by which we recommend, for an intermediate period, to continue using the existing HA-based flu vaccines concomitantly with the M-001 universal flu vaccine as a primer. This approach will demonstrate efficiency in relatively small-scale economical Phase III clinical trials, leveraging the currently approved HAI-antibody response as a surrogate marker for flu vaccines. The measured HAI response triggered when using M-001 as a primer several weeks prior to the administration of seasonal or pandemic commercial vaccines will serve as an end point for the trial. We have demonstrated that when given as a primer to HA-based flu vaccines, M-001 enhances and broadens the HAI antibody response to the strains contained within the commercial HA-based vaccines and also to other drifted strains. This is manifested by higher levels of protective antibodies, indicating that M-001 essentially provides a safety net to the vaccines.

Following commercialization of the M-001 vaccine as a primer to seasonal and/or pandemic vaccines, real-world data efficacy monitoring will be conducted for several seasons. Incidence of laboratory-confirmed influenza illness in patients primed with the M-001 as compared with those who were not primed will be evaluated. It is expected that the cell mediated immunity (CMI) and the memory responses induced by immunization with M-001 will reduce the severity of illness, especially in years when there is a mismatch between the vaccine strain and the circulating strains. Possibly, the regulator will recommend getting both vaccines as standard of care for at-risk populations.

The next regulatory step will seek approval of M-001 as a standalone universal flu vaccine. For this step, a larger Phase III clinical trial with a clinical efficacy end point demonstrating reduction of illness rates and severity will be conducted. Once approved under this regulatory pathway, the M-001 universal flu vaccine will replace the existing HA-based flu vaccines.

Proposed Phase III trial strategy

M-001 has been tested in five Phase I/II and Phase II clinical trials totaling ~700 adults and elderly (65+ years) participants [10,15]. To further confirm its safety and immunogenicity, Phase III trials will be conducted to support the following indications: M-001 as a primer for seasonal vaccine for the elderly; M-001 as a pandemic primer for pre-pandemic preparedness and national stockpile.

Comprising 90% of all seasonal flu-related deaths, the elderly suffer the most from influenza complications, morbidity and mortality [16]. Prioritizing this population first is supported by health authorities. The trial will include 5000 participants aged 65+ years, and the proposed title of the trial is: "M-001 as a primer to the seasonal influenza vaccine for the elderly".

For safety considerations, regulatory bodies have suggested the trial includes 3000 participants in the experimental group. Accordingly, there will be 1500 participants in the placebo group (a 1:2 ratio). Assuming a 10% drop out rate (3000 + 1500) * 1.1 = 4950 participants, the trial will include about 5000 in total. See **Table 1** for schematic trial design.

Table 1. Proposed trial design.

Arm	N	Administration 1 Day 0	Administration 2 Day 21	Blood for HAI Day 42
Experimental	3350	M-001 (1.0 mg)	TIV vaccine	+
Control	1650	Saline	TIV vaccine	+

Trial outcomes

• Immunogenicity outcomes

Primary: To measure the serum HAI titers toward the influenza strain contained in the seasonal HA-based vaccine on days 0 and 42 (21 days following HA-based vaccine administration) in all subjects. A seroconversion (SCR) response of ≥10% to at least two of the trivalent influenza vaccine (TIV) strains and noninferiority to the third TIV strain will be considered successful. Note that these criteria were accepted by the US FDA in the Sanofi Fluzone High Dose flu vaccine marketing authorization [17].

Secondary 1: To demonstrate the difference in cellular immune responses on days 0 and 21 in a subset of subjects from each group. A statistically significant difference will be considered successful and indicative of the universality of the vaccine.

Secondary 2: To measure the serum HAI titers toward drifted strains not contained in the HA-based vaccine used for boosting in all groups on days 0 and 42. An SCR response of ≥10% to representative strains will be considered successful.

• Safety outcomes

Primary 1: To record the solicited adverse events (AEs) in all subjects up to 21 days after dosing of the study vaccine (M-001). An observation of no statistically significant difference in drug-related AEs between study arms will be considered successful.

Primary 2: To record the unsolicited AEs and all serious AEs in all subjects during the entire study period. An observation of no statistically significant difference in drug-related AEs between study arms will be considered successful.

For immunogenicity and efficacy considerations, as described above in the trial's primary outcome to be 10% SCR elevation, statistical calculations indicate that 900 participants (600 in the experimental arm: M-001 and TIV and 300 in the control arm: placebo and TIV) would suffice to consider SCR elevation of 10% as statistically significant.

The trial size of 5000 allows the evaluation of M-001's efficacy with boost immunization by major commercial HA-based flu vaccine brands, thereby enabling approval of M-001 as a primer to all those TIV brands.

Gradual go-to-market strategy

As stated above, the introduction of a novel pharmaceutical product, particularly one targeting large and healthy populations, should be a gradual process. As shown in **Figure 1**, the proposed introduction of M-001 features steps that initially allow its evaluation as a primer to currently available vaccines.

Upon finalizing the Phase III trial for the seasonal primer for the elderly indication, that is based on safety and immunogenicity outcomes, we plan to receive conditional marketing authorization for the elderly. Additional trials will then be conducted to test the efficacy of the same prime-boost vaccination approach in other at-risk populations such as pregnant women and children. In young children an initial vaccination by three administrations could be implemented, parallel to all other pediatric vaccine.

Ultimately, when sufficient safety and efficacy data are accumulated from field observations and using healthcare data resources including real-world data for the seasonal primer, we expect to obtain permission to conduct Phase IV clinical efficacy trials with M-001 as an independent standalone vaccine to seasonal influenza. These will be large-scale trials involving tens of thousands of participants with an efficacy end point of reduction of influenza illness rate and severity following vaccination with the M-001 as an independent vaccine compared with TIV. With careful trial design, we also plan to show the extended duration of protection that may be achieved with M-001, thereby positioning M-001 as a broadly protective, extended duration, universal influenza vaccine.

This stepwise regulatory approach was developed based on M-001's characteristics (e.g., its inherent priming capabilities), regulatory and scientific precedents and discussions with leading regulatory agencies. BiondVax plans to apply for an FDA-accelerated approval pathway which includes getting conditional marketing approval based on a limited-sized Phase III clinical trial. In parallel, BiondVax will submit

a marketing authorization application to the EMA.

It is important to stress that this plan complies with the revised EU guidelines on influenza vaccines, nonclinical and clinical module, which became effective in February 2017 [18]. This guideline explicitly allows comparative analysis of immunogenicity parameters supplemented by postmarketing effectiveness studies.

An alternative regulatory pathway toward permanent marketing authorization may include a postmarketing Phase IV clinical efficacy study evaluating illness rate and severity. In this case, flu incidence would be considered when calculating sample size.

Discussion/conclusion

Vaccines have been the most efficient tools in public health to date, and have led to the eradication or drastic reduction of many severe infectious diseases. Yet, influenza still represents a major threat to public health. This is more concerning in the last decade in view of potentially virulent avian pandemic strains which have emerged and infected human hosts, causing high morbidity and mortality. Although there is an effort to vaccinate large portions of the population annually, this is not sufficient mainly because the currently available influenza vaccines are strain-specific. A universal flu vaccine will provide an effective protective and preventive solution with the potential to achieve eradication of influenza after establishing sustained community immunity. Indeed, several approaches have been attempted in recent years to generate a universal vaccine for influenza, based on the stem of the HA molecule [19], or the matrix protein M2 [20], which are rather conserved in many influenza strains, or on conserved T-cell epitopes [21], or the M-001 described in this commentary. However, an inherent difficulty in developing a universal vaccine is the regulatory issue: Since at any point in time when a single clinical trial is run, there are only one or two major circulating strains and so even if a high level of protection is shown, it is only against these strains, and does not prove the claim of universality. For the approval of a universal vaccine, the inability to predict future mutations of flu strains will lead to the inefficient process of repeated clinical trials to be conducted for a number of consecutive years, showing protection against a range of different circulating strains. Under current regulatory requirements, this would be an expensive, years-long process which would be impractical for any company seeking to develop such a vaccine. Furthermore, with infection rates of ~5%, clini-

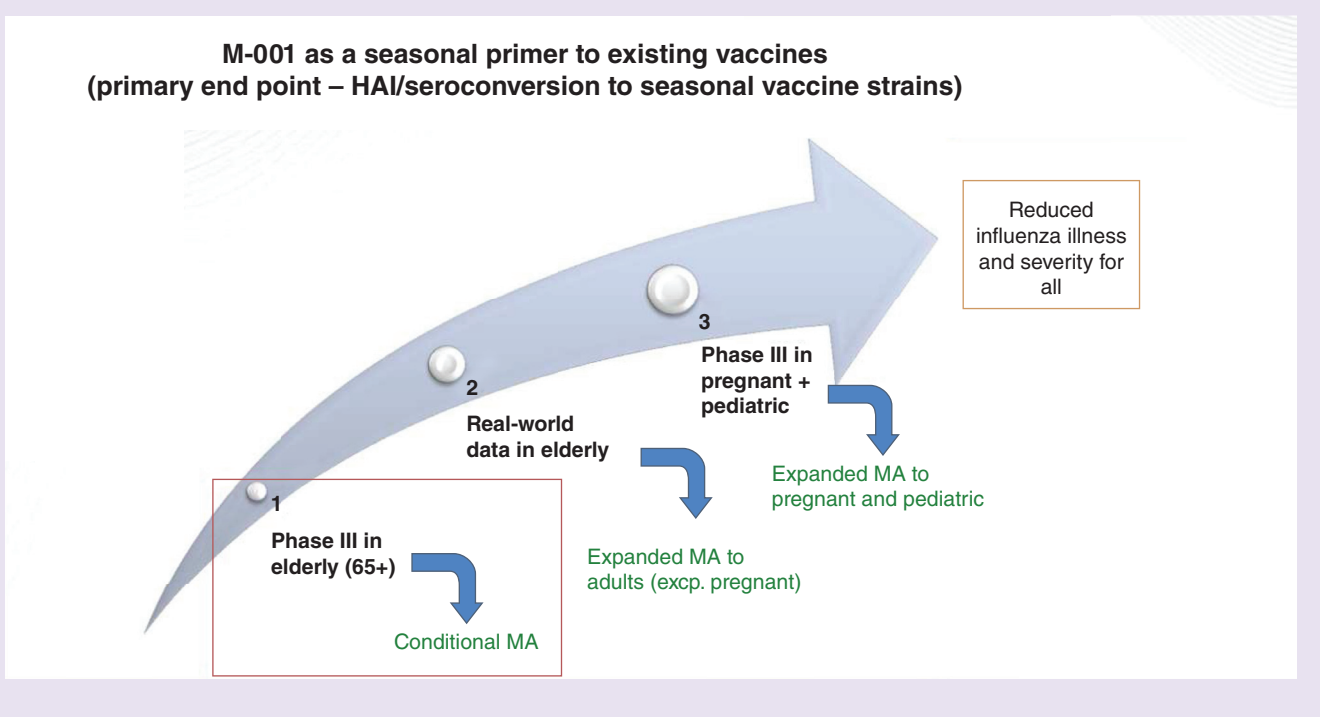

Figure 1. Gradual licensure scheme for M-001.
M-001: Multimeric-001; MA: Market authorization.

cal trials with a huge number of participants are required to show a significant effectiveness.

The prime-boost concept described in this commentary should overcome this regulatory challenge. In a single trial it allows researchers to empirically test, using HAI antibodies, the seroprotective effect of priming with M-001 against many strains of influenza, thus demonstrating its M-001's universality.

Once approved as a primer by regulatory authorities and administered for several years, in many thousands of individuals, M-001's efficacy as a standalone standard-of-care vaccine may also be demonstrated.

Additional advantages of BiondVax's M-001 because of its action against any new and future seasonal and pandemic strain, extended duration of activity and its year-round production cycle, include the ability to stockpile M-001 for immediate use whenever and wherever the need arises.

Future perspective

A universal flu vaccine that is based on conserved viral epitopes has a unique CMI-based mode of action and hence HAI or neutralizing antibodies do not always serve as a correlate of protection. A gradual innovative regulatory pathway for the approval of such vaccines is presented in this commentary. It is expected that several universal vaccine candidates that are currently in clinical trials Phase I and II will advance into Phase III trials within the coming years. Evaluation of cell-mediated immunity in such trials will result in defining new immunological correlates of protection and the regulator will have to set a route for approval of these new-generation vaccines.

Financial & competing interests disclosure

All authors are either employees or related to the BIONDVAX pharmaceuticals company that is developing M-001. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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EXECUTIVE SUMMARY

- There is need for a better influenza vaccine to overcome the morbidity and mortality resulting from the disease especially in at-risk populations.
- M-001 is a recombinant protein, epitope-based universal vaccine candidate that contains conserved influenza A and B peptides.
- M-001 mode of action is based on induction of cell mediated immune responses. When used as a primer before HA-based vaccines, it enhances and broadens the hemagglutination inhibition responses as compared with the HA-based vaccine alone.
- A gradual regulatory strategy is presented: initial approval as a primer, in other words, in combination with current strain-specific vaccines followed by approval as a standalone vaccine based on efficacy clinical trials.

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Future VIROLOGY

EDITORIAL

How close are we to a respiratory syncytial virus vaccine?

"WHO has ranked respiratory syncytial virus) as the number one target pathogen for pipeline vaccines."

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KEYWORDS
• clinical trials
• lower respiratory tract infection
• respiratory syncytial virus
• targeted immunizations • vaccines

As presented at the International Respiratory Syncytial Virus Symposium, WHO has ranked respiratory syncytial virus (RSV) as the number one target pathogen for pipeline vaccines. Renewed interest in RSV vaccines has been spurred by a better understanding of the global impact of the virus and by innovative vaccine discovery. RSV is a leading cause of childhood lower respiratory tract infection and hospitalization in children under the age of 5 years [1,2] and the elderly [3]. There are three major target populations for RSV vaccines: pregnant women (to protect newborns via passive immunization), seronegative children from approximately 4–6 months of age until 24 months of age and the elderly. Multiple vaccines are in clinical development in these target populations, and additional candidates are in preclinical stages. The recent failure of a Phase III trial of an F-protein-based vaccine in the elderly underscores remaining challenges and suggests that we are not as close to a licensed RSV vaccine as thought. However, progress is being made toward prevention of RSV disease in newborns (maternal immunization and novel monoclonal antibodies [mAbs]), the broader pediatric population (active immunization) and the elderly.

The virus

RSV is a prototype orthopneumovirus in the recently classified Pneumoviridae family and has a negative-sense, nonsegmented ssRNA genome of approximately 15 kb which encodes 11 proteins. The two major surface glycoproteins are: the attachment glycoprotein (G) and the fusion glycoprotein (F), which are responsible for attachment of the virus to host cells and fusion of the viral membrane to that of the host cell, respectively. RSV is further classified into subgroups: A and B based on the sequence of the G protein, and both subtypes can circulate simultaneously during an RSV season. The F and G proteins are antigens that induce virus-neutralizing antibodies (nAb), and therefore vaccine efforts have been concentrated on these two proteins. The F protein is the primary

"Longer-lasting, more potent, less expensive respiratory syncytial virus monoclonal antibodies are important part of the respiratory syncytial virus pipeline, especially if maternal immunization is not highly effective."

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vaccine target because F is more conserved than G and nAb against F are thus far measured to be more potent. The prefusion conformation of the F protein (pre-F) is the target of most nAb [4,5]. For this reason, pre-F holds promise for novel RSV vaccines.

"In recent studies, although the adult populations and vaccine doses differed, immunogenicity of pre-F and post-F protein vaccines appeared roughly comparable."

Disease burden

RSV infects most children in the age of 2 years, and reinfection is common due to incomplete immunity following natural infection. RSV infection is associated with bronchiolitis and bronchopneumonia in children <5 years of age and in young infants and children with comorbidities such as chronic heart and lung disease. RSV incidence and mortality can vary widely from year to year, between regions and within countries. In 2005, an estimated 33.8 million new cases of RSV-associated acute lower respiratory tract infection (ALRI) cases occurred worldwide in children <5 years of age with at least 3.4 million of these cases necessitating hospitalization which resulted in about 200,000 deaths in this population [1,6]. The rate of RSV hospitalization in the USA is highest in infants 0–3 months of age, however the total number of hospitalizations is greater in the 3–24-month-old range than in 0–3-month-old range [7]. Infants with RSV-associated lower respiratory tract infections have a higher risk of developing wheeze and asthma during childhood. Elderly and immunocompromised individuals are at increased risk of severe RSV disease, with approximately 177,000 hospitalizations and over 10,000 RSV-associated deaths in adults over 64 years of age recorded annually [3].

Correlates of protection

Serum nAb titers are the most useful correlates we have for protection against RSV disease. Serum nAb titer >6 $\log_2$ correlated with protection from RSV hospitalization [8]. A detailed protocol was recently published [9], and RSV nAb assay standardization would benefit the field. Infants with higher levels of maternally derived antibody (Ab) develop infection at a later age during childhood, and infants infected in the presence of maternally derived Abs develop milder disease than infants with a lower Ab titer [10]. Maternal Ab affords early protection, evidenced by infants <1 month of age having a lower rate of RSV infection than infants of 1 month of age [7]. Recently, two studies demonstrated that the half-life of maternally derived RSV Ab is between 36 and 38 days in infants [11,12]. Other correlates of protection may be important for RSV. For example, RSV-specific nasal IgA correlated more strongly with protection against challenge in human volunteers than did serum nAb [13]. Cellular immunity likely contributes to clearance and correlates with recovery.

Vaccine-enhanced disease

Formalin-inactivated RSV vaccine led to enhanced disease and death among vaccinated infants following natural RSV exposure. Subsequently, protein-based RSV vaccines and vectored vaccines demonstrated vaccine-enhanced disease in animal models [14]. RSV vaccine enhanced disease is highly reproducible in animal models, yet still poorly understood. RSV vaccine enhanced disease is primed in the RSV-naive setting and not thought to occur in seropositive individuals. The cotton rat model of RSV vaccine enhanced disease is widely accepted as being sensitive to reporting enhanced disease, but a negative result (no enhanced disease) in this model is not considered predictive and therefore does little to de-risk clinical development. The history of RSV vaccine enhanced disease led to the development of live-attenuated vaccine candidates for seronegative children and high regulatory hurdles for nonreplicating vaccines for this population.

Live attenuated

Live-attenuated RSV vaccines remain most clinically advanced in the pediatric target population. In general vaccinology, live-attenuated vaccines are more immunogenic than nonreplicating vaccines, but care must be taken to achieve safety, and an effective balance of attenuation and immunogenicity has yet to be clearly demonstrated for RSV despite years of effort. Although young infants readily mount nAb responses to natural infection [15], vaccine strains generated by passage have not been sufficient, potentially related to incomplete natural immunity. Novel mutagenesis strategies for RSV vaccine strains attempt to attenuate the virus and simultaneously enhance immunogenicity. For example, deletion of the viral M2-2 gene preferentially favors viral genomic transcription rather than replication resulting in the synthesis of viral proteins which stimulate protective Ab response with limited infectious virus production [16]. The Laboratory of Infectious Diseases at NIAID, NIH has several RSV live-

attenuated candidates, mostly based on M2-2 deletion, in Phase I studies. Another example of rational vaccine design is the attenuation of expression of viral interferon antagonist proteins NS1 and NS2 [17,18]. RSV live attenuated vaccines given through the intranasal route, have been given to infants as young as 2 months of age, and are being targeted to the seronegative (<24-month-old) pediatric population.

Vectored

There are multiple adenovirus (Ad) vector-based RSV vaccine candidates in clinical trials. Janssen Pharmaceuticals is developing replication-deficient Ad35 and Ad26 RSV F vectors. GlaxoSmithKline, by acquisition of Okairos, is developing chimpanzee Ad vectors expressing the viral F, N and M2-1 proteins [19]. Vaxart is pursuing an oral Ad5 vector that expresses RSV F and a dsRNA adjuvant. Bavarian Nordic is developing a modified vaccinia Ankara (MVA) vectored RSV vaccines that expresses the N, M, G and F proteins. The adenovirus and MVA vector strategies result in cellular and humoral immune responses due to induction of both MHC class I and II processing, and the platform technologies are established.

Subunit

Novavax has taken the lead in developing protein-based RSV vaccines. Their post-F protein vaccine has three target populations, the elderly (>60 years, Phase III), infants (via maternal immunization, Phase II) and older children (24 months to 5 years, Phase I). This recombinant postfusion conformation F protein vaccine was engineered to expose antigenic site II of the F protein, the same site that paluvizumab targets, and assembles into a rosette particle. Although this vaccine was shown to be immunogenic and to elicit paluvizumab-competing Ab, recent Phase III results for the nonadjuvanted version of this vaccine in the elderly were negative. The reasons include but are not limited to, low vaccine efficacy and/or lower than expected attack rate in the population. The Phase II study in pregnant women demonstrated a rise in transferred nAb. GlaxoSmithKline is developing a pre-F protein subunit vaccine targeted for pregnant women. Maternal RSV F protein vaccines are designed to boost immunity during the third trimester of pregnancy to enhance maternal transfer of Ab to infants. In recent studies, although the adult populations and vaccine doses differed, immunogenicity of pre-F and post-F protein vaccines appeared roughly comparable [20,21]. Additional clinical data will shed light on the promise of pre-F protein as a vaccine antigen.

Novel prophylactic antibodies

A humanized version of mAb D25 (MEDI8897, MedImmune) is an mAb that targets pre-F and is more potent than palivizumab [22]. MEDI8897 also harbors mutations in the Fc portion (252Y/254T/256E) that increase Ab half-life [23]. This mAb is aimed at preventing RSV disease in pediatric populations and is currently being evaluated in an ongoing Phase II clinical trial. Another mAb, REGN2222 (Regeneron, NY, USA) is currently being assessed in a Phase III trial in preterm infants. One goal of novel prophylactic anti-RSV mAbs is to reduce the cost of goods for manufacturing and expand beyond the current high-risk target populations (compared with palivizumab). Longer-lasting, more potent, less expensive RSV mAbs are important part of the RSV pipeline, especially if maternal immunization is not highly effective. Also, maternal immunization will not likely apply to very premature infants, those born before maternal vaccines are administered in the third trimester. Limitations of mAbs are efficacy and breakthrough (resistance).

Conclusion

The WHO presented two priorities for RSV vaccines at the 2016 Symposium: maternal/passive immunization to prevent RSV disease in infants less than 6 months old and pediatric vaccines to prevent RSV disease in infants once maternal antibodies wane. Given the number of active RSV vaccine programs, vaccines that will one day be licensed have likely been generated. The field has benefited from an influx of academic and pharmaceutical groups and the diverse expertise and technologies they bring to bear on the problem. The field has also benefited from involvement of additional stakeholders likes the Bill and Melinda Gates Foundation and PATH. How close are we to an RSV vaccine? We are closer than ever before. Challenges ahead are viewed as surmountable. Remaining questions include the following: Will the pre-F conformation of the F protein be a game changer for immunogenicity? Can a parenterally administered protein protect the lung despite modest immunogenicity of RSV protein vaccines tested in the elderly and pregnant women [24]? Can novel strategies

"The Phase II study in pregnant women demonstrated a rise in transferred virus-neutralizing antibodies. GlaxoSmithKline is developing a pre-F protein subunit vaccine targeted for pregnant women."

for live-attenuated candidates reach an effective balance of potency and safety? Can safety of a nonreplicating RSV vaccine in seropositive subjects de-risk the vaccine for seronegative infants? An extensive list of current vaccine candidates can be found here [25].

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REVIEW

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Future VIROLOGY

Vaccines against chikungunya, Zika and other emerging *Aedes* mosquito-borne viruses: unblocking existing bottlenecks

Giovanni Rezza*

The emergence of *Aedes* mosquito-borne viral diseases is a global public health challenge. Since mosquito control programs are not highly efficient for outbreak containment, vaccines are essential to limit disease burden. Besides yellow fever vaccines, a vaccine against dengue is now available, while research on vaccines against Zika has just started. Several vaccine candidates against chikungunya are undergoing preclinical studies, and few of them have been tested in Phase II trials. To overcome hurdles and speed-up the development of vaccines against these viral diseases, several actions should be planned: first, the 'animal rule' could be considered for regulatory purposes; second, public–private partnership should be stimulated; third, countries, international organizations and donors commitment should be strengthened, and potential markets identified.

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KEYWORDS
• arbovirus • chikungunya
• neglected infections
• vaccine • viral disease
• Yellow fever • Zika

Background

Arthropod-borne viruses (arboviruses), in particular those transmitted through the bite of *Aedes* spp. mosquitoes, are emerging in previously naive areas of the world, expanding their geographical area of activity and spreading among human populations. Most of these viruses belong to the *Flavivirus* genus of the Flaviviridae family and to the *Alphavirus* genus of the Togaviridae family. The former group includes the yellow fever virus, the dengue virus (DENV) and the Zika virus (ZIKV), and the latter group includes the chikungunya virus (CHIKV) and other geographically restricted viruses; these viruses present an important challenge for the global public health [1]. Mosquito control activities may be successful in controlling local outbreaks occurring in temperate areas but do not appear to be able to mitigate large epidemics in tropical areas. Thus the availability of safe and effective vaccines is essential in order to reduce the burden of disease.

Currently there is a vaccine against dengue which has just been approved but there are no vaccines available against other neglected viral diseases, such as chikungunya and Zika. This may appear quite surprising, since technological advances are likely to allow the delivery of such vaccines. However, there are several bottlenecks that need to be identified and unblocked in order to promote, expand and accelerate research and development (R&D) activities [2]. We discuss these issues below, targeting our review on *Aedes* mosquito-borne viruses.

• The yellow fever vaccine: a successful story

Few vaccines against arboviruses have been licensed so far. The prototype is represented by the vaccine against yellow fever, which has been available since the 1930s. Yellow fever is a zoonotic disease, caused by a flavivirus, which is endemic in the tropical regions of Africa and South America, where nonhuman primates and mosquitoes are involved in the sylvatic cycle. Spill-over infections may occur, causing human outbreaks in urban areas, where *Aedes aegypti* is the dominant vector. Since

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1982, after the demise of an attenuated vaccine obtained from 176 passages of a 'French neurotropic virus' strain in neonate mouse brain, the only available vaccine is a live-attenuated vaccine derived from the 17D strain (obtained after 204 passages of the Asibi Ghana strain in mouse brain) and from a strain called 17DD (obtained after 195 passages of the Asibi strain) [3–6]. The yellow fever vaccine succeeded a variety of challenges, from attenuation of the virus in tissue culture to its large manufacture in eggs, and to the elimination of contaminants. The WHO, in conjunction with the Global Alliance for Vaccines and Immunization considers the yellow fever vaccine as essential for both preventive and emergency use [4]. The utilization of the yellow fever vaccine in emergency settings may rely also on its high immunogenicity, which drove WHO's decision to recommend the use of a lifelong single dose [7]. A recent example of the yellow fever vaccine being used in outbreak control is represented by the large vaccination campaign conducted in Angola. During this urban outbreak, millions of doses were administered in a few months to protect the population [8].

• The dengue vaccine: when innovation meets public health

Nowadays dengue is the most important and widespread arboviral infection. Infection with one of the four dengue serotypes does not prevent infection with the other serotypes. In fact subsequent infection by the other serotypes increases the risk of developing severe dengue, due to the induction of so-called antibody-dependent enhancement [9]. All of the four dengue virus serotypes have been detected in most tropical and subtropical areas of the new and old world, and co-circulation of different serotypes in the same area is a common finding. Moreover, clusters of cases and small outbreaks have also been reported in Mediterranean Europe [10,11].

A recombinant, live attenuated, tetravalent dengue vaccine based on the yellow fever 17D vaccine strain (CYD-TDV) has completed Phase III trials, with partially successful results. In particular, the findings of a IIb trial conducted in Thailand showed an efficacy of about 30% which differed by serotype. The vaccine protected against three of the four serotypes, but not against DENV2 [12]. This finding was rather disappointing since the serotype 2 is predominant in many affected areas of the world where DENV is present. A Phase III trial conducted in Asia showed that vaccination may reduce incidence of symptomatic infection and hospital admission with 56.5% efficacy. However, the protective efficacy against serotype 2 was confirmed to be quite low of around 35% [13]. In another Phase III trial conducted in Latin America, the vaccine proved to be effective against all the four serotypes. Overall, vaccine efficacy was 65%, ranging from 78% for serotype 4 to 42% for serotype 2 [14]. However, there are five other vaccine candidates against dengue currently in human clinical trials, two in Phase II and three in Phase I [15].

Nowadays policymakers are designing their vaccination strategies by taking into account vaccine efficacy and safety data as well as data on the burden of disease. An analysis of a trial conducted in Latin America estimated a high vaccine preventable disease incidence, which is a measure of the expected burden reduction. This means that in order to prevent a single event (i.e., one case of disease), the number of people needed to vaccinate is low [16], indicating that vaccination in similar contexts would be highly cost effective. In this regard, an important issue is represented by the identification of the target population; for example, in accordance with WHO recommendations, countries should consider introducing the vaccine only in geographic settings with high endemicity. Up until now, the dengue vaccine has been licensed by regulatory authorities in Mexico, the Philippines and Brazil. However, the marketing authorization is limited to the population living in these countries and the vaccine is not yet prequalified by WHO [17].

• A vaccine against chikungunya: a new challenge for global health

CHIKV is an RNA alphavirus belonging to the Togaviridae family, causing a self-limiting illness characterized by high fever, headache and long-lasting severe joint pain, sometimes accompanied by maculo-papular skin rash and itching. Severe complications, such as encephalitis, may occur in the elderly and individuals with comorbidity [18].

CHIKV was first identified in Tanzania in 1952 [19]. Since its identification, sporadic cases and outbreaks were reported in several African countries, on the Indian subcontinent and in southeast Asia [18,19]. During the last decade, CHIKV has expanded its range of activity, conquering new territories and becoming an important global health threat. In particular,

CHIKV re-emerged in 2004 with a series of large outbreaks, which started on the coasts of Kenya and then ravaged Indian Ocean islands in early 2005 and the Indian subcontinent in 2005/2006 [20,21], causing more than one and half million cases of disease. Surprisingly an outbreak of CHIKV occurred in northeast Italy in the summer of 2007 [22], while two and 11 autochthonous cases were also identified in Mediterranean France in the summer of 2010 and 2014, respectively [23,24].

Although research on vaccines against CHIKV has been scanty, several vaccine candidates have been tested so far – both inactivated and attenuated vaccine candidates have shown promising results in human Phase I/II trials.

Initially, formalin-inactivated vaccines against CHIKV were found to induce neutralizing antibodies [25–27]. However, to contain production costs and to reduce the risks associated with handling large quantities of nonattenuated virus prior to inactivation, vaccine research was then directed toward the development of live attenuated candidates.

The first attenuated vaccine candidate was a serially passaged, plaque-purified live chikungunya vaccine [28], which was evaluated in a Phase II clinical study [29]. All vaccinated individuals developed neutralizing antibodies; however, 8% of them had side effects such as transient, mild joint pain, a commonly observed sign of chikungunya fever [30]. Although the risk of transmission of the attenuated CHIKV strain to *Aedes* spp. mosquitoes was considered remote, due to the low and transient levels of viremia [31], the occurrence of arthralgia suggested insufficient and/or unstable attenuation, and later studies indicated that the attenuation was probably mediated by two point mutations [32]. Moreover, seroconversion rates for CHIKV were rather low (36%) among individuals who had been previously vaccinated with other live attenuated alphavirus vaccines, such as a vaccine against Venezuelan equine encephalitis virus, suggesting immunological interference between these vaccines [33]. Because of unconvincing results, scarcity of funding and concerns about the potential market [29,34,35], this vaccine candidate did not advance to Phase III trials [2].

To maintain the advantages of attenuated vaccines consisting of single-dose protection and long-lived immunity, novel attenuated CHIKV vaccine candidates were developed with the intent to improve safety by providing promising results in animal models [36]. However, since some residual infectivity of the vaccine strains in mosquito vectors could not be excluded [37], genetically engineered vaccines were developed and successfully tested in animal models [38–41], including nonhuman primate models [42].

Chimeric vaccines using alphavirus vectors (Venezuelan and eastern equine encephalitis virus, and Sindbis virus) and replacing the structure genes with CHIKV corresponding genes [43] were found to be highly immunogenic in mouse models [44]. Other chimeric vaccine candidates vectored with adenoviruses, vescicular stomatitis virus, attenuated measles virus and modified vaccine Ankara were also successfully tested [45–48].

Other approaches were also utilized, and interesting results were obtained using DNA vaccine candidates [49–53] and subunits [27,54].

Finally, virus-like particle vaccine candidates were tested in Phase I clinical trials. Neutralizing antibodies were detected in most participants after the first vaccination, and 4 weeks after the second vaccination, remaining detectable 6 months after the third vaccination. No serious adverse events were reported and animal models suggested a humoral mechanism of protection [55–58].

Overall, of the 21 vaccine candidates against chikungunya, two are in Phase I and 2 are in Phase II trials; another two vaccine candidates are scheduled to start Phase I and one Phase II in 2016 [59,60]. Whether any of these vaccine candidates will get the opportunity to be tested in Phase III trials cannot be easily predicted **(Table 1)**.

• The emergence of Zika in the Americas: too early for a vaccine?

ZIKV, a single-stranded, positive-sense RNA flavivirus, member of the Flaviviridae family, was identified in 1947 in monkey rhesus and in *Aedes africanus* in Uganda [61,62], and has since reported to cause sporadic human cases in Asia and Africa. In recent years large outbreaks sustained by *A. aegypti* have occurred in Yap, Micronesia [63], in French Polynesia [64], and in Latin America [65–67]. In humans, ZIKV typically causes a mild and self-limiting illness (Zika fever), accompanied by maculopapular rash, headache, conjunctivitis and myalgia. Neurologic complications, such as the Guillain–Barré syndrome, may also occur in patients experiencing Zika fever. However, ZIKV only started to be considered as a global public health problem when it caused a raging epidemic in northeastern

Table 1. A selection of chikungunya vaccines by phase of development (preclinical, Phase I and Phase II).

CHIKV vaccines	Phase	Ref.
Inactivated (whole virus)	Preclinical, Phase I	[25–27]
Live attenuated:		
– Traditional	Preclinical, Phase I/II	[28–33]
– Engineered	Preclinical	[38–42]
– Vectored	Preclinical, Phase I	[43–48]
DNA	Preclinical	[49–53]
Recombinant proteins-subunit	Preclinical	[27,54]
VLPs	Preclinical, Phase I	[55–58]

Modified from [53,54].
References [34–36] concern general issues, and reference [37] alpha viruses other than chikungunya, thus they are not cited in the table.
Preclinical studies include both mouse models and nonhuman primate models.
Human trials started at the end of 2015 or scheduled for 2016 are not reported as `Phasel/II´ in the table.
VLP: Virus-like particle.

Brazil that was followed by an apparent increase in the number of adverse fetal outcomes, consisting in microcephaly and other brain defects [68].

On 1 February 2016, after gaining evidence of an association between such malformations and ZIKV infection during pregnancy, WHO declared the extraordinary cluster of microcephaly and other neurological complications linked to Zika a 'public health emergency of international concern' [69]. Since then, the race for a Zika vaccine has begun.

There are several points that need to be addressed for the development of a vaccine against Zika [70]: whether the infection leads to lifelong protection; whether cross-reactivity between Zika and other flaviviruses may influence vaccine safety and efficacy [71,72]: vaccine-induced immunity against other flaviviruses, especially yellow fever, might confer some cross-protection, confusing the efforts to evaluate Zika vaccines; Zika vaccination could stimulate the phenomenon of antibody dependent enhancement, having a negative impact on subsequent dengue infection; whether a monkey model can be established; whether an attenuated vaccine may be safely administered to pregnant women; whether an inactivated or subunit vaccine may induce effective and long-term protection. To this regard, although inactivated vaccines have the best chance to obtain regulatory approval for use among pregnant women, they usually require higher amounts of antigen and booster doses. An alternative option could be represented by a vaccine composed of a virus weakened through gene deletion, so that it can replicate but cannot cause disease. Other approaches, such as the production of virus-like particles by the insertion of a DNA circular plasmid holding key viral genes into bacterial cells or chimpanzee adenoviruses expressing ZIKV surface proteins, have also been considered [63]. In any case, an ideal vaccine should provide long-lasting protection after a single dose and be safe to use in pregnancy [73]. However, since Zika virus infection is most detrimental during first 3 months, vaccination during pregnancy may not be effective in preventing neonatal outcome; thus, it would be better to envisage its use before pregnancy.

Up to now, a few experimental studies have been conducted. In particular, both a full length premembrane and envelope DNA vaccine and an inactivated virus vaccine conferred protection against ZIKV challenge in a mouse model. Interestingly, Env-specific antibody titers were found to be key immunologic correlates of protection [74]. Phase I clinical trials on DNA vaccine candidates are set to begin and about 15 projects are under way to create an effective Zika vaccine [75]. However, even though a promising vaccine candidate succeeds in animal experiments and small human studies, large-scale efficacy trials are still a few years away.

• Tackling bottlenecks hindering vaccine development

The recent emergence of CHIKV and ZIKV in the Americas has strengthened the need of a safe and effective vaccine for epidemic containment. Though affected by scarce resources, research on vaccines for *Aedes*-borne viruses has slowly progressed, and a number of vaccine candidates are now available and ready to be further tested in human studies. However, there is a series of obstacles, such as technical problems and financial constraints that need to be overcome in order to develop and make available worldwide vaccines against such emerging neglected diseases.

First of all, methodological problems regarding the feasibility of large Phase III randomized controlled trials (RCTs) should be mentioned. In fact RCTs are widely considered the gold-standard for evaluating vaccine efficacy, but they may be planned and conducted only under certain conditions.

For neglected emerging infectious diseases whose dynamic pattern consists of a sylvatic cycle characterized by a low level of endemicity with sporadic spillover causing human cases around their ecological niche, Phase III trials may not be feasible in interepidemics periods

because of the low expected number of cases; thus, epidemic events with a high number of cases may represent a unique opportunity to ensure study power for testing vaccine candidates in large efficacy trials. However, outbreaks usually occur sporadically and are often unpredictable; for this reason, planning and conducting RCT may be a difficult challenge. Moreover, vector-borne diseases such as chikungunya and Zika are likely to occur in poor-resource countries located in tropical areas where the presence of trained and well equipped clinical sites, which are essential for the conduction of RCT, cannot be ensured. To overcome these constraints, it is important to obtain reliable information on correlates of immune protection, in order to apply the so-called 'animal rule'. This rule, consisting of the use of surrogate end points derived from animal data instead of the results of human trials, could be considered as an alternative option when large efficacy studies on humans, which are usually requested for traditional regulatory approval, are virtually impossible [76]. For example, with regard to CHIKV, the level of neutralizing antibodies could be utilized as a surrogate marker of vaccine-induced protection, since it appears to be strongly correlated with a protective immune response [34] and resistance to infectious challenge in animal models [50,55].

However, for all the reasons reported above, the current spread of CHIKV and ZIKV in the Americas provides an invaluable opportunity for the field evaluation of vaccine candidates. In fact, the width of the area at risk, the size of the susceptible population and the availability of vaccine trial sites with adequate scientific and technical background might ensure the feasibility of RCT with promising vaccine candidates.

Second, vaccine development is an expensive process, where investment returns are not always guaranteed. These financial concerns may delay or even prevent all the phases of vaccine R&D and represent a further obstacle to the conduction of large RCT. To overcome this problem, several strategies may be implemented: the creation of public–private partnership, which may favor the development and evaluation of vaccine candidates. To this end, the culture and the practice of the academic institutions/researchers needs to change, and scientists should be committed to bridge the gap between basic research and the development of vaccines for neglected diseases in the developing world (i.e., the products of academic basic research might be exploited by the industry) [2,77]; the identification of target population groups for vaccination to ensure a potential market. This is key to guarantee possible benefits of the vaccine, in terms of profits for the investors and safeguard of the community. To identify large, long-lasting markets for vaccines against emerging neglected infections is difficult, due to their unpredictable epidemic patterns and, sometimes, to low case-fatality rates. Some consideration merits the military 'market', especially when a large amount of soldiers is settled in areas potentially affected by arbovirus infections [2]. Travelers and tourists might represent another priority in terms of return of investments. In this regard it is important to underline how recent outbreaks of dengue, Zika and chikungunya mostly occurred in touristic areas, such as Indian Ocean islands, Pacific islands and the Caribbean [2,34]. The availability of vaccines against these viral infections would be a useful tool to protect tourists, to save local economies, and to prevent the importation of the infection in tourists' countries of origin; the commitment of donor agencies and affected/donor countries, which is key in supporting both the development and the availability of vaccines against neglected tropical diseases like chikungunya [70]. As already mentioned, most R&D projects do not deliver a licensed vaccine for routine or targeted immunization, mostly not because of scientific barriers but due to political and economic obstacles [77]. In fact, neglected diseases disproportionally affect poor and marginalized populations, and vaccines may have low returns, so commercial firms may be reluctant to commit themselves to the expensive development of vaccine candidates. Developing a human vaccine from the preclinical phase to registration requires an increasing average investment (approximately US$200–500, or even up to 900 million) [77,78]; thus, the development of vaccines for neglected infectious diseases may not be considered convenient, because of low probability of market entry, limited market size, and even long timelines [2,35,78]. To overcome these hurdles, public and private commitment is essential in order to minimize investment risks, increasing the chance of access to new vaccines to local communities affected by neglected viral diseases [2,79]; for example, Bill and Melinda Gates and/or other foundations may be interested in funding R&D activities, and the Global Alliance for Vaccines and Immunization supports vaccine markets in eligible countries [34]. Finally, Government support would be also desirable in

order to ensure the sustainability of clinical trials costs and the utilization of innovative vaccines.

Conclusion & future perspective

Aedes-borne neglected diseases represent an important global health challenge, and vaccines are surely a useful tool to contain their disease burden. The emergence of chikungunya and Zika in Latin America, and the discovery of the devastating effects on the fetus brain, will probably speed-up R&D of safe and effective vaccines against these diseases, in particular against Zika. However, there are several obstacles that should be overcome to make vaccines available and sustainable. To achieve these goals, a series of actions involving countries, health authorities, international agencies, private foundations and the industry, should be planned and implemented. In particular, public–private partnership should be stimulated in order to translate basic research into effective products. Innovative rules to introduce new vaccines should be considered in case of insurmountable methodological problems. Finally, when a market cannot be ensured, the commitment of public and private donors to refund in part the investments of vaccine companies should be obtained. Such global convergence, involving different actors and stakeholders, may accelerate the process of development of vaccines against chikungunya, Zika and other neglected viral diseases which mainly affect resource-poor areas of the world.

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EXECUTIVE SUMMARY

- *Aedes* mosquito-borne viral diseases are emerging in several areas of the world, causing large outbreaks in central and southern America.
- Yellow fever is still a public health problem in many tropical countries where the virus is endemic, whereas other viruses such as dengue, chikungunya and Zika are threatening previously naive geographical areas.
- Mosquito control activities are not always highly efficient, thus susceptible populations need to be protected through the development and the use of effective vaccines.
- An effective vaccine against yellow fever is available since the 1930s, while a vaccine against dengue has been licensed in several countries, though the protection conferred against dengue serotype 2 is considered, to some extent, suboptimal.
- R&D of vaccine candidates against the recently emerged Zika virus infection has just started with promising results, while several candidates against chikungunya are already under preclinical and clinical evaluation.
- To overcome hurdles and speed-up the development of vaccines against these viral diseases, several actions should be planned:
- The 'animal rule' could be considered for regulatory purposes when Phase III trials are not feasible.
- Public–private partnership should be stimulated, to increase the number of vaccine candidates.
- Countries', international organizations' and donors' commitment should be strengthened and potential markets identified to ensure a return to economic investments.

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