Vaccine Research
ISSN: 2423-4923
eISSN: 2383-2819

Vaccine Innovation for Dengue, Chikungunya, Zika and Yellow Fever: Accelerating Global Development Agenda and Partnerships in Post-COVID Era

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ARTICLE INFO

Research Article

VacRes, 2022
Vol. 9, No.2, 1- 17
Received: June 11, 2021
Accepted: July 02, 2021
Pasteur Institute of Iran

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KEYWORDS: Vaccine, Dengue, Chikungunya, Zika, Yellow Fever

ABSTRACT

Introduction: Vaccines for emerging arboviral diseases that should be a global priority, in a combined strategy with vector control, are being neglected, affecting particularly the poorest populations worldwide. The object of our investigation is to identify the breakthroughs in vaccine innovation for four leading emerging neglected arboviral diseases (Dengue, Chikungunya, Zika and Yellow Fever). From this perspective we examine vaccine patent document applicants by stage of technological development of different vaccine platforms, including the different partnerships established by these applicants. **Methods:** From a patent landscape approach, we defined methodological strategies for a hybrid search in two different databases (Derwent Innovation Index and Cortellis Drug Discovery Intelligence) that allowed us to identify the stages of technological development of the vaccines to prevent/treat these four arboviral diseases, making it possible to identify the successful ones that go to clinical trials and the partnerships among private and public institutions, which promote continuity of the development/production process. **Results:** Our results confirm these gaps for Dengue, Chikungunya, Zika and Yellow Fever vaccines, with only 13% of these vaccines still under active development and prospect to reach the final stages of development. They also indicate that, in spite of this constraint, innovative platforms such as RNA-based vaccines are increasingly being used to prevent/ treat these diseases. **Conclusion:** We propose urgently strengthening funding and incentive mechanisms in a Global Strategic Plan supported by new business models and community participation to accelerate vaccine development for these four neglected arboviral diseases, a dramatic and concerning global issue in post-COVID-19 era.

Citation:

INTRODUCTION

Emerging Neglected Arboviral Diseases, such as Dengue, Chikungunya, Zika and Yellow Fever, are concentrated in tropical areas of the globe, predominantly affecting the poorest neglected communities [1]. Despite their enormous impact on health systems worldwide, they are often overlooked by policy and decision makers. Vaccines for these diseases which should be a global priority, in a combined strategy with vector control, are being neglected, affecting particularly the poorest populations worldwide. The COVID-19 pandemic has severely affected essential health services, for these Neglected Tropical Diseases (NTD) including: 1. Delays in diagnosis/treatment and manufacture, shipment and delivery of drugs used to their treatment; 2. Suspension of mass treatment interventions and 3. Reassignment to the pandemic response of the personnel in health services. Therefore, it is not sufficient to develop low quality rapid diagnostic tests for NTD, it is urgent to provide effective vaccines in high risk-areas and immediate treatment and care for these diseases. In addition, effective vector control measures must be put in place and continued [2]. The simultaneous circulation of different arboviruses, Dengue virus (DENV); Chikungunya virus (CHIKV), Zika virus (ZIKV) and Yellow Fever virus (YFV), in middle-income countries such as Brazil, implies cocirculation/coinfection of these viruses which contributes to these diseases' severity, a major public health concern [3]. These diseases require an effort from the governments and to associate universities with companies for the continuous development of vaccines (Triple Helix). This

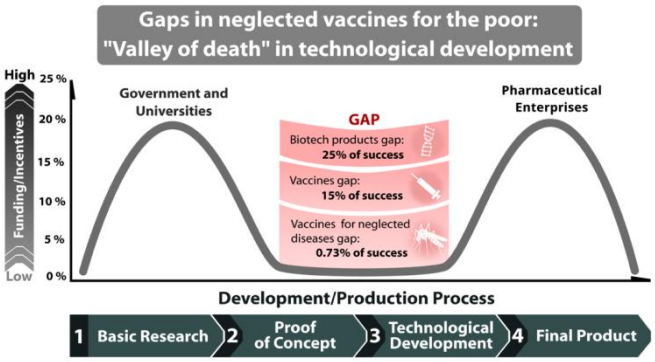
[DOI: 10.61186/vacres.9.2.1]

dramatic and complex epidemiological scenario requires urgent and sustained investment in technologies for the development of new pharmaceutical products, especially those that constitute disease prevention tools, such as vaccines. Moreover, it is important to associate adequate urban planning addressing public health issues, such as the incorporation of mitigation methods for prevention/control strategies to these arboviral diseases (vector borne RNA viruses) through effective vector control of *Aedes aegypti* and *Aedes albopictus* (in a lesser extent) in urban areas [4, 5]. These vector control strategies should be extensive and include trash collection, sanitation and water drainage landscaping to reduce vector breeding sites as well as engineering solutions to improve their effectiveness. These arboviruses are responsible by different febrile syndromes. Some are neurotropic (ZIKV), with the risk of severe neurological syndromes and other (DENV, ZIKV, YFV) are able to cause visceral diseases (vascular compromise, hemorrhagic syndromes and liver failure), with long-term disability or death [5]. Furthermore, these four diseases require urgently more attention: the need of global surveillance to generate data about the distribution of vectors and hosts, due to their dislocation from forest to urban areas and to climate and large-scale ecological changes [6]. Considering the huge impact of these neglected emerging arboviral diseases for the global epidemiological scenario, it is imperative to better understand the vaccine's development/production process, due to their dramatic impact on life quality in low and middle-income countries. In severe cases of DENV infection, the patients can die and there is no treatment, only symptoms management. In 2019, cases of dengue exceeded 3 million in the Americas, severely impacting many countries in the region: Colombia with 127,553 reported cases, Nicaragua (186,173); Mexico (268,458), Honduras (112,708) and Brazil (2,241,974 – with this country accounting for 50% of the DENV deaths in the region) [7]. In 2015, the association between microcephaly and Zika was reported in Brazil, with many cases reported. Congenital Zika Syndrome resulted in Brazil and other countries in the region in severe disabilities and defects among babies who were infected with ZIKV during pregnancy [4, 8-9]. Vaccine innovation for these four arboviral diseases should thus be understood from a broad perspective in this complex epidemiological scenario.

Conceptual framework

Vaccines are recognized as the most important and low-cost contribution to human health. Immunization is a medical intervention than can reach any individual in the globe if adequate access policies are provided. Innovative vaccines require entrepreneurship, policy and decision-making, program implementation and finally, the best of science. They can improve quality of life and reduction of mortality globally and are the most effective tool to prevent infectious diseases [10-12]. Nevertheless, despite the extraordinary recent advances in vaccinology, crucial to ensure sustainable population immunity, most vaccine candidates for NTD will probably never be developed as a final product for the community unless radical changes in global and local policies occur. NTD attract very limited investments from private sectors due to low probability of return, low market value and lack of political will from governments in middle income countries, where most of these diseases are endemic, to invest the minimum amounts necessary to develop the final product. Even though these products cannot survive to the valley of death (due to lack of a business model to develop new vaccines and drugs against neglected diseases) [13-15], an alternative for promoting vaccine development is through government investment in venture capital and also through technological transfer to universities/companies with guaranteed purchase of vaccines for neglected diseases.

These risk costs have been largely neglected and there is a low probability of success for many drug candidates to scale-up to final development stage. Fig. 1 illustrates the gaps for biotechnological products, vaccines and vaccines for neglected diseases. Based on the gaps presented in Fig. 1 (25% for biotechnological products, 15% for vaccines and 0,73 for neglected diseases vaccines) we can hypothesize that most of the vaccines against neglected emerging arboviral diseases in our study might never survive the "valley of death[1]" , due to the very low investments and incentives for R&D and the very high cost for reaching the final development and production stages



[16, 17, 18]

Fig. 1. Conceptual framework: development/production process of neglected vaccines.

Source: Elaborated by the authors. For rates of success, information from [15, 17-19].

Considering the conceptual framework in Fig. 1, one of the ways to verify in our study if vaccines for the four neglected emerging arboviral diseases (Dengue, Chikungunya, Zika and Yellow Fever) have not become "dead" is if they have left Research Institutions and become processes/products of interest to companies for development, partnerships and licensing. This is an indication that the product will be able to fulfill all the steps of development/production process: 1. basic research; 2. proof of concept; 3. technological development 4. clinical studies and final product.

For technological analysis of patent documents, diverse vaccines platforms under investigation and development were used, including vaccine platforms consolidated during the COVID-19 pandemic such as Nucleic-acid and viral-vector platforms, presented in the following categories. **Group 1: Live attenuated vaccines** (with weakened live (replicating)/functional bacteria or virus) and Inactivated vaccines (without live (replicating) bacteria or virus); **Group 2: Subunit vaccines** (with sugar - **pure polysaccharide vaccines** - or proteins derived - **protein vaccines** - from the organism

[1] The valley of death for pharmaceutical products includes the development stages from discovery to effective proof-of-concept phases which includes phase II clinical trial development [13].

[DOI: 10.61186/vacres.9.2.1]

which cause the disease); **Group 3: Recombinant vaccines**[2] (replicating and non-replicating, developed through recombinant DNA technology, that use viral particles to deliver the genetic material encoding the antigen into the cell) or **Group 4. Nucleic acid vaccines**[3] (using DNA or RNA encapsulated into glycol-proteic lipid nanoparticle that mimics a viral particle to deliver the genetic material into the cell to produce the antigen) [20, 21] (Fig. 2).

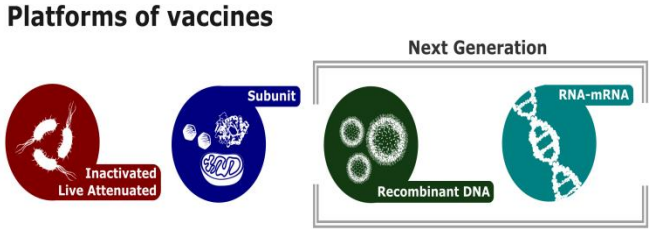


Fig. 2. Platforms of vaccines. (Source: Elaborated by the authors)

In this chapter we provide an overview of vaccines from different platforms, i.e., live attenuated, inactivated, subunit, recombinant, and nucleic acid vaccines direct to specific arboviruses: CHIKV, DENV, YFV and ZIKV, besides the analysis of immunotherapies strategies to the infections caused by the mentioned arboviruses. As seen in Fig. 1, the applicants will be identified at each stage of development and our results will be presented and discussed in three steps: First, we provide an overview of vaccine innovations from the applied documents' analysis (patent data's analysis), to reveal scientific, business and technological trends *(i.e.* Patent Landscape) [22]. This one was also presented by assignees/applicants, besides the presentation of the licensor in order to present ultimate ownership, respectively [23] which will provide the development scenario of products to treat/prevent the neglected diseases, *i.e.*, CHIKV, DENV, YFV and ZIKV infections, exclusively and not exclusively (more than one neglected disease from the scope of the study). Second, we will discuss the developed immunotherapies to these infections and third, it will present vaccines under active development[4] to treat/prevent these diseases.

MATERIALS AND METHODS

Part 1: Search for Patent Filings

The patent search was carried out in the Derwent Innovations Index database (Clarivate Analytics) to retrieve the patent documents for Dengue, Zika, Chikungunya and Yellow Fever vaccines filed between 2010 and 2020 (priority year[5]). For this purpose, the diseases' synonyms were used, using the terms from MeSH (Medical Subject Headings) accessed in the database PubMed Database[6] . For this goal, 3 different search strategies were used[7] . In order to complement the search for patent filings, it was used the CDDI database for the same infections (*i.e.* Dengue, Zika, Chikungunya and Yellow Fever) and searches were conducted to retrieve patent documents also filed between 2010 and 2020[8] . However, the method is not capable of retrieving the most recent launched/developed vaccines, due to the patent documents, in general, be published only 18 months after applications' earliest priority date. For the search for information from clinical trials, papers and filed patent documents, different databases (Derwent Innovation Index - DII[9]) and Cortellis Drug Discovery Intelligence (CDDI)[10] were chosen to retrieve the documents. This facilitates a hybrid type approach to show different innovative aspects of the vaccines against the four infections, *i.e.*, CHIKV, DENV, YFV and ZIKV infections. This is schematically shown in Fig. 3.

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[2] Recombinant vaccines are produced through recombinant DNA technology, inserting DNA encoding an antigen that stimulates an immune response into cells. There are diverse types of vaccines using this technology, such as live recombinant vaccines, made of live attenuated viral or bacterial strain or using Adeno-associated virus (AAV). In molecular terms, for example, DENV2 is a clone of a virus attenuated in tissue culture by traditional methods and other serotypes using the same backbone only by changing the envelope. So, it can be called "attenuated recombinant". From an immunological point of view, whether the attenuation method was recombinant, or a strain developed "in vitro" makes no difference. Attenuated recombinant vaccines contain viral RNA or DNA to encode the antigen, the difference is that they use the virus to enter the cell and the genetic vaccines use artificial methods.

[3] Nucleic acid vaccines use genetic material from disease causing virus or bacterium to stimulate an immune response. Nucleic acid-based vaccines include DNA as plasmids and RNA as messenger RNA - mRNA vaccines, with extraordinary promising potential in targeting a broad range of diseases.

[4] A product under active development (UAD) is the one in different phases (preclinical testing; investigational new drug – IND filed; clinical; phases 0-III; pre-registered; registered; recommended approval) and reported over the past 12-18 months in biomedical literature, such as congresses/journals, annual reports, clinical trial register and company press releases. According to CDDI database: "If the product is commercialized, the highest phase is always Launched whether it is under active development or not". Moreover, "Launched drugs that are not being investigated for new conditions, in new regions or by new organizations are not considered Under Active Development". For the present study the UAD launched vaccines were not considered/retrieved, such as the vaccine DENGVAXIA from Sanofi Pasteur (from France and launched in 2016) [24].

[5] The year of the first filing of the patent in the host country, regional or via Patent Cooperation Treat (PCT).

[6] MeSH (Medical Subject Heading) descriptor is used for indexing articles from the Pubmed Database (National Center for Biotechnology Information) (https: // www. ncbi.nlm.nih.gov/mesh).

[7] The first strategy was based on the International Patent Classification (IPC) from the World Intellectual Property Organization (WIPO) A61K39*, related to "Medical preparations containing antigens or antibodies", crossing with selected diseases and their synonyms: Chikungunya, zika, ZikV, dengue, Break-Bone Fever, Yellow Fever; 2. The second strategy used the Derwent Manual Code classification, a classification specific to the database, crossing with the selected diseases. It was observed that there is a specific classification for vaccines, the B14-S11*, whose description is "vaccine [general]"; 3. The third strategy used the word "vaccine" crossing with the selected diseases. The search was carried out on July 21, 2021. Data update in March 2022.

[8] The patent document search was done separately in Patent Field using the terms vaccine and Dengue (or Zika, or Chikungunya or Yellow Fever) in title/abstract and as condition, the terms "Infection, dengue virus" (or "Infection, Zika virus", or "Infection, Chikungunya virus" or "Infection, Yellow Fever") were used. The search was conducted using as priority date period from 01/01/2019 to 31/12/2020.

[9] Derwent Innovation Index (DII) (ClarivateTM): From almost 60 patent-issuing authorities all over the world, the DII covers over 14.3 million basic inventions [25].

[10] Cortellis Drug Discovery Intelligence (CDDI) (ClarivateTM): The platform offers pharmacological, chemical and biological data from phamaceuticals and drugs). It focus on drug and pharma development exclusively [26].

[DOI: 10.61186/vacres.9.2.1]

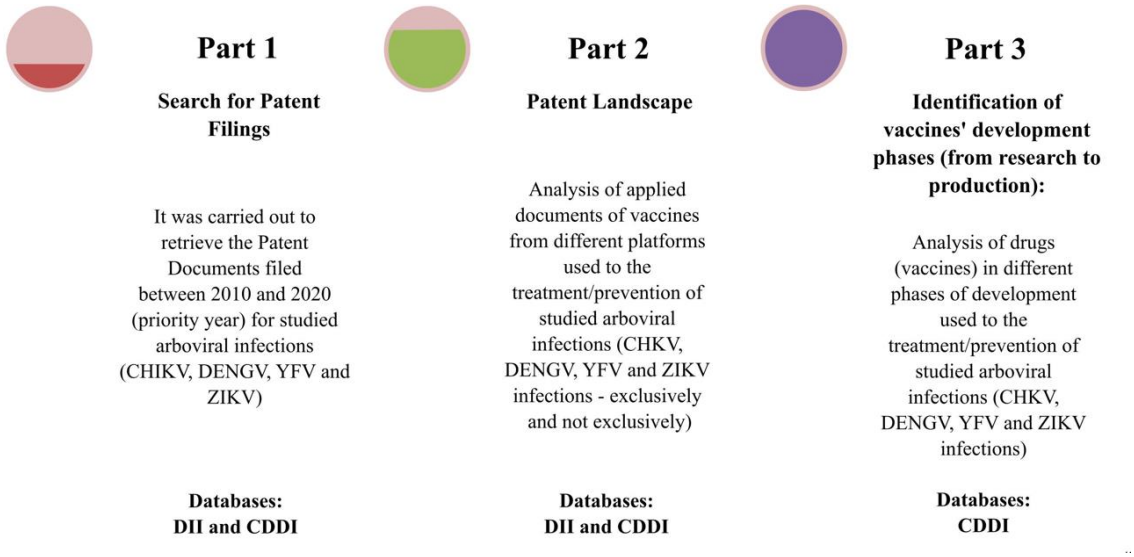

Fig. 3. Methodology's steps in different databases (Source: Elaborated by the authors).

Part 2. Patent Landscape

For the purpose of this study, the following key data were extracted from the filed patent documents: First: The applied technologies/potential innovations were retrieved from the filed patents which were separated into five vaccines platforms: inactivated, live attenuated, subunit, recombinant and nucleic acid vaccines (and immunotherapies) from title/abstract/claims' analysis exclusive and not exclusive to the interest diseases, *i.e*., CHIKV, DENV, YFV or ZIKV infections (Fig. 3). Second: It was analyzed the assignees/applicants (and the licensors[11]) to identify the development scenario for these products (Fig. 1).

Part 3. Identification of vaccines' development phases (from research to production)

Global human vaccines related to clinical studies to Dengue, Chikungunya, Zika and Yellow Fever which were retrieved according to the developed strategies searches using the CDDI from Clarivate Analytics[12] . Here, products and biologics related to vaccines (against the interested diseases) under active development[13] in the highest development phase (clinical, pre-registered/registered) were analyzed. "Thus, the proposed method not only considers the most advanced development stage of the drug ("*highest development phase*") but also accounts for those products which are "*actively moving through the drug* R&D *pipeline from preclinical stages through registration*" [24].

RESULTS

Part 1: Search for Patent Filings

For the present study, the search methodologies resulted in patent documents filed for different technologies used in the development of vaccines for the neglected diseases of interest, *i.e*., CHIKV, DENV, YFV and ZIKV infections. Here, the patent documents for vaccines directed to vaccine platforms (live-attenuated, inactivated, recombinant, subunit and nucleic acid platforms), exclusive and non-exclusive for these diseases, will be presented.

Part 2: Patent Landscape

Exclusive vaccines for the treatment/prevention of neglected diseases:

Chikungunya, Dengue, Yellow Fever and Zika infections

For each group of vaccines will be presented the use of the platform to vaccine´s development (underlined), the technologies and the nature of the involved actors , i.e., assignees/applicants/licensors, including theirs interest related to the development of vaccines, such as research (demonstrated by applications from research institutions/ universities), research and development (demonstrated by documents from companies, including biotechnology companies) and finally, partnerships between these actors (research institutions/universities and companies) that demonstrate the vaccines' technologies (for the treatment/prevention of diseases: CHIKV, DENHV, YFV and ZIKV infections exclusively) that have the possibility of leaving "valley of death" and thus reach the market as final product.

CHIKV Infection

According to the search strategy, the development of vaccines for infectious diseases is object of interest for Institutes and Universities. The **WUHAN INSTITUTE OF VIROLOGY, CHINESE ACADEMY OF SCIENCES**

[11] In the present work the licensor was only described if it was different from the original assignee/applicant, due to the importance of observing if any institution/company is interested in licensing the filed document.

[12] In CDDI Database for Drugs & Biologics', the search strategy used for the field "condition" the terms "infection, dengue virus" (for dengue); "infection, Chikungunya virus" (for Chikungunya); "infection, Zika virus" (for Zika) and "yellow fever" (for yellow fever). For it infection the term was used separately and associated to the word "vaccine" in the field "product category". The search was carried out in August 10th, 2021.

[13] The drug or biologic under development are the ones which the products' development activity has been reported for 12-18 months in conferences, peer reviewed journal articles, company's pipeline chart, mention in annual reports, company press releases or clinical trial registers [24].

[DOI: 10.61186/vacres.9.2.1]

developed CHIKV clone used in compositions of attenuated vaccines against CHIK infection or as an expression vector. The Chinese Institute developed the clone CHIKV -Δ C virus lacking the capsid protein, by a method to delete it (the capsid protein), which indicates that recombinant vector can be used to express proteins from other viruses, may be other alpha viruses. It can be used to express a foreign gene (expression vector) and to induce immune response against the virus (as an attenuated vaccine by recombinant technology) (PN: CN109536464) [27]. Another example is the vaccine applied by **UAB RES FOUND (US) (Licensor[14] : UNIVERSITY OF ALABAMA)** with an attenuated alphavirus (CHIKV, e.g.) particle. This one comprises a "nucleotide sequence encoding the alphavirus nsP2 protein", which its amino acid substitutions are able to disrupt the ability of nsP2 to induce RPB1 inhibition/degradation transcription in the virus (CHIKV). The nsP2 protein is used to prepare a vaccine formulation with the alphavirus particle in a vaccine diluent. The effective amount of the attenuated particle enhances/induces the immune response in a subject (PN: WO2020028749-A1) [28]. No applied document has been retrieved from partnerships between companies and institutions/universities for development of live attenuated vaccines against CHIKV infection. This finding is an important result of our study, since it evidences the low interest of companies in investing in products derived from research (from the universities and institutes) for the prevention of neglected diseases such as CHIKV infection). Notwithstanding, important findings are provided (applied patent documents) from companies and institutions/universities. The French Biotechnology company **VALNEVA SE** developed three live attenuated vaccines. The first one was a lyophilized/liquid frozen live CHIKV vaccine. In this formulation, there is one CHIKV's strain. This stable and safe formulation stimulates immune response in humans against CHIKV after a single dose of the CHIKV-A5nsP3 vaccine (attenuated) and presents tolerability profile. Seroprotection and neutralizing antibody titers were obtained by a single-shot. In fact, the vaccine increases serum antibody titers in a subject about 7/14 days from primary immunization (by at least 1 log relative when it is compared to a control). Moreover, the seroconvertion ("*reaching a CHIKV-specific antibody titer of at least 10*") is stimulated up to 100% withing 14 days of vaccination in subjects (PN: WO2021028406) [29]. The second vaccine from **VALNEVA SE (France)** was a one-shot vaccine developed by the same company used CHIKV-delta5nsP3 (attenuated CHIKV), which is able to express excipients and a protein called "E2 protein" to prevent/treat the infection by the induction of a sustained immune response (it lasts more than 6, 12 or 24 months). Within 7 days or 14 days of vaccination the vaccine stimulates in vaccinated subjects the seroconversion in 25-100% (to neutralize CHIKV antibody titer ≥ 10, preferably 20) (PN: WO2021028407) [30]. Moreover **VALNEVA SE** (France) also developed a process to produce an immunogenic live attenuated CHIKV and a vaccine (in lyophilized form) to produce immune response against the virus. One of the key findings of this formulation (CHIKV-A5nsP3) is that the composition stimulates neutralizing antibodies' production in human even at least 95% at a serum dilution 1:80 (or higher) (PN: WO2019057793-A1) [31]. The **AMERICAN SECRETARY OF ARMY** developed an inactivated, safer and more stable vaccine against CHIKV when compared to live CHIKV vaccines. To people from developing countries, it provides more accessibility, due to its easy transport, storage and does not need refrigeration. The isolated virus offers broad neutralizing and cross-neutralizing activities against different CHIKV's genotypic variants, strains or genotypes. The formulation can offer immune protection against heterologous CHIKV strains, including the strain used in the vaccine (PN: WO2017123932) [32]. Here no partnership from institutions/universities and companied was retrieved. However, companies have developed platforms to treat/prevent CHIKV infection, such as the inactivated vaccine applied by the Indian company **BHARAT BIOTECH INTERNATIONAL LTD**. The stable composition comprises an inactivated CHIKV (mutated) strain which can infect *Aedes albopictus*, enhances the virus' adaptation to *Aedes aegypti* and promotes immunity against CHIKV's genotypic variants. Once again, the formulation offered protection not only against the virus strains used in the vaccine, but also against plural CHIKV's strains, with cross-neutralizing/broad neutralizing activities against different genotypic variants, genotypes and strains (PN: WO2012172574) [33]. Indeed, one of the key findings of the retrieved applied documents was the interest of universities and governmental institutions to develop better vaccines for CHIKV, with novel strategies: the **INDIAN DEFENSE RESEARCH AND DEVELOPMENT ORGANIZATION** applied a preparation (and the process) against CHIKV which consists in an "immunogenic cell-culture-based protein vaccine". The composition promotes cell-mediated and high humoral immune response to prevent the infection (PN: IN200900783) [34]. Here, **GRIFFITH UNIVERSITY** (from Australia) was responsible for a purified, isolated, recombinant or synthetic CHIKV (and Semliki Forest Viral). It should be highlighted that the virus genome is able to encode the mutated capsid protein. Genome, polyprotein, protein or alphavirus are used in a subunit vaccine to prevent disease's development. The vaccine is able to elicit an anti-alphaviral protective immune response and also influences T-cell and cellular responses (PN: IN201817043293) [35]. Once again, the findings indicate the lack of interest in partnerships to the develop subunit vaccines against CHIKV infection. Moreover, the American company (**EMERGENT TRAVEL HEALTH INC**.) applied a virus-like particle (VLP) vaccine with an envelope and capsid protein from CHIKV among other formulation components, such as aluminum hydroxide adjuvant. It is used to induce neutralizing antibody response for treatment of CHIKV infection The neutralizing antibody response by VLP induction of antibodies against heterologous/homologous strains of CHIKV is sustained for one year (at least) (PN: WO 2021081499) [36]. Subunit platforms have supported research of universities and institutions, such as the applications from the **UNIVERSITY INDUSTRY FOUNDATION YONSEI UNIVERSITY WONJU CAMPU** from Korea, which claimed for a recombinant subunit vaccine against CHIKV, using as a main component a fusion protein that provides synergic effect in the induction of immune esponse. The protein comprises toll-like receptor 5 stimulating protein and CHIKV antigenic protein (PN: KR2018042958) [37]. The recombinant vaccines to treat/prevent CHIKV infection are currently under research by companies. **GLAXOSMITHKLINE BIOLOGICALS SA** (from Belgium), which developed a recombinant adenovirus vector to stimulate the immune response against CHIKV. The

[14] For the present results, the licensor will be described if this is different from the original assignee/applicant.

[DOI: 10.61186/vacres.9.2.1]

polynucleotide used as medicament comprises the pharmaceutical excipient[15] and the recombinant adenovirus. It can be used as a vaccine to treat/to prevent/to ameliorate the viral infection. The promoted immune response can be an antigen T cell response (local/systemic). It comprises CD4+ or CD8+ responses (expression of a plurality of cytokines, e.g. TNFalpha, IFNgamma). Furthemore, antigen specific B cell response is elicited with neutralizing antibodies production (PN: WO2019016756) [38]. Moreover, the biopharmaceutical company **ETUBICS CORP. (US) (Licensor: NANT HOLDINGS IP LLC**) developed a "*recombinant adenovirus based vector vaccine*" which contains alphavirus antigen genes to prevent CHIKV infection. The significant protective immune results are interesting and help to justify the ability of recombinant Ad5 [E1-, E2b-] platform based-vaccine. The vector-based vaccine is able to induce higher cell-mediated immune (CMI) responses compared to current ones. Furthermore it is able to induce anti-tumor responses and potent CMI response, besides it can be used in multiple immunization regimens (PN: WO2018014008) [39].This partnership can be seen in the development of a method to the vaccines' production based on virus recombined with polynucleotides ("*recombinant infectious replicative measles vírus*") which is able to encode CHIKV antigens to provide life-long immunity with a single/two administration steps (**INSTITUT PASTEUR and CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE from France/ THEMIS BIOSCIENCE GMBH from Austria**). It elicits cellular immune response (in particular human host) and antibodies directed against CHIKV proteins to treat/prevent CHIKV infection (PN: WO2014049094) [40] (**Licensors: INSTITUT PASTEUR, CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE, MERCK & CO. INC**.).

According to the search strategy, no document was applied on Nucleic Vaccines Technology (DNA/RNA) to treat/prevent CHIKV infection.

DENV Infection

Concerning research development for DENV, BUTANTAN Institute (Brazil), licensed from the NIH, through BUTANTAN Foundation, a technology to develop a recombinant attenuated tetravalent vaccine against DENV and partnered with MERCK to incorporate the necessary technologies for manufacturing this vaccine. It is important to highlight that there are important steps in the process including the Vero cells' amplification in culture for production of Working and Master banks and the infection of the Vero cell from these banks with DENV serotypes. The resulted immune response against the virus is able to reduce/inhibit the infection's severity. The method to induce the immunologic response is done by a heterologous immunization comprising the administration of a non-replicating DNA delivery system followed by a booster immunization with an attenuated virus. The present application comprises the process to obtain the live attenuated DENV vaccine applied by **BUTANTAN FOUNDATION** (PN: WO2017041156) [41]. This recombinant platform has been object of interest of some companies such as **TAKEDA VACCINES, INC.** (from Japan) which applied new methods/compositions against DENV. The composition has non-chimeric live virus to the induction of immune response with a reduced volume dose when compared to subcutaneous injection. This is an advantage – the reduction of pain due to the reduced volume at a site of administration. Moreover the composition induces protective neutralizing antibodies against three or more DENV subtypes (PN: US2018008691) [42].

Another promising finding was a pharmaceutical composition, also applied by **TAKEDA VACCINES, INC.** (Japan) to treat children and young adults, which comprises dengue-dengue chimera and live- attenuated DENV to the induction of immune response against DENV infection. The compositions, uses and methods applied in the document concern to the induction of immune response against four DENV serotypes. When DENV chimeras are combined in a bivalent/trivalent/tetravalent composition against DENV serotypes, it can also be used against other pathogenic flavivirus "*by including nucleic acids encoding one or more proteins from a different flavivírus*" (PN: WO2017179017) [43]. The Japanese company (TAKEDA VACCINES, INC.) (Licensors: **TAKEDA VACCINES, INC & THE UNITED STATES HEALTH & HUMAN SCIENCES**) is an important applicant of this vaccine platform, i.e., recombinant attenuated vaccines against DENV infection. The applied documents confirmed it: a tetravalent composition with live attenuated DENV (live attenuated flaviviruses/or flavivirus chimeras and dengue-2/4 chimera). The vaccine can induce the immune response in a subject (preferably in human) to prevent flavivirus/DENV infections, so it provides strong immunogenicity (PN: US20170049874) [44]. Or the vaccine's application of all four live attenuated DENV from **TAKEDA VACCINES, INC.** In line with the findings of this technology, it can be reported that the composition can induce a cellular immune response when it is introduced via intradermal to modulate the production of neutralizing antibody. Moreover, a single vaccine dose can increase cross protection against all DENV serotypes (PN: US2016129102) [45]. An important partnership was found for development of a tetravalent recombinant live attenuated vaccine with DENV (DENGUE-2/4 chimera), which was applied by TAKEDA VACCINES INC (Japan) and THE US SECRETARY OF THE DEPT OF HEALTH AND HUMAN SERVICE (US). The vaccine has "*live, attenuated DEN-2 virus backbone having one or more structural proteins from a DEN-4 virus, where a nucleic acid construct encoding the live, attenuated DEN-2/4 chimera*". This patent teaches that insertion of homotypic capsid/premembrane junction to the chimeric membrane and envelop proteins into the DEN-2 backbone improved the efficiency of in vitro viral replication, demonstrated by the DENVax-/DEN-4 chimera. (PN: US2019151433) [46]. For this platform, we did not find in the period of our search any application, which corroborates important gaps in vaccine development for Dengue vaccine research. In applied patent documents, very few partnerships for Dengue vaccines between Institutions/Universities and Companies were identified, with most innovations not reaching full scale development, which characterizes, once again, the "valley of death" for Dengue Vaccine platform.

Nevertheless, some advances in this direction were identified. **GLAXOSMITHKLINE BIOLOGICALS SA** (Belgium) applied an inactivated vaccine to treat/ameliorate/prevent diseases caused by DENV, e.g., dengue shock syndrome and hemorrhagic fever. The composition comprises the antigen (dengue virus) with the aluminum-free

[15] Vaccine excipients are substances other than the active pharmaceutical ingredient (API) included in the vaccine delivery system. They include a wide range of products, such as vaccine adjuvants, used in a number of novel vaccines developed and marketed by leading vaccine manufacturers.

[DOI: 10.61186/vacres.9.2.1]

adjuvant which promotes immune response (via T helper) (PN: WO2010094663) [47]. Other institutions/universities also claimed for inactivated vaccine compositions: the **US NAVY/US ARMY** applied an immunogenic composition to treat/ prevent/ ameliorate dengue infection against all DENV subtypes, e.g., Dengue-1 virus, Dengue-2 virus, Dengue-3 virus and Dengue-4 virus to promote cellular and humoral immune response. The whole inactivated DENV composition (vaccine formulation) has the nucleic acid to encode "*dengue virus NS3 helicase and/or its fragments as an additional ingredient*" (PN: US2019015498) [48]. Another American Institution (**US SECRETARY OF THE NAVY**) claimed for a vaccine with one/more inactivated virus (against dengue infection – the four subtypes) which is prepared by **psoralen inactivation** (the virus was exposed to an inactivating psoralen which compound is "*4'-aminomethyltrioxsalen hydrochloride (AMT), 8-methoxypsoralen (8-MOP), 4, 5', 8-trimethylpsoralen (TMP)*" and combinations. Furthermore, the virus is also exposed to an ultraviolet radiation (preselected intensity) to render a noninfectious virus and according to the document "*Psoralen inactivation, by contrast, has the potential to present these epitopes to the host immune system in their native confirmation*" what is crucial to vaccine development (PN: US2015291936) [49].

The **UNIV PLA 3RD MILITARY MEDICAL (China)** developed a microneedle vaccine against DENV infection (inactivated virus). In the microneedle injection the antigen can be delivered in the skin with a high immune signal cell. An advantage of this approach is that the vaccine delivery has a better effect (PN: CN105496986) [50]. About data from research institutions, it is currently under consideration by several institutions the use of epitope polypeptide to develop vaccines: **UNIV PLA 3RD MILITARY MEDICAL (China) (Licensor: UNIVERSITY OF CALIFORNIA)** claimed for an "*epitope polypeptide dengue virus 2 type NS3 protein*", for a reliable and safe vaccine, with high immunogenicity and specificity. The epitope polypeptide derivative is formed by coupling to prevent serotype 2 of DENV to be used in a vaccine composition (PN: CN 102936278) [51]. In addition, it was also claimed by the same institution (**UNIV PLA 3RD MILITARY MEDICAL (China) (Licensor: UNIVERSITY OF CALIFORNIA)** a dengue virus-Japanese encephalitis' recombinant expression vaccine, which is embedded by a pseudovirus particle vaccine to stimulate the human body to produce an immune response with suitable safe, good immune effect and suitable for industrial production (PN: CN 101899466) [52]. **NAT HEALTH RESEARCH INSTITUTES (Taiwan)** claimed for dengue virus peptide (synthetic, isolated or recombinant peptide) to provide immune response against DENV infection, which comprises a sequence of amino acids (103) (PN: US2009074781) [53]. Once again, the lack of applications of subunit vaccines from partnerships with companies is consistent with the findings of few neglected vaccines that reach the market. Companies, however, have submitted applications. Some examples should be highlighted: a tetravalent dengue vaccine was developed by **INT CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY (India)**, comprising "*a recombinant tetravalent mosaic virus-like particle*" with DENV's serotypes (from 1 to 4) which resembles all DENV serotypes with no infection capacity, since the VLP do not contain viral genetic material. Furthermore the viral structural proteins' expression (capsid/envelop) lead to "*the self-assembly of virus like particles*" (IN 201711034626) [54]. Indeed, to treat DENV infection, a new isolated peptide/oligopeptide was claimed **IMMUNOTOPE INC (US)**. The most relevant finding here is that the peptide/polypeptide can be processed to bind one/more class I MHC (major histocompatibility complex) molecules. Indeed, they can bind to one/more class I MHC molecules, and finally this peptide/polypeptide can activate T lymphocyte response. The method can be used to prevent/treat the subject against DENV infection (PN: WO2015175361) [55]. Here we also investigated institutions, such as the **INST OF MEDICAL BIOLOGY CHINESE ACAD OF MEDICAL SCIENCES (China)**, which submitted the application of a method to construct a protein (NS1 recombinant protein) to prepare a quadruple vaccine to prevent and to induce immune response for DENV fever type I-IV. Furthermore, the composition uses aluminium hydroxide adjuvant (vaccine adjuvant), which promotes better immune response with safety. Our most intriguing finding is that when the invention is compared to previous technologies, there are important beneficial such as: in the invention has adopted *"the prokaryote colon bacillus expression*" of four DENV subtypes and produces a higher immune response against DENV subtype IV (NS1 antigen, which plays an important role in this viral infection) (PN: CN106754983) [56]. In addition, the **UNIVERSITY OF NORTH CAROLINE (US)** applied an alphavirus vector, which it is prepared by a recombinant method. The nucleotide sequence can encode DENV protein to induce immune response in subjects against the infection (PN: WO 2012027473) [57]. It is a type of construct where you can use DNA or a viral vector to amplify the production of mRNA for the desired protein. Another **important partnership is TAKEDA VACCINES INC (Japan) and US GOV HEALTH & HUMAN SERV (US)**, which developed the same vaccine mentioned above in live recombinant vaccine. The virus used a cDNA clone to produce the virus of the vaccine. There are indications that they patented the cDNA vector and made other IP for final vaccine, as part of the same package There is one attenuated virus DENV2 produced by passage in dog cells and three chimeras made of DENV1, 3 and 4 applied a new nucleic acid chimera which is prepared by a standard recombinant method. It is able to encode structural proteins (from DENV-1) and non-structural proteins (from attenuated/modified live DENV-2) and can be used as a vaccine against the infection. For the induction of immune response against DENV serotypes, the nucleic acid chimeras and attenuated DENV are used in the vaccine composition (PN: WO2014150939) [58]. Another partnership among **KAGOSHIMA UNIVERSITY, NAGASAKI UNIVERSITY and TOKYO METROPOLITAN INSTITUTE OF MEDICAL SCIENCE** (all of them from Japan) claimed for a recombinant vaccinia virus with immunostimulant and virucide activities. This composition comprises parts or all of cDNA which can encode an expression promoter and a non-structural DENV protein. The recombinant vaccinia virus can suppress the induction of serious disease (dengue hemorrhagic fever, for example) "*by the phenomenon of antibody-dependent enhancement*", which must be highly considered in DENV infection (PN: WO2020184730) [59]. The companies' observations also agree with the previous results from the other platforms. Here, there is an important company's partnership between GREEN BIOMED INC. and KIRIN KABUSHIKI KAISHA (both from Japan). The companies applied a new fusion polypeptide (by a method to produce recombinant protein) to the induction of immune response against viruses such as DENV and influenza A virus). The new fusion

[DOI: 10.61186/vacres.9.2.1]

polypeptide comprises T-cell and B-cell epitope (PN: WO 2019124557) [60]. The results are also interesting and help to understand the innovations involving DNA/RNA vaccines by the inventors (SIMMONS MONIKA; PORTER KEVIN R; RAVIPRAKASH KANAKATTE; SUN WELLINGTON – all of them from the US which claimed for a DNA vaccine: the induction of immune response to DENV by tetravalent DNA vaccine (or tetravalent live attenuated vaccine). The last one was claimed for boosting vaccine prevention. In this approach, the induction of the immune response against the virus is by administration of a tetravalent purified inactivated or tetravalent DNA vaccine and a tetravalent live attenuated viral vaccine (boosting DENV immunogen) (PN: US20120039937) [61]. **UNIV WENZHOU MEDICAL (China)** claimed for a DENV "*general cytotoxic T lymphocyte epitope DNA vaccine*" used to treat/prevent DENV infection with the promotion of secretion of IFN-γ against DENV infected cells in form of transdermal drug, sprays or injections (PN: CN104971362 [62]; PN: CN104971347) [63]. Here no partnership was observed.

YFV Infection

The search strategy also resulted in few applied documents about innovative technologies in YFV vaccines. However, some technologies/innovations developed by universities/institutions were described, such as a live attenuated or inactivated based vaccine used to breast cancer's prognosis and prevention was applied by **UNIV PADOVA (Italy) (Licensor: DE LONGHI S.P.A.)**. The findings indicate that this vaccine can be used as an economical and safe immunopreventive agent in humans. In fact. a single dose of the YFV 17D vaccine in women 40-54 year old can prevent up to 65% of breast cancer's cases (for a period of 6 years since vaccination) and 25% (period of 8 years) (PN: WO2015059114) [64]. Furthermore, live-attenuated flaviviruses (attenuated YFV is the YF 17D) using heterologous antigens provide as well interesting results, with the claimed polynucleotide (bacterial artificial chromosome) that can be used as a vaccine to prevent an infection caused by immunogenic peptide fragment (T cell epitope) or immunogenic protein (T cell antigen) **KATHOLIEKE UNIVERSITEIT LEUVEN (Belgium) (PN: WO2019068877)** [65]. Despite the absence of partnerships to the development of YFV infection (according to the search strategy), the institutions/universities and companies' applications also potentially contribute to the discussion of different technologies involving vaccines: the vaccine applied by **SANOFI PASTEUR (France) (SANOFI AS)**: A live-attenuated YFV strain (the virus is adapted to grow on Vero cells) is used to prepare vaccine against the infection. The strain has the same immunogenic activity, and attenuated in viscerotropism and less neurovirulent when it is compares to its parent 17D YF substrain (PN: WO2019192997) [66]. Once again, it was claimed by **XCELLEREX INC (US) (Licensor: GENERAL ELECTRIC COMPANY)**. It comprises a nucleic acid sequence with a mutation (of an unadapted YFV) and can be used to develop an inactivated virus vaccine. The method implies the serial passage of the unadapted virus in VERO cells which produces an immune response, because there is the increasing of "titer to yield a sufficient antigenic mass" (PN: WO 2012011969) [67]. **KOREA CENTER FOR DISEASE CONTROL AND PREVENTION (Korea) (Licensor: KOREA ADVANCED INSTITUTE FOR SCIENCE AND TECHNOLOGY)** claimed for a YF vaccine which has as the active ingredient a YF virus-like particle to induce immune response against YFV infection. About the invention, the method of administration to the mammals (except for humans) the virus-like particle is able to produce antibody against YFV (PN: KR2015067801) [68]. To prevent/treat YFV infection, **CHENGDU INST OF BIOLOGICAL PRODUCTS CO LTD (China)** claimed for a new YF/Japanese encephalitis "chimeric virus complementary DNA clones" useful to prepare vaccine against YF without toxic side effects. The Chimeri-JYF (chimeric virus) promotes good immune response, with high safety, small toxicity and it can be used to vaccine preparation to replace the live-attenuated YF (17D plant) and ensures immune protection (PN: CN105400799) [69]. **BAXTER INTERNATIONAL INC. (US) and BAXTER HEALTHCARE SA. (Zwitzerland)** developed a new modified and recombinant vaccine against YFV. It comprises a modified recombinant virus Ankara (modified vaccinia virus Ankara – MVA) which can encode an envelope polypeptide of YFV and a precursor of membrane. The recombinant MVA is comprised by "*the YFV-17D prME gene cassette set out in a nucleic acid sequence*" to induce (in a pharmacological composition) the immune response against YFV infection (PN: WO2012040474) [70]. **OSWALDO CRUZ FOUNDATION-FIOCRUZ (Brazil)** developed oligonucleotides used to prepare DNA vaccines against YFV infection. The patent demonstrates the construction of a protein chimera using the prM/E regions of the Yellow Fever protein and a cellular targeting signal from the Lysosomal Associated Membrane Protein-1 to direct the antigen to the lysosomal/exosomal compartment using a codon optimized genetic code. This optimized genetic code can be used in DNA, RNA and viral vector platforms. The oligonucleotides are provided to amplify Pre/M-E and are able to induce T-cell response (PN: WO2011050431) [71]. The company **CUREVAC AG (Germany)** applied an artificial RNA ("heterologous 5'-untranslated region (UTR) and/or heterologous 3'-UTR") to promote immune response to treat/prevent YFV infection (or a disorder related to) (PN: WO2019193183) [72].

ZKV Infection

It is of utmost importance to highlight the very low number of documents against ZIKV infection, with few applications by partnerships between companies/universities, which would be crucial to help Zika vaccines to overcome the "valley of death" in vaccine development. The applicants **SHI PEI YONG, XIE XUPING, SHAN CHAO (all of them from the US)** were responsible for the development of a live attenuated vaccine containing a ZIKV (with a 3'UTR deletion). This vaccine can induce different responses: cellular immune, an antibody and/or a cluster of differentiation (CD)-8+ cell responses (PN: WO2018152158) [73]. In addition, a partnership between **US HEALTH and UNIV JOHNS HOPKINS (US)** has applied a live attenuated ZIKV vaccine developed from ZIKA nucleic acid chimera (with nucleotides sequence to encode 3'untranslated region and nonstructural and structural proteins). The mechanism of attenuation maintains Zika chimera, DEN1, DEN2, DEN3 and DEN4 viruses' proteins with the full capability to induce cellular/humoral responses against ZIKV and each DENV serotypes (non-structural/structural proteins) (PN: WO2017156511) [74]. Moreover, the **US HEALTH (US)** is responsible for an immunogenic composition with at least two bicistronic attenuated flavivirus, one of them against ZIKV to prevent/treat ZIKV infection. The flaviviruses attenuation

[DOI: 10.61186/vacres.9.2.1]

uses the bicistronic rearrangement approach or the dual approach attenuation. The company uses the mechanism of attenuation which also maintains Zika chimera, DEN1, DEN2, DEN3 and DEN4 viruses' proteins with the full capability to induce cellular/humoral responses against ZIKV and each DENV serotypes (non-structural/structural proteins) (PN: WO2018129160) [75]. Once again, we identified a few number of applications by universities, such as the ZIKV inactivated (by polypeptide) vaccine by FUDAN UNIVERSITY (China). In a mouse model, the polypeptide Z2 ZIKV inactivated vaccine showed a protective effect and a great potential as a vaccine candidate (PN: CN110025780) [76]. As for partnerships between Government and Research Institutions, some applications were identified: **THE US SECRETARY OF THE ARMY (US) and UNIV NEW YORK STATE RES FOUND (US)** applied a purified inactivated immunogenic ZIKV to prevent, alleviate the symptoms or treat the infection caused by the flavivirus. The pharmaceutical composition with an acceptable adjuvant promotes in a subject the raising of antibodies that recognize ZIKV (PN: WO2017210215) [77]. The result of **TAKEDA VACCINES INC (Japan) and US HEALTH (US)**'s partnership was an inactivated and isolated ZIKV preparation with formaldehyde to be used as vaccine to treat/prevent the infection which can be used to provide antigens to induce the immune response in a subject (PN WO2019090233) [78]. Moreover, the virus' inactivation method (isolating preparation using depth filtration followed by buffer exchange or/and dilution; and/or exchange chromatography and finally the treatment with formaldehyde) was applied by the same companies. The pharmaceutical composition is immunogenic and therapeutically effective according to the dosage formulation, where the administered quantity depends on the subject, such as desired degree of protection and the immune system of the individual (PN: WO2019108976) [79]. A new inactivated ZIKV vaccine with specific amount of the virus (100-1600 U/mL Zika virus antigen and less than 20 µg/mL total protein) is useful for women before pregnancy (women of childbearing age). The formulation has advantages: it does not cause side effects; it is cost-effective and a simple vaccine and can be used by women before pregnancy (SINOVAC RES & DEV CO LTD (China). The formulation is comprised by "600 U/mL Zika virus antigen and less than 20 µg/mL total protein" to enhance the immunity (PN: CN105749268) [80]. The same company (**SINOVAC RES & DEV CO LTD – (China)** also applied a combined inactivated vaccine (a mixing of encephalitis B virus and ZIKV and additives). The vaccine's advantages are (e.g.): it can avoid the allergic reaction or infection, immunize/prevent/control the encephalitis B virus and ZIKV simultaneously and reduce the number of immunization (PN: CN108187036) [81]. **TAKEDA VACCINES INC. (Japan)** applied a liquid inactivated whole ZIKV and formulation's components (one buffer, an optional polyol) without adjuvant (aluminium salts). The vaccine can induce antibody/cellular and/or secretory-mediated immune response, such as the production of antibodies (immunoglobulins A, D, E, G or M), expansion/suppressor of T cell helper or proliferation of T/B lymphocytes, besides the provision of immunological cells' signals (PN: WO2020226831) [82]. **INSTITUT PASTEUR OF SHANGHAI CHINESE ACADEMY OF SCIENCES (China)** introduced a further innovative subunit ZIKV vaccine which was expressed by yeast cells to treat/prevent diseases related to ZIKV. The immune response is induced by a sufficient amount of virus-like particles in the administration path (PN: CN108503696) [83]. The French institutions **INSTITUT NATIONAL DE LA SANTÉ ET DE LA RECHERCHE MÉDICALE; INSTITUT DE RECHERCHE POUR LE DÉVELOPPEMENT; CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE and the UNIVERSITE DE LA REUNION SAINT DENIS (Licensor: CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE and INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE)** developed an isolated polypeptide with 500 amino acids against ZIKV envelope protein to induce immune response. According to the inventors: "*this is the first report demonstrating the antigenicity of a fragment of the Envelope protein of a flavivirus, within Domain I (E-DI), which explicitly includes the glycan-loop itself*" (PN: WO2021144363) [84]. Once again, one of the key findings was most of the applications were from Research Institutions such as "*recombinant vesicular stomatitis virus vector*"(rVSVV) used as vaccine against ZIKV infection from **UNIVERSITY OF MIAMI (US)**. It was observed that rVSVV (rVSV based vectors) can provide protection against ZIKV. In addition, immunoglobulin to the ZENV protein is generated when mice are inoculated with rVSV (PN: WO2017192856) [85]. Another application, from **HARVARD COLLEGE** also applied a RVSVV, which comprises the nucleic acid sequence to encode ZIKV envelope protein or ZIKV envelope and it can induce immune response against ZIKV infection (PN: WO2018075751) [86]. Based on the search strategy, once again no partnerships were found between companies and research institutions, with most of them are from the last ones. Notwithstanding, applications from companies alone were found, such as the one from **GEOVAX INC (US)**, for a "recombinant modified vaccinia Ankara vector". This strategy is used to induce celular and/or humoral response against ZIKV infection and it can limit the diseases' severity and/or an infection (PN: WO2017136419) [87]. Another company, **ETUBICS CORP (US) (Licensor: NANT HOLDINGS IP LLC)** applied compositions to produce vaccines to promote immune response against flaviviruses. The compositions comprise a "*recombinant adenovirus based vector vaccine*" and can prevent ZIKV infection. The generation of immune response against the target flavivirus antigens is reached by the administration of an adenovirus vector to the individual (PN: WO2018014006) [88]. Nucleic vaccines are also researched by universities, as in the application on a ZIKV DNA vaccine with recombinant plasmid pCMV-SZIKA-ME (with DNA fragments to encode ZIKV prM/E and human matrix metalloproteinase-9) – from **UNIV SHAXI (China)**. The results in mice demonstrate the adequacy of the vaccine for induction of specific antibody against ZIKV E protein/prM generate by the "gene vaccine atomizing immune Balb/c mouse" (PN: CN112023034) [89]. The **US DEPARTMENT OF HEALTH & HUMAN SERVICES (US)** developed a nucleic acid molecule (to encode a polyprotein), virus-like particle or protein to detect in a sample anti-ZIKV antibodies prepare a medicament and to induce immune response against ZIKV infection (PN: WO2018052549) [90]. Two companies, **CUREVAC AG (Germany) and SANOFI PASTEUR SA (France)** applied an artificial nucleic acid with one code region (at least) to treat/prevent ZIKV infection or a disorder related to this infection. It can be produced at a storage stable and an industrial scale. The artificial nucleic acid elicits an adaptative immune response to treat/prevent ZIKV infections or any disorder related to ZIKV (PN: WO2017140905) [91].

[DOI: 10.61186/vacres.9.2.1]

Non-exclusive vaccines for the treatment/prevention of neglected diseases: Chikungunya, Dengue, Yellow Fever and Zika infections

We retrieved in our study as well the vaccines' technologies (for the treatment/prevention of neglected emerging diseases: CHIKV, DENV, YFV and ZIKV infections not exclusively). Here, the preventive;therapeutic compounds retrieved from the applied documents are described:

UNIV ILLINOIS FOUND (US) claimed in its applied patent document a method to promote immune response using virion or live-attenuated virus adsorbed in highly porous (HPAC) against diseases, including **Chikungunya, Zika and Dengue**. Thus, in the drug delivery system there is the adsorbed to HPAC in an amount that can reduce viral spread or inhibit virus entry into a cell (PN: WO2020210376-A1) [92]. **TAKEDA (Japan)** developed a tetravalent DENV vaccine which comprises the four live attenuated DENV strains (serotypes 1-4). This composition can be used to prevent, not only **DENV infection, but also YF infection, pertussis, diphtheria and hepatitis A**. There are particular advantages to the use of this method, such as the induction of "*cross reactive multitypic antibody and/or cell mediated immunity*". Moreover, it avoids dengue NS1 toxicosis and the risk related of Dengue Shock Syndrome and Dengue Hemorrhagic Fever caused by DENV serotypes (DENV 1- DENV4) (PN: US2021023204 [93]; US2020230230) [94]. The vaccine applied by **BHARAT BIOTECH INTERNATIONAL LTD. (India)** comprises Arbovirus antigens against **CHIKV and ZIKV** (and Japanese encephalitis virus) in prime boost strategy, where the first candidate is an inactivated vaccine[16] (PN: WO2017009873) [95]. When the vaccine, which is suitable for humans, is administered in mammals in a single dose/two or more doses it elicits Th1/Th2 immune response against the mentioned viruses. A subunit vaccine against **ZIKV, YFV, DENV or West Nile Virus was claimed by the UNIV MINNESOTA (US) and the company KANPRO RES INC (Licensor: UNIVERSITY OF MINNESOTA – THE REGENTS OF)**. Here, the subunit composition with a pharmaceutically carrier comprises the antigen amino acid sequence with amino acid residues. Furthermore, the vaccine does not promote immune interference, antibody-dependent enhancement (PN: WO2020123759) [96]. Concerning research institutes, **TEXAS TECH UNIVERSITY SYSTEM (US)** developed a multivalent arbovirus virus- like particles vaccines with the viruses' structured proteins. The multivalent vaccine can combine different virus-like particles from the selected arbovirus. Therefore, it is important to highlight that the cell line which is transduced with a lentiviral vector can make a virus-specific virus like particles. These ones are purified and mixed in different combinations to prepare the multivalent vaccine against many viruses, including **CHIKV, YFV and ZIKV (PN: WO2020081759)** [97]. **EMERGEX VACCINES HOLDING LTD (United Kingdom)** developed a vaccine with flavivirus peptide (with one/more of the CD8+ T cell epitopes) to stimulate immune response against West Nile virus infection, **ZIKV infection (African ZIKV), DENV infection** without adverse effects associated to this kind of vaccine (PN: WO2019186199) [98]. Moreover, a more effective and safer flavivirus vaccine (virus-like particle immunogenic composition) was claimed by **TECHNOVAX INC (US)**. The virus-like particles have a combination of **ZIKV, CHIKV and DENV** to provide immune response, where the particles feature conformational epitopes to enhance neutralizing immune response against the virus (PN: WO2016210127) [99]. Other applied patent documents reinforce the general belief about the subunit platform for vaccine development. A vaccine developed by **EMERGEX VACCINES HOLDING LTD (United Kingdom)** that in the composition there is flavivirus peptide from ZIKV, YFV, DENV, West Nile virus and/or Japanese encephalitis virus . The peptide comprises "*one or more of the cluster of differentiation (CD)8+ cell epitopes*" (PN: WO2019135086) [100]. **OXFORD UNIVERSITY INNOVATION LTD. (England)** applied a method to treat/prevent CHIKV and ZIKV infection: a recombinant vector vaccine, which comprises the nucleic acid to encode the virus structural antigen. The composition (combination of viral vectors) has a strong potential to induce antibody responses, due to (at least similar to individual viral vectors) the expression of viruses' structural proteins (PN: WO2018020271) [101]. **WASHINGTON UNIVERSITY (US)** applied a vaccine of a "*dendritic cell target adenovírus*" and a "*structural gene of a heterologous virus*" (at least) to induce immune response against viruses or flaviviruses, including **CHIKV, DENV, ZIKV or YFV** (PN: WO2018201025) [102].

The partnership between **WISTAR INST (US) and INOVIO PHARMACEUTICALS INC (US) (Licensors: UNITED STATES HEALTH & HUMAN SERVICES and INOVIO PHARMACEUTICALS INC)** has as a product an isolated nucleic acid molecule that has some advantages: safety enhanced translation, absent/low innate immunogenicity and increased stability. The composition can induce immune response against the combination of DENV, CHIKV and ZIKV which developed an efficacious vaccine to reduce public health infections caused by mosquito-borne viruses such as CHIKV and additional virus antigen (ZIKV, DENV or its combination). The nucleic acid vaccine comprises an isolated molecule (nucleic acid) to induce immune response in a subject and for delivery (PN: WO2019148086) [103]. Furthermore, the companies **CUREVAC AG (Germany) and SANOFI PASTEUR (France)** claimed for an artificial nucleic acid to treat/prevent flavivirus infections, such as DENG and YF, which promotes an antigen-specific immune response. Furthermore, the effective, cost-effective, and safe immunogenic composition can be produced at large quantities and cold chain is not required (PN: WO 2019115635) [104]. **MODERNATX INC (US)** applied a RNA vaccine (formulated within lipid nanoparticles) against CHIKV, DENV and ZIKV which encodes immunogenic fragments or antigenic peptides to immunizes for more than 2 years with an efficacy greater than 60% (PN: WO2017015463) [105].

Therapeutics Compounds

The partnership between **AGENCY SCI TECHNOLOGY&RES (Singapore) and NOVARTIS AG (Switzerland)** applied a method to determine the absence/presence of DENV serotypes, to treat subjects with severe dengue infection (medicament's preparation) and to determine dengue 's outcomes in subject (PN: WO2012125125-A1) [106]. Therapeutic compounds can be used as immunomodulators: **AURIGENE DISCOVERY**

[16] The boost used can be the inactivate one or other platforms, such as vaccine from synthetic Zika virus, chimeric Zika virus vaccine, DNA vaccine, live attenuated virus vaccine, virus like particles, vectored vaccine, inactivated Zika vaccine or recombinant subunit vaccine (PN: WO2017009873).

[DOI: 10.61186/vacres.9.2.1]

TECHNOLOGIES LIMITED (India) (Licensor: DR. REDDY'S LABORATORIES LTD.) developed a modified peptide derivative to diseases' treatment, such as DENV, HIV, Cancer, Influenza, etc. by *"programmed cell death 1 (PD1) signaling pathway inhibitor"* (mechanism of action) (PN: WO2012168944-A1) [107]. **AURIGENE DISCOVERY TECHNOLOGIES LIMITED (India) and CURIS INC (US) (Licensors: CURIS INC and DR. REDDY'S LABORATORIES LTD.)** claimed for a method to modulate the immune response, to inhibit metastasis or/and tumor cells' growth and to treat infectious diseases (including **DENV and YF infection**) or cancer (PN: WO2019087092-A1) [108]. It is also important to highlight the development of oral adjuvants of pharmaceutical composition to treat Th1-mediated diseases, viral and bacterial infections. The composition has an excellent curative rate and among the claimed diseases, there are rheumatoid arthritis, bacterial meningitis and **Dengue Hemorrhagic Fever (BIOCOPEA LTD – England)** (PN: WO2012056251-A1) [109]. Furthermore, **GEISTLICH PHARMA AG (Switzerland)** developed a method (oxazinane derivative) to inhibit, prevent or reduce an increase of one or more cytokines caused by infection conditions, including DENV fever (PN: WO2020234830-A1) [110]. Immunotherapy using stem cells was claimed by **WHITE OAK IND INC (US).** The applied document presented a method to immunomodulate/immunostimulate cells in vitro conditions. Among the virus used, there are adenoviruses, herpes simplex viruses, or Dengue Fever. (PN: US2016160178-A1) [111]. Gene therapy was the topic studied in the document applied by **SYNTHETIC GENOMICS INC (US)** about methods/compositions to enhance gene expression. In fact, the nucleic acid is useful to produce the polypeptide of interest in the cell. From the claimed components' composition, there are the alphavirus species, including **CHIKV** (PN: WO2018106615) [112].

Part 3. Identification of vaccines' development phases (from research to production)

The products under active development, reported over the past 12-18 months in the CDDI database, were described here in different phases of development. From the research strategy, a few numbers of documents related to vaccines for the NTD of interest were retrieved. For **dengue**, from 154 drugs & biologics related to dengue vaccine retrieved by the search strategy, 135 were not under active development, while **18** were under active development. For **Zika,** from 142 drugs & biologics related to vaccine against Zika retrieved from the search strategy, 125 were not under active development, while **17** were under active development. For **Chikungunya,** from **34** drugs and biologics related to vaccine against Chikungunya retrieved from the search strategy, **25** were not under active development, while **9** were under active development. Finally, for **Yellow Fever**, from **24** drugs & biologics related to vaccine against Yellow Fever retrieved from the search strategy, **21** were not under active development, while **3** were under active development. Overall, the number of drugs/biologics under active development is very low: only 13% are still under active development, while 87% stopped in any development phase. Fig. 4 presents an overview of these products (vaccines and candidates) which are in the "valley of death" or crossing the "valley of death" to prevent /treat CHIKV, DENV, YFV and ZIKV infections for each phase of the development status.

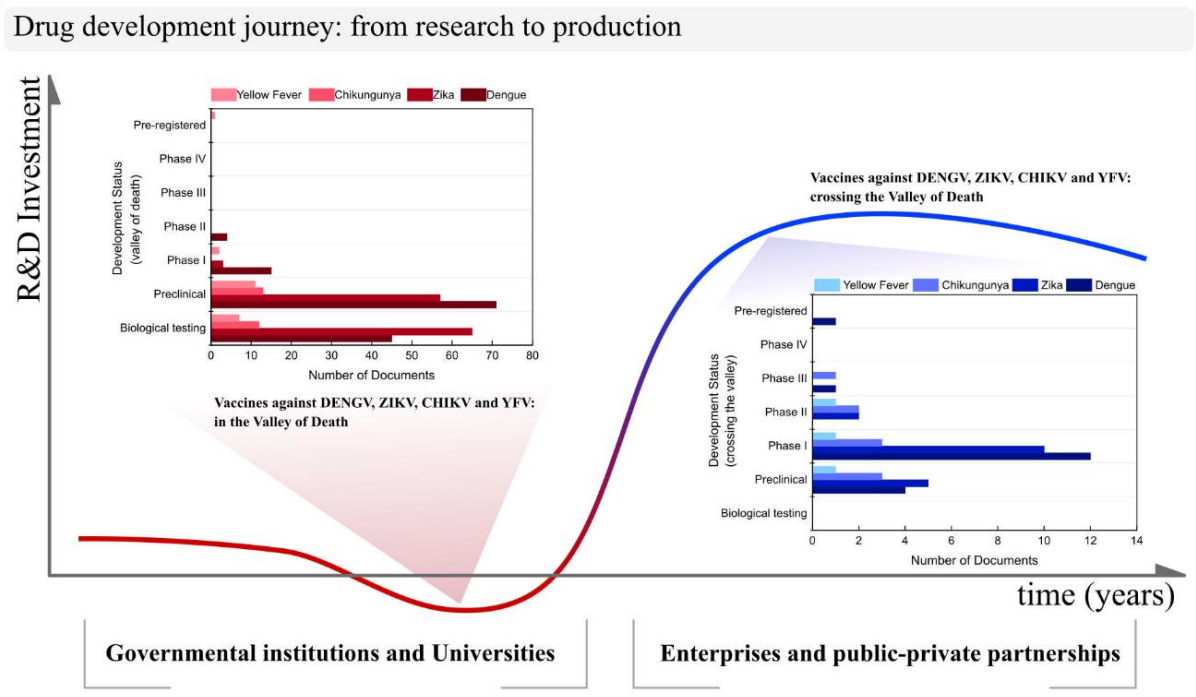

Fig. 4. Drugs & Biologics (in the valley of death and crossing the valley of death) for vaccines (and candidates) to prevent CHIKV, DENV, YFV and ZIKV infections for each phase of the development status.
(*) Drugs or Biologics in Clinical trials in phase I/II were grouped in phase I in the present study.
Elaborated by the authors from the source CDDI from Clarivate Analytics.

[DOI: 10.61186/vacres.9.2.1]

DENV: The live attenuated tetravalent dengue vaccine (TDV) was developed by Takeda -TAK-003 (DENVax) to prevent dengue in individuals ages 4 to 60. It was first registered in the European Union (under regulatory review) [26]. According to
the search strategy, 37 clinical studies were associated to this vaccine. In United States, the vaccine was granted fast track designation to prevent dengue infection (CDDI). In 2017, a clinical trial was enrolled to assess the safety and immunogenicity of sequential/concomitant administration of both TDV and yellow fever vaccine) in adults [113]. According to George and collaborators [114], the randomized, double-blinded phase 1 clinical trial to assess the immunogenicity of the TDV demonstrated that, despite the need of further developments studies (e.g. in multiple age groups), the vaccine was well tolerated in the studied group. Furthermore, it induced broader or trivalent antibodies against dengue virus in most volunteers who are flavivirus-naïve. In 2017, Inviragen (Takeda – Japan) announced in a press release the DENVax‘s advances into an ongoing phase 2 clinical study to evaluate initial safety data in multiple age groups. The study offered additional safety and immunogenicity data in children (as young as 18 months) [115]. The efficacy of TAK-003 (dengue candidate vaccine) in children and adolescents (4-16 years old) in the second year after vaccination was conducted, with no occurrence of adverse effects during this year [116]. Moreover, a phase 3 randomized trial [117] showed that TAK-003 demonstrated efficacy in regions of Latin America and Asia (endemic regions) against symptomatic dengue. A phase 3 study was conducted to evaluate the efficacy of TDV (2 doses) in this age group [118]. The Chimeric yellow fever-dengue tetravalent vaccine has 84 clinical studies associated to it. Tetravalent Dengue Vaccine (TDV) developed with recombinant technology by Sanofi-Pasteur induced full serum conversion in adults (flavivirus-naïve) against all serotypes with three doses [119]. The vaccine was launched in Mexico in 2016 and registered in European Union (2018) and The United States (2019). Moreover it was withdrawn from Philippines in 2019 [26]. Capeding and collaborators [120] demonstrated that TDV is also efficacious to children of endemic areas in Asia (aged 2-14 years old) with three doses at months 0, 6 and 12. Meanwhile, Qiao and collaborators [121] investigated the candidate TDV (live attenuated dengue vaccine) - infectivity, safety and immunogenicity data - in a controlled and open phase IIa study to analyze the effect of yellow fever immunity and pre-existing dengue, which increase the immune response to a single injection of TDV (mainly against serotypes 1 and 2) (Table 1).

ZIKV: ZIKV vaccine development was stimulated by its spread to Western Hemisphere, besides the severe congenital disease caused by the virus, due to the vertical transmission (mother to fetus) [5, 122]. The analysis of vaccines under active development revealed that most of them were in the earliest clinical development phases. Most of the vaccines were in phase I clinical trials (Fig. 4) (KD-406 (from KM Biologics), ChAdOx1 Zika (Oxford University Innovation; University of Oxford); MV Zika RSP (Merck & Co.); mRNA-1893 (Moderna; Washington University and TAK-426 (Takeda)). The vaccine in the most advanced stages of development was BBV-121 from the Indian company Bharat Biotech and it is an inactivated Zika virus vaccine to prevent Zika virus infection [26]. From the company’s pipeline, the Zika virus vaccine candidate is in phase II clinical development [123]. Another vaccine candidate is the DNA-vaccine candidate (from US National Institute Allergy Infectious Diseases) which is able to encode structural immunogenic proteins of ZIKV, tested in animal models and humans). When Maciejewski and collaborators [122] compared the ZIKV candidate vaccines: VRC5288 and VRC5283 (amino acid sequence similarity), one of the results was related to the level of neutralizing antibody activity to protect from viremia by the last candidate (VRC5283) which was substantially lower when compared with the level required by VRC5288 vaccine. Meanwhile, 100% of the participants (14) who received two doses of VRC5283 presented positive antibody responses. This vaccine candidate, advanced into phase 2 clinical trial, showed the most robust T-cell responses and robust neutralizing antibody [124] (Table 1).

CHIKV: There is a very low number of drugs &biologics under active development for CHIKV. In clinical trials, the following vaccines were identified: in phase 1, three vaccines (BBV-87 from Bharat Biotech; ChAdOx1 Chik from University of Oxford; mRNA-1388 from Moderna). In phase 2 development, MV-CHIKV from Institut Pasteur and VLP CHIKV from National Institute Allergy and Infectious Diseases/National Institutes of Health. In the highest phase of development (Phase 3), a vaccine candidate is the the live attenuated vaccine: VLA 1553 - from Karonlinska Institutet and Valneva. For this vaccine, there is a partnership with Butantan Institute, Brazil. The Brazilian producer of biologic compounds signed a final agreement to develop, manufacture and market Valneva Chinkungunya vaccine for low and middle income countries [125]. In 2020, the company Valneva announced the end-of-phase 2 clinical trial of the single-shot Chikungunya vaccine (VLA1553) [126], considering the excellent immunogenicity results after a single shot, with 100% seroconversion announced by the company in 2019 [127] (Table 1).

YFV: For Yellow Fever, only three products were under active development: the live attenuated vaccine YF-S0 from Rega Institut in preclinical phase; A Sanofi Pasteur Yellow Fever vaccine in phase 1/2 stage. And finally in the highest phase, the virus vaccine produced in Vero cells. In July 2021, the company Sanofi through its press release announced its vaccine had moved into Phase 2 clinical trial. Finally, among the new announced products, another Vero cell candidate vaccine against Yellow Fever, is the SP0218 vaccine in adults in the United States [128] (Table 1).

Concerning the results of table 1, an interesting observation can be made, related to the neo-evolutionary model of the relations among University-Industry-Government (Triple Helix Model for innovation) launched in 1996 by the Professors Henry Etzkowitz and Loet Leydesdorff. It is worth remarking here that these actors (Research Institutions, Companies and Government) are fundamental for innovation and this relationship in the Triple Helix is complex and contributes to a better understanding of the dynamics of the ongoing transformations in the vaccine innovation scenario. Thus, in our view, the key strength of this research lies in providing evidences for existing gaps and urgent need to support partnerships among these three actors as a path to innovation and a facilitator for technological development of vaccines. It is certainly the most feasible way to accelerate innovation and key

[DOI: 10.61186/vacres.9.2.1]

for the success in development and implementation of new technologies [129, 130].

Table 1. Vaccines (or candidates) crossing the valley of death to prevent D

Brand name	Code name	Highest phase	Product category (Number of related clinical studies)	Description	Results	Organizations
DENGUE VACCINE						
DENVax	TAK-003	Pre-registered	- Live attenuated vaccine - Recombinant vaccine (37)	*"Tetravalent dengue vaccine comprising live attenuated dengue virus serotype 2 (DENV-2) PDK-53 strain and three chimeric virus (DENV-2/ 1, DENV-2/3 and DENV-2/4), generated by replacing the DENV-2 PDK-53 structural genes (prM and E) with genes of DEN-1, DEN-3 and DEN-4 respectively"*	Prevention in individuals ages 4 - 60 against any DENV serotypes (under regulatory review in European Union)	Takeda and Centers Disease Control Prevention (Originators) Inviragen (Takeda)
ZIKA VACCINE (candidate)						
-	-VCR-5283 -VRC-ZKADNA090-00-VP	Phase 2	-DNA vaccine (4)	"*Zika virus (ZIKV) vaccine consisting of a DNA plasmid encoding a codon-optimized chimeric ZIKV (H/PF/ 2013 strain)/Japanese encephalitis virus (JEV) precursor transmembrane (prM) and envelope (E) protein, with the prM signal sequence exchanged with the analogous sequence from JEV; under the control of cytomegalovirus (CMV) immediate early promoter"*	*"The vaccine is in phase 2 clinical trials at National Institute Allergy Infect Dis"*	-National Institute Allergy Infec. Dis. (Originator)
Zikavac	BBV-121	Phase 2	-Inactivated (killed) Vaccine (0)	*"Zika virus vaccine consisting of inactivated Zika virus"*	The vaccine is in phase 2 clinical trial at Bharat Biotech	-Bharat Biotech (Originator)
CHIKUNGUNYA VACCINE (candidate)						
-	VLA 1553	Phase 3	-Live attenuated vaccine (12)	*"Chikungunya (CHIKV) vaccine consisting of live attenuated CHIKV clone LR2006-OPY1 comprising 183-bp deletion (1656-1717 aa of the P1234 polyprotein) in the C-terminal hypervariable region of nonstructural protein 3 (nsP3); produced in Vero cells"*	The vaccine is candidate in phase 3 at Valneva and received the PRIME designation in the European Union in 2020	- Karonlinska Institutet/ Valneva (Originators) -Instituto Butantan
YELLOW FEVER VACCINE (candidate)						
-	SP 0218	Phase 2	-Vero cell vaccine (0)	*"Yellow fever virus vaccine produced in Vero cells"*	The vaccine is in phase 2 clinical trial at Sanofi to prevent YFV in adults	Sanofi (Originator)

Source: Elaborated by the authors from CDDI (Clarivate Analytics) [26].

[DOI: 10.61186/vacres.9.2.1]

DISCUSSION

Our results indicate a concerning global scenario for vaccines candidates for DENV, CHIKV, ZIKV and YFV infections, with only 13% of them still under active development, while 87% stopped in any development phase. This scenario of virtual non-existence of vaccine products for emerging neglected arboviral diseases, mainly affecting the poorest populations, contrasts with recent breakthroughs and advances in Vaccinology 4.0, particularly for innovative COVID-19 vaccines.

Moreover, the results of our research based on reported vaccine platforms to prevent or treat DENV, CHIKV, ZIKV and YFV indicate that the traditional ones such as the attenuated or inactivated vaccine platforms are still used by many companies and research institutions for the development of new processes or products. Notwithstanding, a key contribution of the present research relies on the fact that new innovative platforms, such as RNA-based vaccines are also increasingly being used to prevent or treat these emerging arboviral diseases. From this perspective, outstanding innovative strategies by the German company CUREVAC AG's for the prevention and treatment of YFV infection using artificial RNA illustrate well the recent scientific and technological advances in novel vaccine platforms. The promising potential of these innovative platforms to target a broad range of diseases has been also a goal of the same company in partnership with the French company SANOFI PASTEUR for the development of a RNA vaccine within lipid nanoparticles with 60% of efficacy for immunization for more than 2 years against CHIKV, DENV and ZIKV. From this perspective, new knowledge on the immune system and innovative research based on RNA platforms are accelerating the processes, leading to faster development of more effective vaccines, consistent with recent vaccine advances in post-COVID era.

A combined approach integrating vaccine innovation and access policies into vector control strategies will require comprehensive and effective international and national plans. Our results indicate that an intersectoral global "One Health" perspective will thus be crucial to support these Plans and achieve these goals, with clear priority setting, adequate investments in local vaccine research, development and manufacturing and community participation. Finally, it important to note that vaccines are just a component of a broader and more comprehensive eco-social approach necessary to prevent emerging neglected arboviral diseases. Arboviruses are getting closer to big cities due to unrestrained deforestation, with people increasingly exposed to a great variety of emerging arboviruses as they invade forests for work, tourism and housing, a global scenario aggravated by uncontrolled urbanization and accelerated populational mobility. Vaccines are therefore very important but not sufficient. A combined strategy is necessary, integrating innovative vaccines into other strategies such as vector control and effective urban planning and sanitation, which are critical to prevent arboviral diseases. Although innovative vector control measures, such as the control of *Aedes aegypti* with *Wolbachia* have proved effective, they have also some limitations. They work well within the urban perimeter but when closer to the forest or the waterfront their impact decreases. For this reason, a combined eco-social approach to arboviral diseases is necessary and urgent, integrating vaccines, vector control, urban planning and environmental policies into a new and more effective global strategy to prevent these diseases.

Our results of just 13% of vaccines for Dengue, Chikungunya, Zika and Yellow Fever under active development reinforce the alert for an urgent need for new strategies from international agencies, governments, G-7 and G-20 countries to accelerate vaccine innovation and overcome gaps in the "valley of death" in the vaccine development process. Another relevant finding is that RNA-based vaccines are increasingly being used to prevent or treat these arboviral diseases, in contrast with dominant traditional platforms, such as attenuated or inactivated vaccine platforms. Despite these extraordinary advances, there are still few companies involved in these innovative platforms, namely CUREVAC AG and SANOFI PASTEUR. International and national innovation policies and incentives are thus necessary to urgently change this scenario. Developing countries, particularly the ones more affected by Dengue, Chikungunya, Zika and Yellow Fever, such as Brazil, India and African countries, should search to integrate the actions of their Ministries of Health and Science and Technology, aiming the elaboration and implementation of National Strategic Plans for Development of Innovative Vaccines for Emerging Arboviral Diseases by 2030. These plans should be supported by an exponential increase in global and national funding and incentive mechanisms, new business models and novel strategies for integrating research, development and production of vaccines into their sustainable development goals. These global and national Plans must be urgently implemented in a synergistic way and closely monitored with indicators for achieving their goals, in line with Agenda 2030 Sustainable Development Goals and with the WHO recommendations and roadmap for tropical neglected diseases 2021-2030 [131].

FINANCIAL SUPPORT

The authors thank Bio-Manguinhos Fiocruz, the Chemistry Departament of the Federal University of Rio de Janeiro and the National Institute of Industrial Property for the support.

AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Alessandra Oliveira, Adelaide Antunes and Suzanne Schumacher. The first draft of the manuscript was written by Cristina Possas, Adelaide Antunes, Akira Homma and Ernesto Marques. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The authors declare that they have no conflict of interest.

[DOI: 10.61186/vacres.9.2.1]

ACKNOWLEDGEMENTS

The authors thank Bio-Manguinhos of Oswaldo Cruz Foundation, the School of Chemistry of the Federal University of Rio de Janeiro and the National Institute of Industrial Property for the support. We also thank Rafael Cavalcante dos Santos, M. Sc., School of Chemistry, Federal University of Rio de Janeiro for the figures and final art.

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[DOI: 10.61186/vacres.9.2.1]

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[DOI: 10.61186/vacres.9.2.1]

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