# New vaccines against Zika virus

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## 1 Introduction to Zika virus[1]

The Zika virus (ZIKV) belongs to the *Flaviviridae* family and it's transmitted mainly by mosquitoes of the *Aedes* genera. The virus has a +ssRNA genome with a single ORF encapsulated in an icosahedral capside and surrounded by a lipid membrane [Fig.1].

The most recent outbreak was in Brazil in 2015, when it was first discovered the association of ZIKV infection with severe complications and vertical and sexual transmission.

**OBJECTIVE**: The aim of this study is to review the new vaccine candidates that are being researched and tested at the moment, with main focus on the ones that progressed to clinical trials, and conclude which ones would fit best each situation.

**METHODOLOGY:** All the information for this project was extracted from scientific articles and reviews, WHO reports and reliable webpages. All graphics are elaborated with Microsoft Power Point.

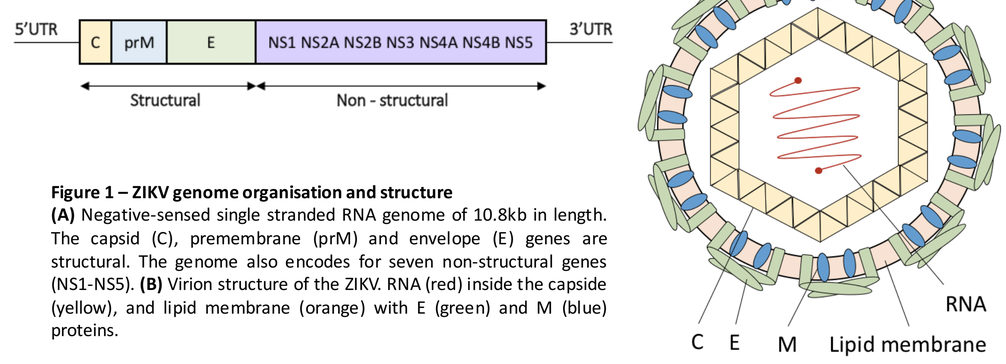


**Figure 1 – ZIKV genome organisation and structure**
**(A)** Negative-sensed single stranded RNA genome of 10.8kb in length. The capsid (C), premembrane (prM) and envelope (E) genes are structural. The genome also encodes for seven non-structural genes (NS1-NS5). **(B)** Virion structure of the ZIKV. RNA (red) inside the capside (yellow), and lipid membrane (orange) with E (green) and M (blue) proteins.

## 2 Considerations for developing a vaccine against ZIKV

The main reason for the need of a vaccine is to avoid vertical transmission and the associated neurological disorders. Therefore, the main target population should be women who are pregnant or at a reproductive age. Due to that, safety requirements are unique. It has to be taken into account that there's a lack of epidemiological data and studies about the infection, disease and complications. Hence, different vaccine platforms should be developed to target different situations.

## 3 Vaccine candidates

### A. DNA vaccines[2]

It consists in plasmids encoding prM and E genes under the control of a promoter [Fig.2]. In this case, WNV vaccine was used as the backbone, since there was evidence that the vaccine was immunogenic and safe. When the proteins express in mammalian cells, they assemble into subviral particles, non-infectious but immunogenic, and trigger an immune response.

**PROS**:
- DNA is stable
- Stablished manufacturing process
- Elicit antibody and cellular response

**CONS:**
- Loss of immunity overtime.
- Could lead to integration in chromosomes (insertional mutagenesis)

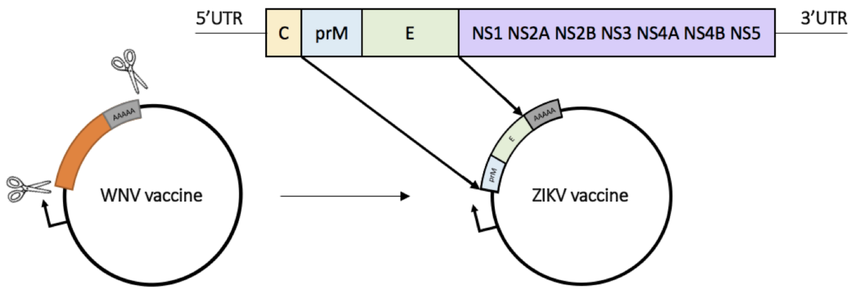


**Figure 2 – Construction of a DNA vaccine against ZIKV**
Construction of a DNA vaccine from a West Nile Virus vaccine backbone. The main coding sequence of the WNV vaccine is enzymatically deleted and substituted by the prM and E proteins from wild type ZIKV.

### B. Live - attenuated vaccines (LAV)[3]

They use an attenuated form of ZIKV, alive but with reduced virulence. Attenuation is achieved by deletions in the 3'UTR, which slows down ZIKV genome replication [Fig.3]. Moreover, mutant 3'UTR viruses are more sensitive to interferon-ß inhibition. They generate E-protein-expressing infectious non-virulent viruses that will trigger a long lasting immune response.

**PROS**:
- Single dose
- Long-lived protection
- Rapid and robust immune response
- Protect against vertical transmission

**CONS:**
- Need to be kept in cool
- Contraindicated for immunocompromised patients and pregnant women.

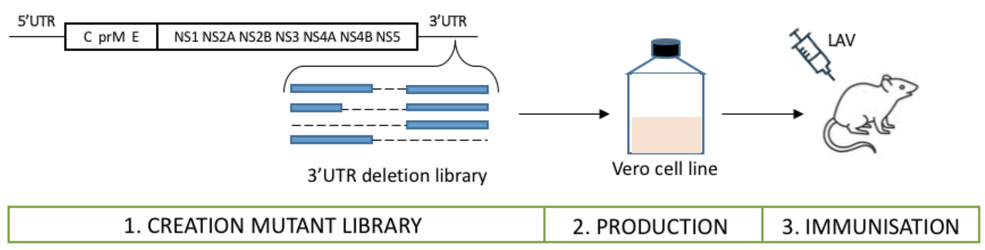


**Figure 3 – Construction of a live-attenuated vaccine against ZIKV**
Steps for the construction of a LAV from a mutant 3'UTR library of wild type ZIKV. Clones with different deletions are generated and then cultured in a Vero cell line. Later on, they are harvested, selected, and administered to mice.

### C. Purified inactivated vaccines (PIV)[4]

They contain the killed ZIKV. The virus follows a process of purification and inactivation to obtain non-infectious and non-virulent subviral particles that will be administered as a vaccine [Fig.4]. The inactivated viruses are easily administered, elicit robust neutralising antibody titers and have no significant adverse effects. PIV have been licensed against JEV and TBE, therefore ZPIV remains a promising candidate.

**PROS**:
- Good safety profile
- Effectiveness demonstrated with JEV
- Optimised manufacture (cDNA clone)

**CONS:**
- Immunity is not as strong as live vaccines
- Require more than one dose or booster shots

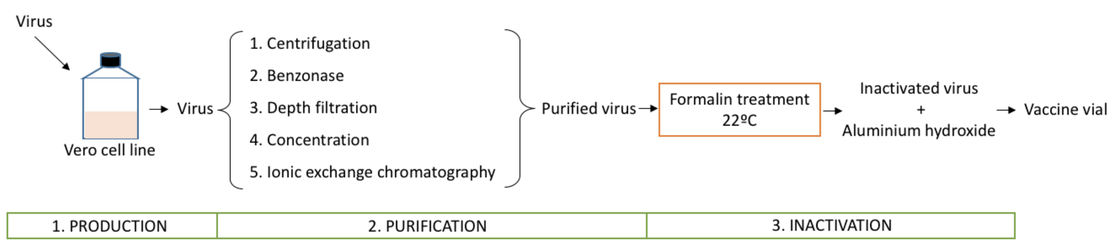


**Figure 4 – Construction of a purified inactivated vaccine against ZIKV**
Steps for the construction of a PIV. First viruses are cultured and harvested, then purified and later on inactivated by formalin treatment. The combination with the inactivated virus with an adjuvant, in this case aluminium hydroxide, constitutes the vaccine.

### D. mRNA modified vaccines[5]

It consists of prM and E genes encoded under the control of a promoter. mRNA vaccine is delivered via optimised lipid nanoparticles (LNPs) [Fig.5]. mRNA translates directly intro a protein in the cytoplasm, so it bypasses the nuclear step. Sub-viral particles assemble and mimic the ZIKV usual cycle and trigger a rapid and potent immune response. They overcome the safety risks associated with live virus vaccines.

**PROS**:
- Single low doses
- Very versatile and cost-effective
- High-safety profile
- Protect against vertical transmission

**CONS:**
- mRNA is not as stable as DNA

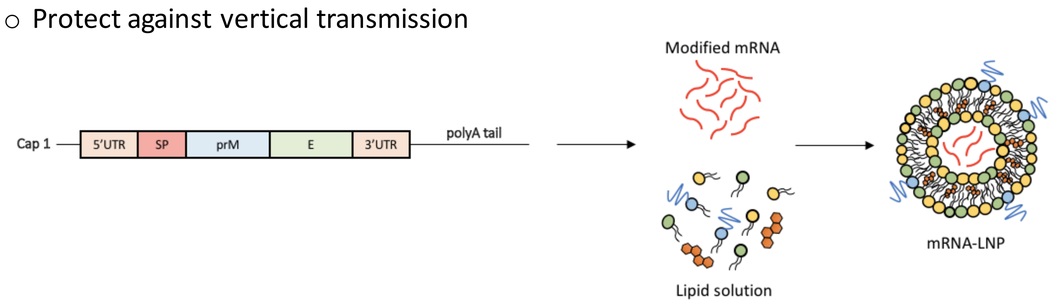


**Figure 5 – Construction of a mRNA modified vaccine against ZIKV**
Steps for the construction of a prM-E mRNA-LNP vaccine. The mRNA is modified to the desired construction and encapsulated in a self-assembly process by mixing the modified mRNA solution with an ethanol solution containing the LNPs constituting lipids.

## 4 Conclusions

All of the newest vaccine candidates elicit high titers of neutralising antibodies that protect against ZIKV challenge, viremia and tissue viral burden; however, mRNA-LNPs vaccines might be the best option for at-risk populations, since they protect against vertical transmission, are safe and immunogenic. Although more time is needed to see if the conferred protection is sustained overtime, it seems that we are on the right path for de development of an effective vaccine against ZIKV.

Relevant references:
[1] Who.int. (2019). *Enfermedad por el virus de Zika*. [online] Available at: https://www.who.int/es/news-room/fact-sheets/detail/zika-virus [2] Gaudinski, M., Houser, K., Morabito, K., Hu, Z., Yamashchikov, et al. (2018). Safet, tolerability, and immunogenicity of two Zika virus DNA vaccine candidates in healthy adults: randomised, open-label, phase 1 clinical trials. *The Lancet*, 391, pp.552-562. [3] Shan C, Muruato A, Nunes B, Luo H, Xie X, Medeiros D et al. A live-attenuated Zika virus vaccine candidate induces sterilizing immunity in mouse models. Nature Medicine. 2017;23(6):763-767. [4] Modjarrad K, Lin L, George S, Stephenson K, Eckels K, De La Barrera R et al. Preliminary aggregate safety and immunogenicity results from three trials of a purified inactivated Zika virus vaccine candidate: phase 1, randomised, double-blind, placebo-controlled clinical trials. The Lancet. 2018;391(10120):563-571. [5] Pardi N, Hogan M, Pelc R, Muramatsu H, Andersen H, DeMaso C et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature. 2017;543(7644):248-251.