In one example, the present disclosure refers to a pharmaceutical composition comprising (i) CHIKV-Δ5nsP3 particles expressing an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2; (ii) CHIKV-Δ5nsP3 particles expressing an E2 structural protein having at least one mutation compared with the amino acid sequence of SEQ ID NO: 2; and (iii) optionally a pharmaceutically acceptable excipient.

In one aspect, the present disclosure refers to a pharmaceutical composition for use as a vaccine comprising (i) CHIKV-Δ5nsP3 particles; and (ii) optionally a pharmaceutically acceptable excipient; wherein at least 30% of the CHIKV-Δ5nsP3 particles present in the composition express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2; and wherein 1-50% of the CHIKV-Δ5nsP3 particles present in the composition express an E2 structural protein having at least one of the immunogenicity-reducing mutations G55R or E168K in the amino acid sequence of SEQ ID NO: 2.

In another aspect, the present disclosure refers to a process for producing a pharmaceutical composition comprising CHIKV-Δ5nsP3 particles which express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2, comprising the step of:
-growing CHIKV-Δ5nsP3 with a polynucleotide sequence as defined by SEQ ID NO: 1 on Vero cells in such a way as to minimize the presence of immunogenicity-reducing mutations G55R and/or E168K, especially E168K, in the E2 structural protein of the virus, comprising the step of passaging the CHIKV-Δ5nsP3 less than five times after viral rescue.

In another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein, in the manufacture of a medicament for treating or preventing a Chikungunya virus infection.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings are not intended to be drawn to scale. The Figures are illustrative only and are not required for enablement of the disclosure. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

CLAIMS

1. A pharmaceutical composition for use as a vaccine comprising (i) CHIKV-Δ5nsP3 particles; and (ii) optionally a pharmaceutically acceptable excipient; wherein at least 30% of the CHIKV-Δ5nsP3 particles present in the composition express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2; and wherein 1-50% of the CHIKV-Δ5nsP3 particles present in the composition express an E2 structural protein having at least one of the immunogenicity-reducing mutations G55R or E168K in the amino acid sequence of SEQ ID NO: 2.

2. The pharmaceutical composition for use according to claim 1, wherein 5-50%, 10-50%, 10-30% or 10-20% of the CHIKV-Δ5nsP3 particles present in the composition express an E2 structural protein having at least one of the immunogenicity-reducing mutations G55R or E168K, preferably E168K, in the amino acid sequence of SEQ ID NO: 2.

3. The pharmaceutical composition for use according to claim 1 or 2, wherein said composition induces neutralizing antibodies against CHIKV-Δ5nsP3 in a mouse immunized with said pharmaceutical composition resulting in a serum comprising said neutralizing antibodies and wherein said serum neutralizes CHIKV infection of cells in an *in vitro* neutralization assay, at least 50%, preferably at least 60%, preferably at least 70%, more preferably at least 80%, even more preferably at least 90%, most preferably at least 95% at a 1:80 serum dilution, wherein the *in vitro* assay is a BHK-21 cell-based Luciferase assay assessing neutralization of LR-CHIKV virus replicon particles (VRPs).

4. The pharmaceutical composition for use according to any one of claims 1 to 3, wherein said CHIKV-Δ5nsP3 in (i) is defined by the polynucleotide sequence of SEQ ID NO: 1.

5. The pharmaceutical composition for use according to any one of claims 1 to 4, wherein said pharmaceutical composition comprises an effective amount of CHIKV-Δ5nsP3 particles which express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2, wherein said effective amount is defined as at least 10^2, at least 10^3, at least 10^4, at least 10^5, at least 10^6, preferably at least 10^3 CHIKV-Δ5nsP3 particles which express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2.

6. The pharmaceutical composition for use according to any one of claims 1 to 5, wherein said pharmaceutical composition is a one-shot pharmaceutical composition; i.e., confers protection with only one vaccination.

7. A process for producing a pharmaceutical composition comprising CHIKV-Δ5nsP3 particles which express an E2 structural protein as defined by the amino acid sequence of SEQ ID NO: 2, comprising the step of:
-growing CHIKV-Δ5nsP3 with a polynucleotide sequence as defined by SEQ ID NO: 1 on Vero cells in such a way as to minimize the presence of immunogenicity-reducing mutations G55R and/or E168K, especially E168K, in the E2 structural protein of the virus, comprising the step of passaging the CHIKV-Δ5nsP3 less than five times after viral rescue.

8. The process according to claim 7, wherein the frequency of said immunogenicity-reducing mutations in an E2 structural protein of CHIKV-Δ5nsP3 having the amino acid sequence of SEQ ID NO: 2 is reduced compared to the frequency of immunogenicity-reducing mutations in an E2 structural protein of CHIKV-Δ5nsP3 obtained by passaging CHIKV-Δ5nsP3 with a polynucleotide sequence as defined by SEQ ID NO: 1 on host cells in culture five or more times.

9. The process according to claim 7 or 8, wherein the frequency of said immunogenicity-reducing mutations is reduced by at least 10%, at least 20%, at least 30% or at least 50% compared to passaging five or more times.

10. The process according to any one of claims 7 to 9, wherein the frequency of said E168K mutation of the E2 structural protein is less than 90%, less than 80%, less than 70%, less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, preferably less than 50% in the total pool of harvested CHIKV-Δ5nsP3.

11. The process according to any one of claims 7 to 10, wherein CHIKV-Δ5nsP3 with a polynucleotide sequence as defined by SEQ ID NO: 1 is passaged on Vero cells in culture, wherein CHIKV-Δ5nsP3 is passaged in the process less than four times after viral rescue, preferably less than three times, preferably less than two times, more preferably only one time, most preferably at most three times.

12. The process according to any one of claims 7 to 11, wherein the Vero cells are grown at a temperature of between 28°C to 37°C, preferably 35°C.

13. The process according to any one of claims 7 to 12, wherein the Vero cells are infected between day 2 and day 5 after seeding, preferably at day 4 after seeding.

14. The process according to any one of claims 7 to 13, wherein the CHIKV-Δ5nsP3 particles are harvested at day one and/or day two after Vero cell infection.

15. The process according to any one of claims 7 to 14, wherein the multiplicity of infection (MOI) used to produce said CHIKV-Δ5nsP3 particles or said pharmaceutical composition comprising CHIKV-Δ5nsP3 particles is less than 0.1, preferably between 0.1 and 0.001, more preferably between 0.09 and 0.0011, even more preferably between 0.05 and 0.005, most preferably 0.01.

16. Use of the pharmaceutical composition of any one of claims 1 to 6, in the manufacture of a medicament for treating or preventing a Chikungunya virus infection.