SUMMARY

The present disclosure relates to a pharmaceutical composition comprising a sufficient amount of immunogenic Chikungunya virus to elicit a neutralizing immune response in a subject; i.e., an immune response that is protective against infection with and/or disease caused by Chikungunya virus. In particular, the disclosure provides a pharmaceutical composition comprising live attenuated CHIKV-Δ5nsP3 particles wherein the percentage of said viral particles with immunogenicity-reducing mutations, particularly immunogenicity-reducing mutations in the E2 protein, are minimized. The disclosure further provides a process for producing a pharmaceutical composition comprising a live attenuated CHIKV-Δ5nsP3, wherein the process minimizes the presence of immunogenicity-reducing mutations in the viral genome, particularly mutations at E168 of viral E2 protein and/or other E2 residues and/or residues in other structural or non-structural CHIKV proteins. The current disclosure further provides pharmaceutical compositions comprising an immunogenic live attenuated Chikungunya virus obtainable by the process of the disclosure.

Efforts to develop a vaccine against Chikungunya virus are currently underway. One of the most advanced vaccine candidates provides a chimeric construct in a measles virus platform (see http://www.themisbio.com/#/news). The vaccine, currently in Phase 2 trials, is delivered in two doses (https://clinicaltrials.gov/ct2/show/NCT02861586?term=themis&recrs=a&rank=2). A one-shot vaccine would represent a distinct advantage in the field.

Accordingly, in one example, it is an object of the current disclosure to provide a stable, well-defined, safe and effective pharmaceutical composition such as, e.g. a vaccine, against Chikungunya virus, preferably an improved vaccine conferring protection with only one vaccination; i.e., a so called “one-shot” vaccine at industrial scale using common cell substrates, to provide processes to generate such a stable, well-defined, safe and effective vaccine and methods and uses for said stable, well-defined safe and effective vaccine.

In one aspect, 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 another aspect, the present disclosure refers to a pharmaceutical composition 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.

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 of the virus, carried out at least by passaging the virus in culture less than five times.

In another aspect, the present disclosure refers to the pharmaceutical composition as disclosed herein, for use in 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 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.

2. A pharmaceutical composition 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.

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

4. The pharmaceutical composition according any one of claims 1 to 3, 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, e.g. Vero 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.

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

6. The pharmaceutical composition according to any one of claims 1 to 5, wherein said at least one mutation in the E2 structural protein is selected from E168K and G55R, preferably E168K.

7. The pharmaceutical composition according to any one of claims 1 to 6, 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 an amount sufficient to prevent Chikungunya virus viremia in a vaccinated

subject and/or 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.

8. The pharmaceutical composition according to any one of claims 1 to 7, wherein said pharmaceutical composition is a one-shot pharmaceutical composition.

9. 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 of the virus, carried out at least by passaging the virus in culture less than five times.

10. The process according to claim 9, wherein a passage 2 (P2) CHIKV-Δ5nsP3 virus is used.

11. The process according to claim 9 or 10, 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.

12. The process according to claim 11, 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.

13. The process according to any one of claims 9 to 12, wherein said immunogenicity-reducing mutations are located in the E2 structural protein as defined by the amino sequence of SEQ ID NO: 2, wherein said immunogenicity-reducing mutations located in the E2 structural protein are selected from G55R and E168K, preferably E168K.

14. The process according to claim 13, 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.

15. The process according to any one of claims 9 to 14, wherein CHIKV-Δ5nsP3 with a polynucleotide sequence as defined by SEQ ID NO: 1 is passaged on host cells in culture less than four times, preferably less than three times, preferably less than two times, more preferably only one time, most preferably at most three times.

16. The process according to any one of claims 9 to 15, wherein the Vero cells are grown at a temperature of about 28°C to 37°C, preferably about 35°C.

17. The process according to any one of claims 9 to 16, wherein the Vero cells are infected between day 2 and day 5 after seeding, preferably at day 4 after seeding.

18. The process according to any one of claims 9 to 17, wherein CHIKV-Δ5nsP3 particles are harvested at day one and/or day two after host cell infection.

19. The process according to any one of claims 9 to 18, 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 about 0.1 and 0.001, more preferably between about 0.09 and 0.0011, even more preferably between about 0.05 and 0.005, most preferably about 0.01.

20. The pharmaceutical composition of any one of claims 1 to 8, for use in treating or preventing a Chikungunya virus infection.