Design and *In-Silico* Evaluation of a Novel Multiepitope-based Recombinant DNA Vaccine Candidate against Lassa Hemorrhagic Fever

Abubakar Ojone Woziri[1,2], Paul Habila Mamman[1], Khadijah Sulaiman Ukashah[3], Emmanuel Oluwadare Balogun[2,4], Anyebe Bernard Onoja[5], Oladipo Elijah Kolawole[6]

[1]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria; [2]Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria, Nigeria;
[3]Department of Nursing, Ahmadu Bello University Teaching Hospital, Shika, Nigeria.
[4]Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria
[5]Department of Virology, College of Medicine, University of Ibadan, Oyo state, Nigeria.
[6]Department of Microbiology, Adeleke University, Ede, Osun State, Nigeria.

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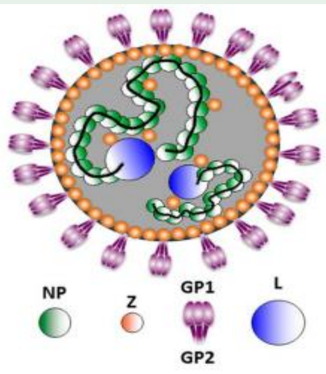
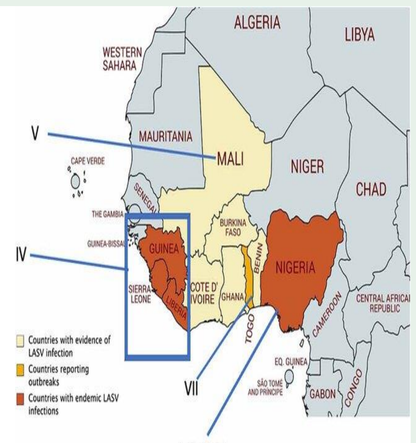
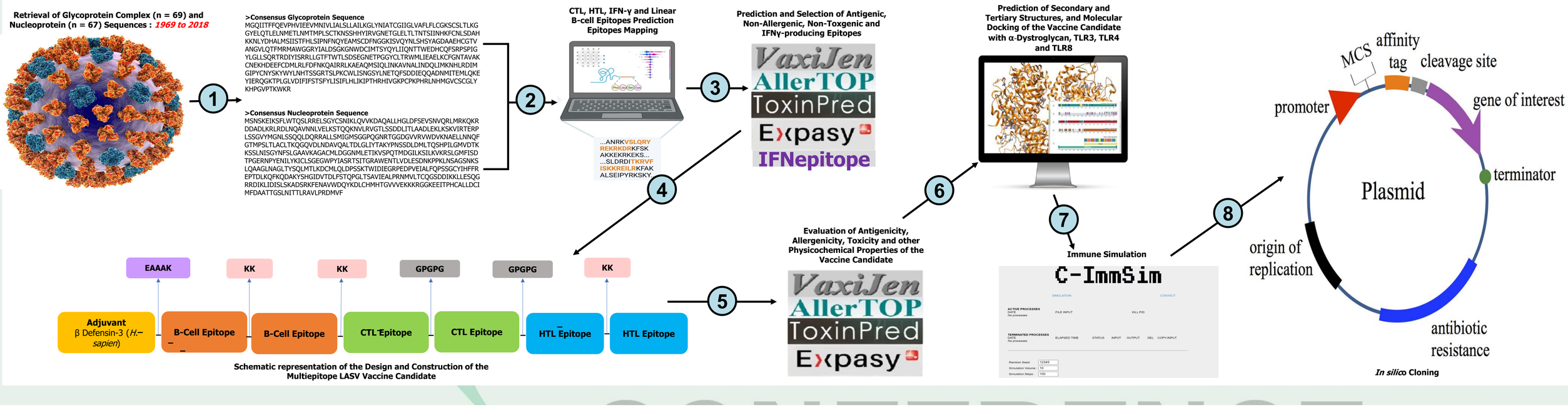
Background



- The genomic diversity of the virus, as well as the expanding geography and species distribution of its reservoir hosts, has made Lass fever (LF) a foremost endemic neglected zoonosis in Nigeria, with no available vaccine for its prevention globally.
- Lassa virus (LASV) glycoprotein complex (GPC) and nucleoprotein (NP) are critical for virus entry and replication.
- The GPC and its components prove an interesting target for potential therapeutics owing to its location on the surface of the viral envelope and presents the sole target for neutralizing antibodies.
- The nucleoprotein (NP) is essential for both transcription of viral mRNA and replication of the genome.
- On-going outbreaks of LF in Nigeria have resulted in unprecedented morbidities and mortalities, making it pertinent to develop a cross-protective and highly immunogenic LASV vaccine candidate for the prevention of LF in Nigeria.
- However, the only LASV vaccine candidate that has reached preclinical trial in Africa is based on glycoprotein sequences of the Josiah strain from lineage IV.
- This study therefore aimed to develop a multi-strain, multi-lineage, cross protective, multiepitope-based recombinant DNA Vaccine candidate against Lassa Virus strains circulating in Nigeria.



Methods



Results

Table 1. Predicted T-cell (CTL and HTL) Epitopes from the Glycoprotein Precursor Complexes (GPC) of Lassa Virus strains Circulating in Nigeria.

S/N	Epitope Sequence	Epitope Length	Antigenicity Score	Percent of protein sequence matches at identity = 100%	Immunogenicity Score
	CTL				
1	RTRDIYISR	9	1.647	82.61% (57/69)	0.1809
2	VMNIVLIAL	9	0.939	65.22% (45/69)	0.2968
	HTL				
1	PSPIGYLGLLSQRTR	15	1.776	92.75% (64/69)	NA
2	RPSPIGYLGLLSQRT	15	1.988	92.75% (64/69)	NA
3	IGYLGLLSQRTRDIY	15	1.837	78.26% (54/69)	NA
4	MNIVLIALSLLAILK	15	0.919	43.48% (30/69)	NA
5	NIVLIALSLLAILKG	15	0.829	43.48% (30/69)	NA
6	EVMNIVLIALSLLAI	15	1.007	43.48% (30/69)	NA

Table 3. Predicted Linear B-cell Epitopes from both Glyco- and Nucleoproteins of Lassa Virus strains Circulating in Nigeria.

S/N	Epitope Sequence	Epitope Length	Antigenicity Score	Percent of protein sequence matches at identity = 100%
	Glycoprotein			
1	DHCQFSRPSPIGYLGL	16	0.924	95.65% (66/69)
2	TEAQMSIQLINKAVNA	16	0.844	60.87% (42/69)
	Nucleoprotein			
1	VVVEKKKRGGKEEITP	16	1.084	58.21% (39/67)

MKKTAIALTIAALAAASVAQAAPQANTFYVGAKAGWASFHDGLNKLNYSNDNVSFSTKNDSVTYG
VFGGYQITDHFAVELGYDDFGRAKLKLTPKENAQAPVGSMTIAKHTNHGAHLSLKASYPVLEGLDV
YARVGAALVRSDYKIADELAAIGEIVRNHSLKVSPVFAGGVEYAFIPELALRVEYQWLKGVGKYKTA
SGHQVDYSPSIGSVTAGLSYRFGQTVAMPEIVSKTFTLNSDVTFGFDKADLKPAAQNVLDGIYGEI
AQLKAASVAVSGYTDRLGADAYNLKLSQRRADTVANYLVAKGVAQNAIMATGHGEANPVTGNKC
DAVKGRKALIACLADDRRVEIAVKGNKEAAAKTEAQMSIQLINKAVNAKKDHCQFSRPSPIGYLGL
KKVVVEKKKRGGKEEITPKKRTRDIYISRAAYVMNIVLIALSLLAAYALTDLGLIYAAYLGLIYTAKYAAYP
SPIGYLGLLSQRTRGPGPGRPSPIGYLGLLSQRTGPGPGIGYLGLLSQRTRDIYGPGPGMNIVLIALS
LLAILKGPGPGNIVLIALSLLAILKGGPGPGEVMNIVLIALSLLAIGPGPGNISGYNFSLGAAVKAGPG
PGLNISGYNFSLGAAVKGPGPGSLNISGYNFSLGAAVGPGPGQGRRDIKLIDISLSKKKHHHHHH

Figure 1. Primary structure of the Lassa Virus Vaccine Candidate (LFV-NG): Mol. Weight = 32 kDa; Antigenicity Score = 0.813; CAI (*C. porcellus*) = 0.92; CAI (*E. coli* K12 = 0.95)

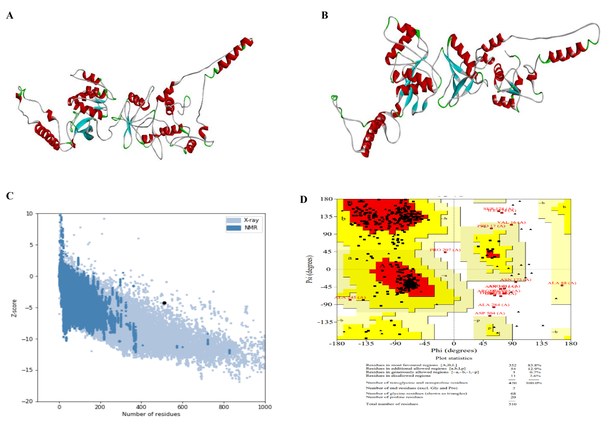


Figure 2. Tertiary structures of the designed vaccine candidate before (A) and after refinement (B). Validation of the refined structure indicating the Z-score (C), and the Ramachandran Plot showing the distribution of the various amino acids that composed the tertiary structure (D).

Table 2. Predicted T-cell (CTL and HTL) Epitopes from the Nucleoprotein of Lassa Virus strains Circulating in Nigeria.

S/N	Epitope Sequence	Epitope Length	Antigenicity Score	Percent of protein sequence matches at identity = 100%	Immunogenicity Score
	CTL				
1	ALTDLGLIY	9	1.284	100.00% (67/67)	0.1244
2	LGLIYTAKY	9	1.091	98.51% (66/67)	0.0703
	HTL				
1	NISGYNFSLGAAVKA	15	0.883	97.01% (65/67)	NA
2	LNISGYNFSLGAAVK	15	1.003	85.07% (57/67)	NA
3	SLNISGYNFSLGAAV	15	0.876	85.07% (57/67)	NA
4	QGRRDIKLIDISLSK	15	1.670	40.30% (27/67)	NA

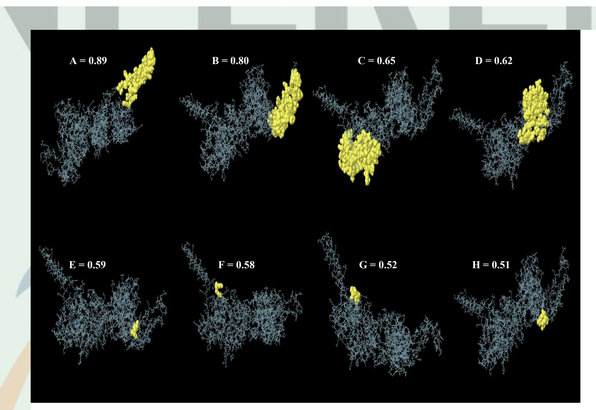


Figure 3. The Synthetic Vaccine Construct (LFV-NG) showing the predicted discontinuous B-Cell epitopes (yellow color) with their various Protrusion Indices (A - H).

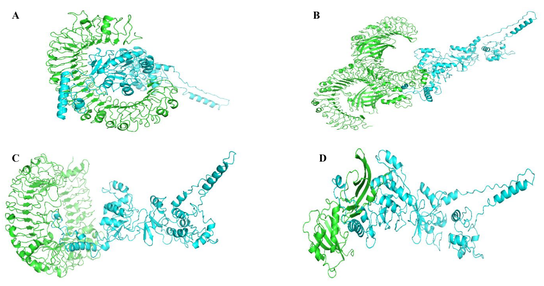


Figure 4. Docked complex of Lassa virus vaccine construct (LFV-NG) and human TLR3 (A), TLR4 (B), TLR8 (C) and α-Dystroglycan (D). LFV-NG is colored blue and the human immune receptors colored Green.

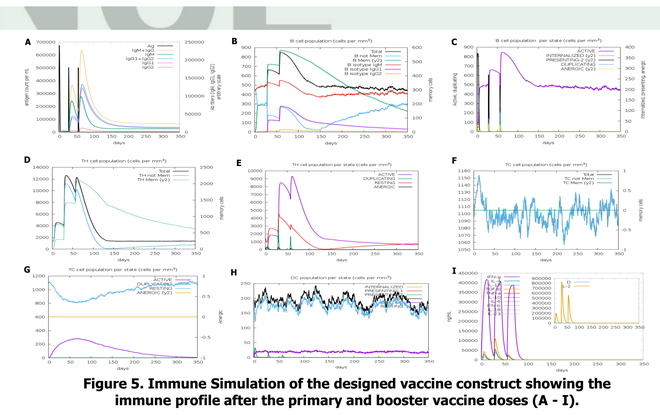


Figure 5. Immune Simulation of the designed vaccine construct showing the immune profile after the primary and booster vaccine doses (A - I).

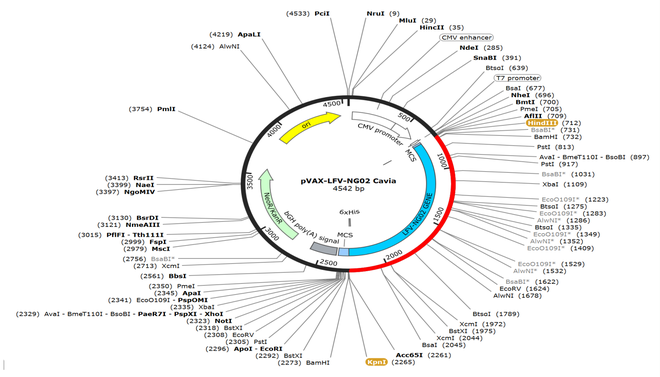


Figure 6. Molecular restriction cloning of the optimized LFV-NG gene showing the cloned LFV-NG gene of 1,530 nucleotides (red color) and the two restriction enzymes (gold color).

Conclusions and Recommendations

- This study was able to design and construct a highly antigenic LASV vaccine candidate from the circulating strains in Nigeria.
- This study provides a viable platform for discovering a novel vaccine candidate for the strains of LASV circulating in Nigeria, and offer the opportunity to develop a country-specific, cost-effective, safe and extensively immunogenic vaccine towards tackling the menace of Lassa hemorrhagic fever in Africa.
- **LFV-NG** should be enlisted as a Lassa Fever Vaccine Candidate for downstream *in vitro* and *in vivo* validations and Proof of Concept experiments in the sub-region.

Contact: Dr. Abubakar Woziri
Africa Centre of Excellence for Neglected Tropical Diseases and Forensic Biotechnology (ACENTDFB), Ahmadu Bello University, Zaria, Nigeria.
E-mail: woziriabubakar@gmail.com **Tel: +2348036152637**