

Communication

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[Misaki Wayengera](#) *

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Communication

De Novo Assembly of H3L and A17L Epitopes for the Rapid Development of Clade 1b MPOX Countermeasures for Africa

Misaki Wayengera

Unit of Genetics & Genomics, Departments of Immunology, Molecular Biology & Pathology, School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala, Uganda; waymisaki@gmail.com

Abstract: Background: An outbreak of Mpox disease caused by a mutant clade 1b virus (MPXV) in Kamituga area, South Kivu Province, DRC has rapidly grown to involve neighboring countries. This now has the authorities worried, majorly due to the limited supply of Mpox medical countermeasures world over. As an emergency local response to this challenge, we set out to identify epitopes of clade 1b MPXV surface proteins as targets for the rapid research and development (R & D) of Mpox medical countermeasures. Methods: The H3L (OPG108–heparin binding protein) and A17L (OPG143–myristylated protein) were selected as targets by virtue of their surface localization, stability and known role in MPXV host cell fusion entry. Within the immune epitope database analysis resource (IEDB-AR), surface accessibility, antigenicity and 3-D localisation (DiscoTope) were measured to identify > 9-nomer epitopes Results: Three (3) and six(6) candidate >9-nomer epitopes were respectively derived for H3L and A17L. Across all parameters, the two best identified epitopes were as follows:—H3L (28-HINDQKFDDVDKDNEVMQEKR-47 and 236-YVEHDPRLVAEH-247) and A17L (104-QNEVTPEYIKDLKYATDYIA-123;) 270-KKDYLLGDSDSVAKCCSKTNTKHCPKIFNNNYKTEHC-290). Conclusions: These epitopes—whether alone, multiplexed (say as a nano-spiked virus-like particles, VLPs) or fused as chimeric recombinant protein arrays form immunogens for the rapid production of protein sub-unit vaccines. Inversely, derivative monoclonal antibodies (mAbs) from the same are precursors for therapeutic cocktails and or antigen, rapid diagnostic tests (RDT).

Keywords: MPox; MPXV; H3L; A17L; Medical Countermeasures; Vaccines; Diagnostics; Therapeutics

1. Background

A rapidly evolving outbreak of MPOX disease caused by a mutant clade I virus (now dubbed 1b) that started around Sept 2023 has persisted in the South and North Kivu Provinces of Eastern DRC, with confirmed isolated cases now reported in the neighboring countries of Burundi, Rwanda and Uganda [1,2]. Epidemiological profiling in DRC shows a predilection for (a) children <15 yrs, (b) PLWHA and (c) pregnant women [1, 3, 4]. Although the observed general case fatality rate is an acceptable 4.6%; this is higher among children <5yrs, PLWHA with uncontrolled viremia, and pregnant women. The East And Central African Great lakes region represents one of the global regions with poorly controlled HIV viremia; lagging behind the UN-SDG 3 targets of 95:95:95 by 2030. Only upto 75% PLWHA here have optimally controlled viral loads. This increases the risk of having severe cases of MPOX in this region, necessitating the use of medical countermeasures (MCMs) to combat the epidemic [1-3].

Whereas there are several MCMs with demonstrated prior efficacy against MPOX, not only is the global capability to rapidly manufacture large doses of vaccines and therapeutics limited, but the impact of the mutations that have been observed in the circulating clade 1b remains unknown. That said, history has proven that—in the face of a global pandemic, the needs of the resource-limited countries of the South will not be prioritized, over those of the wealthier North. The lack of

transparency and equitable access to COVID19 vaccines, revealed Africa’s vulnerabilities and limited capacity for research and development (R & D) of its own homegrown MCMs against priority pathogens. This forced the African Union (AU) to establish the Partnerships for Vaccine Manufacturing (PAVM) under the Africa Centers for Disease Control [5, 6] . Although positive, the impact of the PAVM is yet to be realized, necessitating more agility in tackling the MPOX needs of MCM on the continent [7].

Our group has over the past 2 decades acquired and built expertise to identify, map and validate epitopes against high consequence, priority pathogens for diagnostic, monoclonal antibody (mAbs) therapeutic and vaccine research and development ((R & D) [8-14]. As an emergency local response to the on-going MPOX challenge, we set out to identify ideal epitopes of clade 1b MPXV as targets for the rapid research and development (R & D) of Mpox medical countermeasures.

Two MPXV proteins:— H3L (OPG108–heparin binding protein) and A17L (OPG143–myristylated protein), were chosen as targets by virtue of their surface localization, stability and known role in MPXV host cell fusion entry [16,17].

2. Methodology

Design: Immuno-informatics

Site: Division of Pathogen OMICS, Unit of Genetics & Genomics, Depts of Immunology, Molecular Biology & Pathology, School of Biomedical Sciences, College of Health Sciences, Makerere University, Kampala, Uganda

Materials and accession: Two MPXV proteins:— H3L (OPG108–heparin binding protein) and A17L (OPG143–myristylated protein) obtained from Uniprot, with the following swissport accession #s:

- >sp|A0A7H0DN80|PG108_MONPV Envelope protein OPG108 OS=Monkeypox virus OX=10244 GN=OPG108 PE=2 SV=1
- >sp|A0A7H0DNB5|PG143_MONPV Virion membrane protein OPG143 OS=Monkeypox virus OX=10244 GN=OPG143 PE=3 SV=1

Interventions: Within the immune epitope database analysis resource (IEDB-AR), the two epitopes were examined for surface accessibility, antigenicity and 3-D localisation (DiscoTope) [15].

Measurable variables: positive > 9-nomer epitopes

3. Results & Discussion

We identified three (3) and six(6) candidate >9-nomer epitopes for H3L and A17L respectively, that met the criteria for surface accessibility, antigenicity and 3-D colocalization. For details, see Tables 1 and 2, Figures 1 and 2, and Supplementary File S1, respectively. Overall, across all 3 analyzed parameters, the two best identified epitopes for each target were as follows:—H3L (8-HINDQKFDDVKDNEVMQEKR-472 and 236-YVEHDPRLVAEH-247) and A17L (104-QNEVTPEYIKDLKYATDYIA-123;) 270-KKDYLLGSDSDSVAKCCSKTNTKHCPKIFNNNYKTEHC-290).

Table 1. List of 3 epitopes for MPXV heparin binding protein, H3L (OPG108).

Epitope #	Position and amino acids composition
1	17-PPSETFPNVHEHINDQKFDDVKDNEVMQEKRDRVVIVNDDPDHY-59
2	28-HINDQKFDDVKDNEVMQEKR-47
3	236-YVEHDPRLVAEH-247

Table 2. List of 6 epitopes for the MPXV myristylated protein, A17L (OPG143).

Epitope #	Position and amino acids composition
1	11-KIETGIADIRD-21
2	44-YMYYYETSPGEI-55
3	104-QNEVTPEYIKDLKYATDYIA-123
4	270-KKDYLLGDSDSVAKCCSKTNTKHCPKIFNNNYKTEHC-290
5	291-CKYVGCTINVNSL-303
6	110-EYIKDLKYA-118

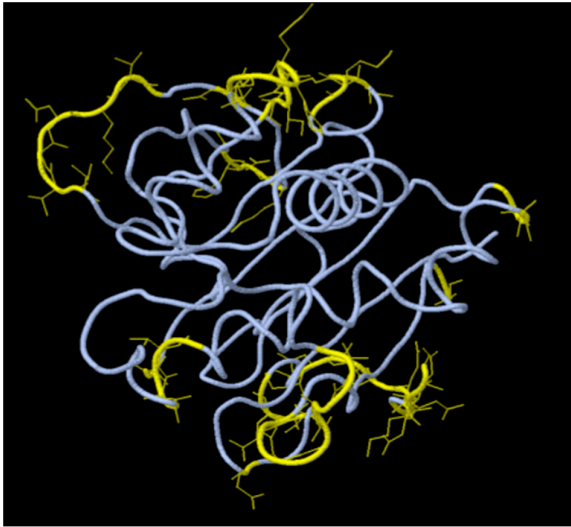


Figure 1. Map of epitopes on 3-D crystal structure of H3L. This figure shows the map of epitopes on 3-D crystal structure of H3L as predicted by Discotope.

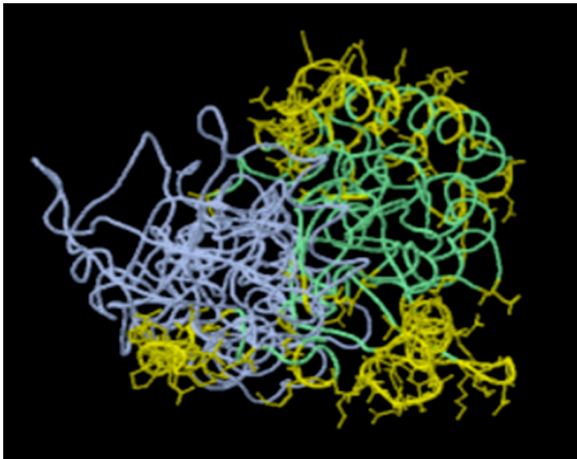


Figure 2. Map of epitopes on 3-D crystal structure of A17L. This figure shows the map of epitopes on 3-D crystal structure of A17L as predicted by Discotope.

Interestingly, prior studies have highlighted H3L and A17L as ideal vaccine candidates, and separate epitopes of both surface proteins have been derived [16,17]. de Araújo LP, et al. (2024) listed the A17L as one of the 6 host cell entry proteins for MPXV (alongside A26L/A30L, A33R, H2R, L1R) that can be targeted for vaccine R & D. This team identified 138 epitopes for A17L, all of which overlap

with our 6 target epitopes. Some of these epitopes are shared with Alaskapox viruses [18]. Disturbingly, Masirika LM, et al. (2024) [1] found A17L to be one of the 7 proteins emerging as hot spots mutations based on the consensus inframe deletions, frameshift variants, synonymous variants, and amino acids substitutions among isolates of clade 1b taken from Kamituga area, South Kivu, DRC. The location of most of the referenced mutations, however, lay outside the listed epitopes in Table 2, Supplementary File S1. That said, it remains to be examined, what the impact of these mutations will be on the real world effectiveness of derivative MCMs.

Elsewhere, Wang Y, et al. (2023) [17] highlighted several potential vaccine targets for MPXV, emphasizing the relevance of 6 potent immunogens inclusive of H3L (alongside M1R, E8L, A29L, A35R, and B6R). Interestingly, residues of the epitopes identified by this group (13-VIDRLPSETFPNVHEHHINDOKF-34; 231-DNAAKYVEH-239) overlap and traverse the target regions of the priority epitopes we identify for the H3L (8-HINDOKFDDVKDNEVMQEKR-472 and 236-YVEHDPRLVAEH-247) (Table 1). They conclude that these targets can be integrated into diverse vaccine platforms to elicit powerful B-cell and T-cell responses, thereby providing protective immunity against MPXV infection [17].

Lastly, we have mapped residues of these epitopes on the available 3-D crystal structures of the corresponding proteins in Uniprot. Notable is that, only the structure of the vaccinia virus H3L (PDB entry # 5EJ0) exists. No 3-D structure corresponding to the VACV A17L protein was found, except the structure of the vaccinia virus A16/G9 sub-complex from the orthopoxvirus entry-fusion complex (PDB entry # 8GP6) [19]. The predictions by DiscoTope are shown in Figures 1 and 2 in color yellow, revealing surface accessibility for presentation as vaccine immunogens. This is consistent with findings by Yefet R, et al. (2023) who found that Monkeypox infection elicits strong antibody and B cell response against A35R and H3L antigens [20]. Indeed, Zuiani A, et al. (2024) recently found that multivalent mRNA monkeypox virus vaccine (BNT166) that expresses H3L as one of the antigens protects mice and macaques from orthopoxvirus disease [21]. Moreover, there is evidence for cross reacting protective immunity against other members of the genera *orthopoxviridae* [20,21].

In conclusion, we identify epitopes of two MPXV surface proteins involved in fusion entry which, whether expressed alone, multiplexed as nano-spiked virus-like particles (VLPs), or fused as chimeric recombinants form immunogens for the production of protein sub-unit vaccines. Inversely, derivative monoclonal antibodies (mAbs) from the same are precursors for therapeutic cocktails and or antigen, rapid diagnostic tests (RDT).

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, S1: Details of methodology for epitope mapping presented.

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Conflicts of Interest: The author declares no sources of competing interests to declare.

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