INVESTIGATING THE IMPORTANCE OF SUDAN VIRUS GLYCOPROTEIN EPITOPES TO AID VACCINE DEVELOPMENT

Lucy Evans, George Hooper, Edward Wright

University of Sussex, Department of Life Sciences

SUDAN EBOLAVIRUS (SUDV)

- One of **four** Ebola virus species that impacts humans, causing **Ebola Virus Disease**
- Localised to **South Sudan/Uganda** (in green on the map)
- **Eight** outbreaks have occurred since **1976**
- The case **fatality** rate is between **53% and 100%**
- There are currently **no treatments or vaccines** for SUDV

AIMS OF THE PROJECT

Determine the **immunogenic hierarchy** of epitopes present on the **glycoprotein** (GP) of SUDV

Identify the most **immunogenic epitopes** that could be included in more **effective vaccines**

SUMMARY OF METHODS

Identify potential epitope sequences → Design **primers** to **mutate** the epitope(s) → Run PCRs, **mutating** the **epitope sequences** → **Clone** the mutated SUDV GP in to **pCAGGS** → Create **lentiviral pseudotypes** bearing the **mutated SUDV GP** → **Titrate** PV and run **neutralisation assays** to assess impact of epitope mutation

RESULTS

IDENTIFYING EPITOPES

To identify epitopes, we conducted a **literature review,** and inputted the GP sequence into **BepiPred** to identify **potential epitopes.** **Three** epitopes of interest were identified- **'140', '157' and '614',** named based on the first amino acid of the epitope in the SUDV GP sequence.

Figure 1 (left)- X-ray crystallography structure of SUDV GP (incomplete), PDB entry 3S88, with multiple potential epitopes highlighted. 140 is red, 157 is pink, and 614 is orange.

EPITOPE MUTATION

To examine the individual epitopes, amino acids in these were mutated into **alanines.** The **overlap-PCR** method was used, which utilises **pairs** of **internal** and **external primers** which are **specific** for each **epitope.** The internal primers introduce the **mutation,** while the external primers allow the sequence to be **cloned** into the **pCAGGS** plasmid**.** **Gel electrophoresis** is completed to check the amplicon and ensure the **correct product** has been amplified.

CLONING MUTATED GLYCOPROTEINS

Once the PCRs were completed, the amplicons were **ligated** into the **pCAGGS** plasmid, then **transfected** into *E. coli* bacteria, grown overnight, and the **plasmid** was **extracted.** The plasmids were then **sequenced** to ensure the mutation was present, as shown in figure 2. At this stage we had **one successfully mutated epitope**- the **614** epitope.

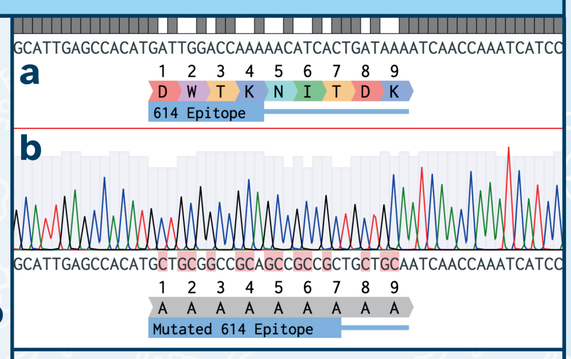

Figure 2- A diagram of the 614 mutation showing (a) the initial wild type sequence and (b) the mutated sequence with Sanger chromatogram.

VIRAL PSEUDOTYPE PRODUCTION

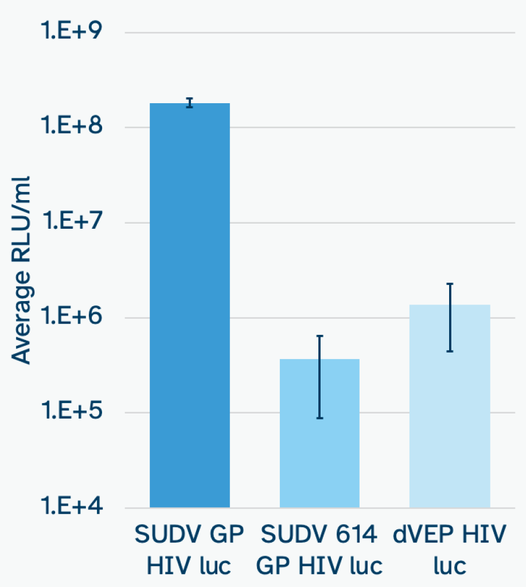

Figure 3- Average viral activity in RLU/ml for the three study viruses Error bars set to ±1S.D of the mean.

Pseudotypes were made using **three** plasmids- **pCAGGS** (GP), **pCSFLW** (reporter), and **p8.91** (PV core). These were **transfected** into virus-producing cells (HEK293T/17), with virus being **harvested** 48 and 72 hours later. The viruses- **wild-type GP PV, mutated GP PV,** and a **control virus**- were **titrated** in a 96-well plate. A reporter in pCSFLW allowed us to read a **luminescent signal (RLU),** equating to viral activity. As shown in figure 3, the the mutated virus **did not show sufficient viral activity** to proceed with the neutralisation assay.

STRUCTURAL IMPACT OF MUTATION

Given the titration results show a lack of viral activity, we used **AlphaFold** to **predict** the structure of the mutated SUDV GP to determine if the mutation **affected its structure.** The model created (Figure 4) shows a **large variation** between the wild-type SUDV GP (figure 1) and the mutant. However, it is important to note that Figure 4 is a **predicted structure.** Examining data from the literature also showed that the 614 epitope is within the **transmembrane domain.** This may explain why the mutation had such a large impact.

Figure 4- Structural prediction of the SUDV GP with the 614 mutations (orange).

CONCLUSIONS

From the results of the titration, alongside the predicted structure of the GP, we believe it is likely that the 614 epitope is **too integral to the structure** of the SUDV GP to enable assessment using PV, with the mutation resulting in **a lack of infectivity in the pseudotype**. Thus, other assays would be required to assess the suitability of this epitope for **inclusion in an optimised vaccine**.

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