

# An mRNA-based FimH ferritin nanoparticle vaccine against UPEC is highly immunogenic in rodents

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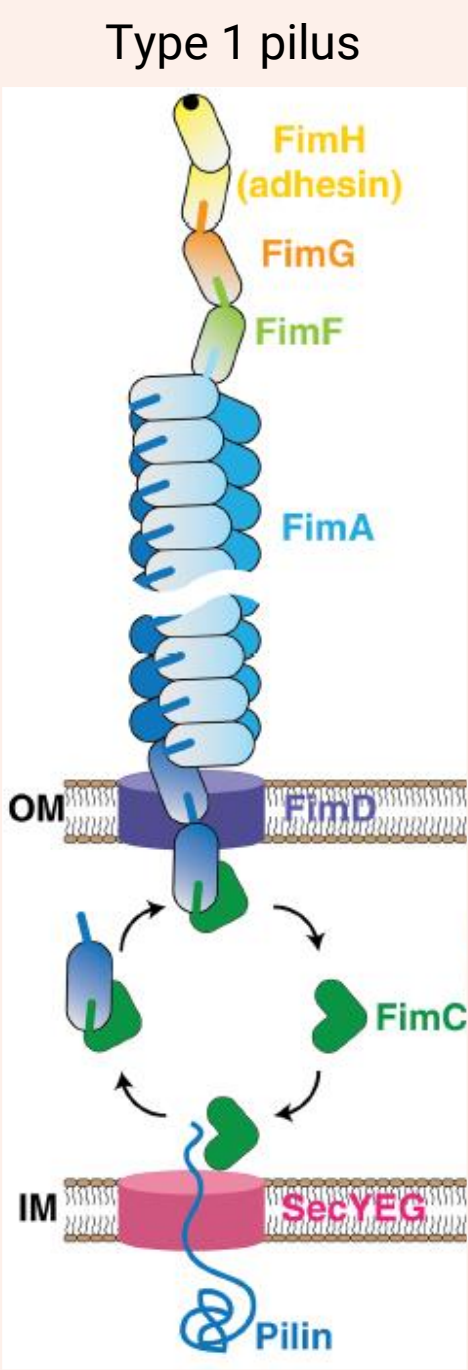
Poster #126

## Uropathogenic *E. coli* (UPEC)

- UPEC is the most common cause of urinary tract infections (UTIs)<sup>1</sup>
  - Transmission to the urinary tract occurs by fecal shedding
  - UPEC enters the urinary tract and invades and colonizes host bladder and kidneys tissues
  - The increasing antibiotic resistance among UPEC warrants the development of an effective vaccine

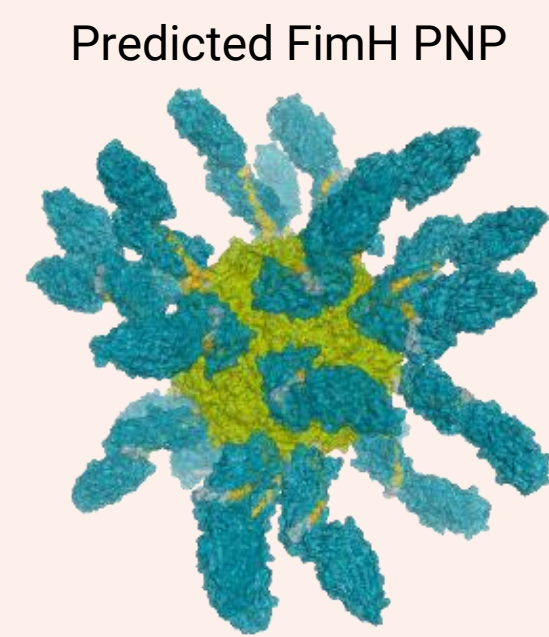


- Type 1 pilus is a key virulence factor for UPEC<sup>2,3</sup>
  - Mediates bacterial adhesion to host tissues and biofilm formation
  - FimH at the tip of the type 1 pilus is responsible for interaction with the host cell receptor



- FimH is the target antigen for our mRNA vaccine development

- FimH<sub>D6</sub>-Ferritin mRNA-based candidate vaccine consists of stabilized FimH linked to a *H. pylori* ferritin protein nanoparticle (PNP)-forming domain to allow clustering of FimH on the PNP surface
- Each PNP is expected to consist of a ferritin core (green) displaying 24 copies of FimH (blue) stabilized in a pre-binding conformation



## METHODS

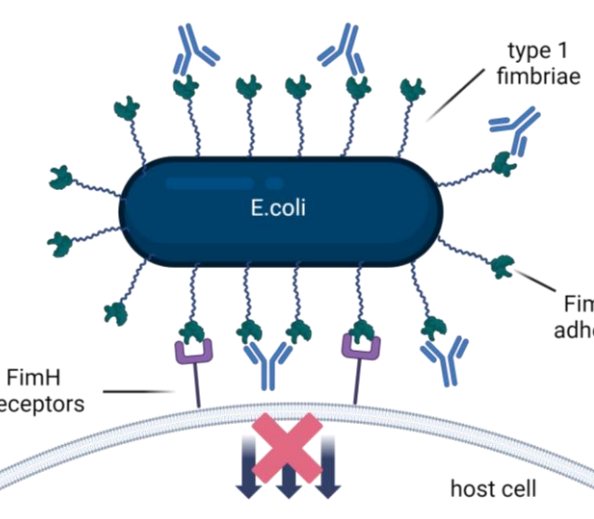
### Immunization

- The vaccination studies included one mouse and two rat immunogenicity studies with three intramuscular vaccinations on days 0, 21 and 35 and ex vivo on day 49
- LNP formulation was performed using LNP technology from Acuitas Therapeutics

### Assays

(Lower limit of quantification (LLOQ) is shown by a dotted line in all figures)

- Bacterial adhesion inhibition (BAI) assay
  - Used to analyze functional serum antibody responses
  - The UPEC strain UT189 used for in vitro functional readout was kindly provided by Prof. T. E. Andersen, University of Southern Denmark<sup>4</sup>
  - Samples with response below the LLOQ were set to 0.5 (Fig. 3), 1.5 (Fig. 7), or 3.5 (Fig. 10)
- Enzyme-linked immunosorbent assay (ELISA)
  - IgG in serum and urine from vaccinated mice and rats was detected by ELISA using plates coated with the lectin binding domain of FimH (FimH<sub>L</sub>)
- Intracellular cytokine staining (ICS)
  - Mouse splenocytes were stimulated with a FimH peptide library and tested for the induction of CD4<sup>+</sup> and CD8<sup>+</sup> T cells secreting IFN $\gamma$  and TNF



## RESULTS

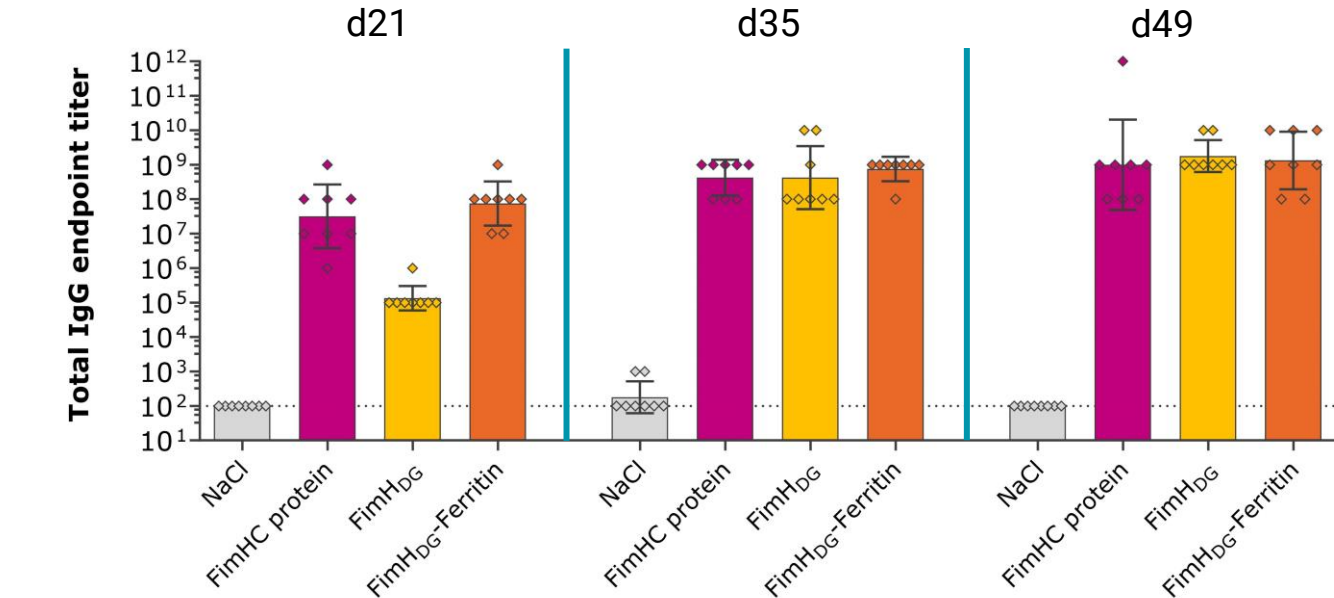
### Immunogenicity of FimH<sub>D6</sub> and FimH<sub>D6</sub>-Ferritin mRNA-based candidate vaccines in rodents

#### Naïve female BALB/c AnNRj mice

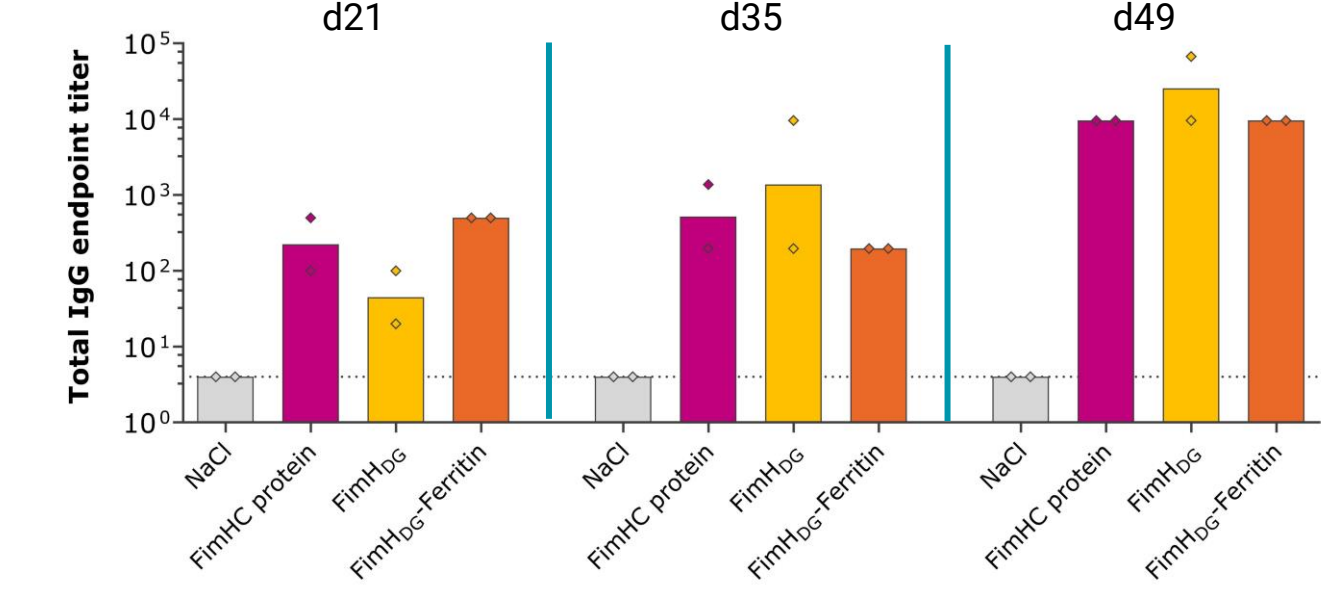


- Aim:** to compare the immunogenicity of 2  $\mu$ g FimH<sub>D6</sub> and FimH<sub>D6</sub>-Ferritin with that of 2  $\mu$ g PHAD-adjuvanted FimHC (FimH + FimC) recombinant protein vaccine

- Figure 1.** Immunization with FimH<sub>D6</sub>-Ferritin mRNA vaccine showed similar levels of FimH<sub>L</sub>-specific total IgG binding antibodies in serum compared with the protein vaccine

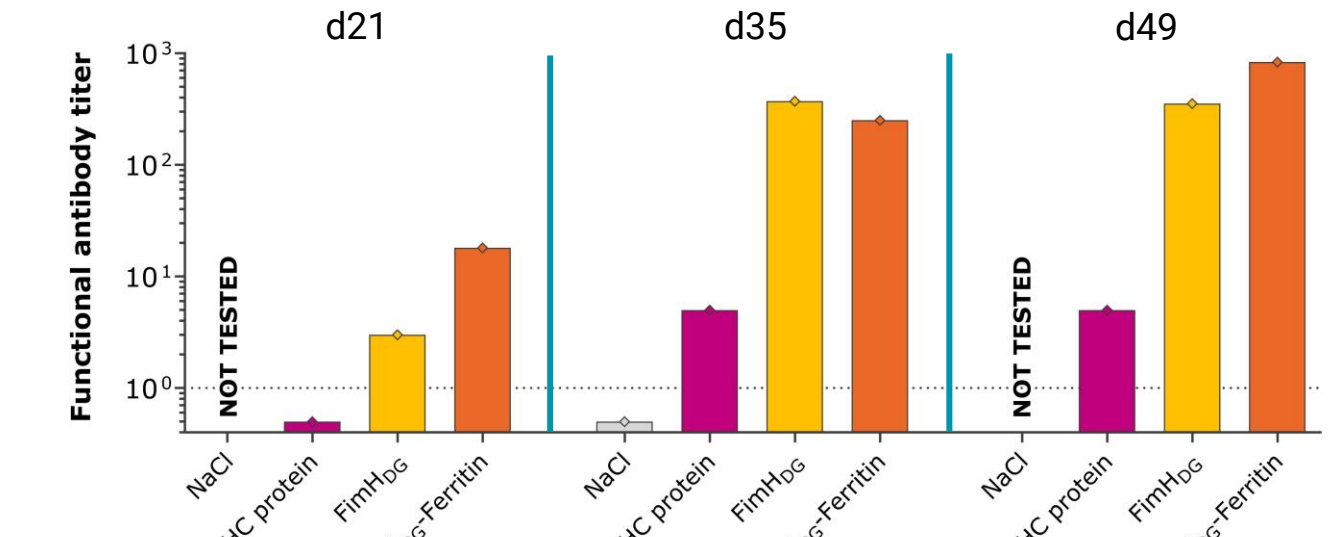


- Figure 2.** Immunization with FimH<sub>D6</sub>-Ferritin mRNA vaccine showed similar levels of FimH<sub>L</sub>-specific total IgG binding antibodies in urine compared with the protein vaccine



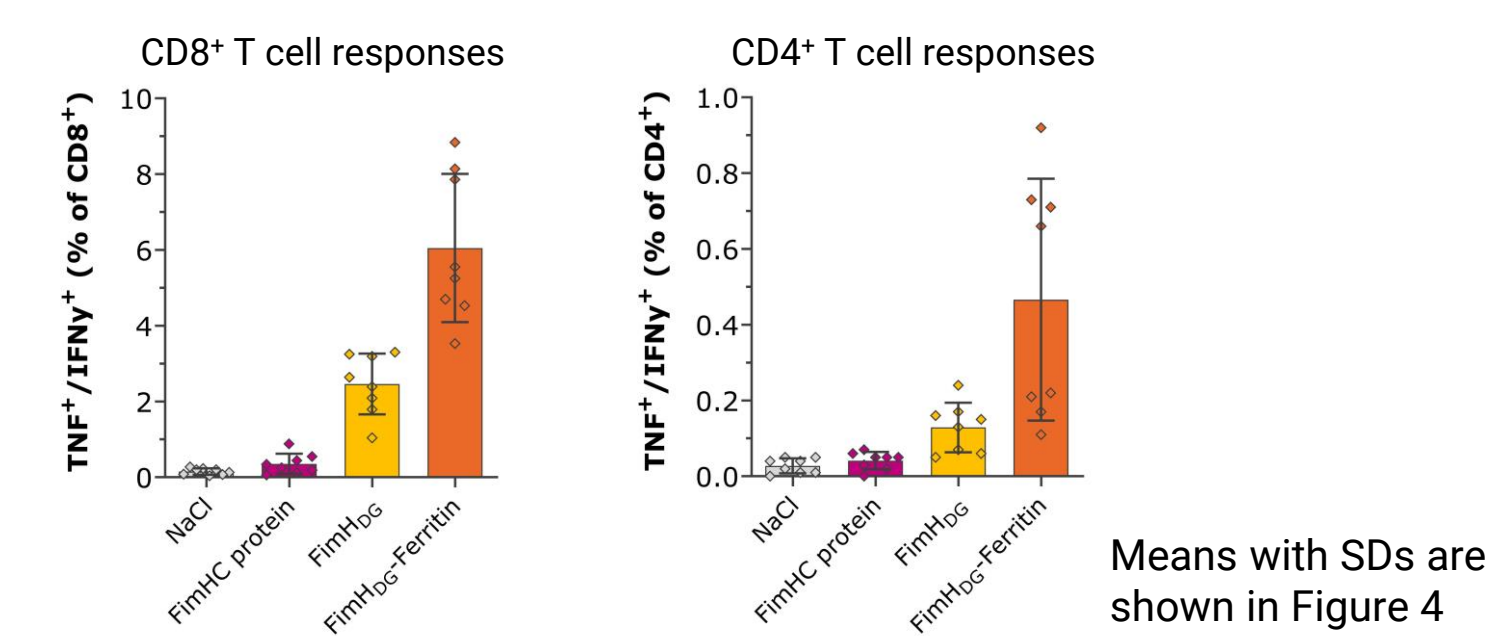
Geometric means (GMs) with geometric standard deviation (geo SDs) are shown in Figure 1 & 2

- Figure 3.** Functional serum antibody responses were highest in the group immunized with the FimH<sub>D6</sub>-Ferritin mRNA vaccine



Results from BAI assay on pooled samples are shown in Figure 3

- Figure 4.** FimH<sub>D6</sub>-Ferritin mRNA vaccine induced FimH-specific CD4<sup>+</sup> T helper cells and CD8<sup>+</sup> cytotoxic T cells producing IFN $\gamma$  and TNF

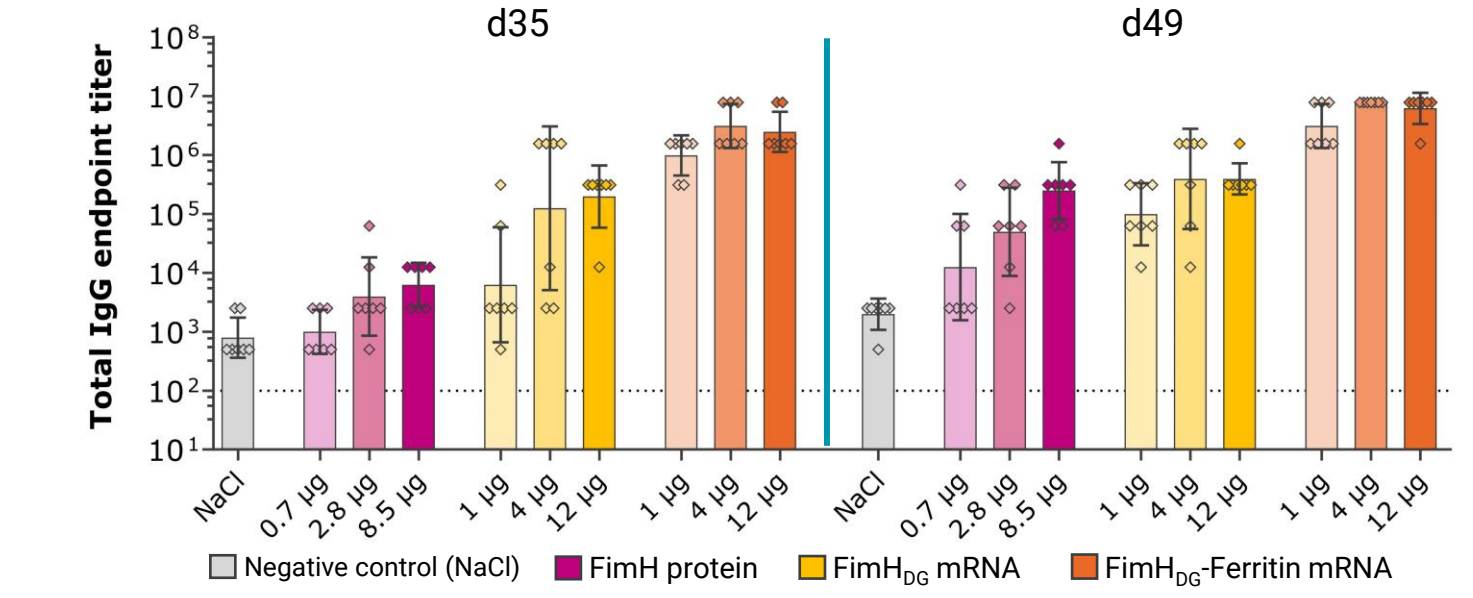


#### Naïve female Wistar rats

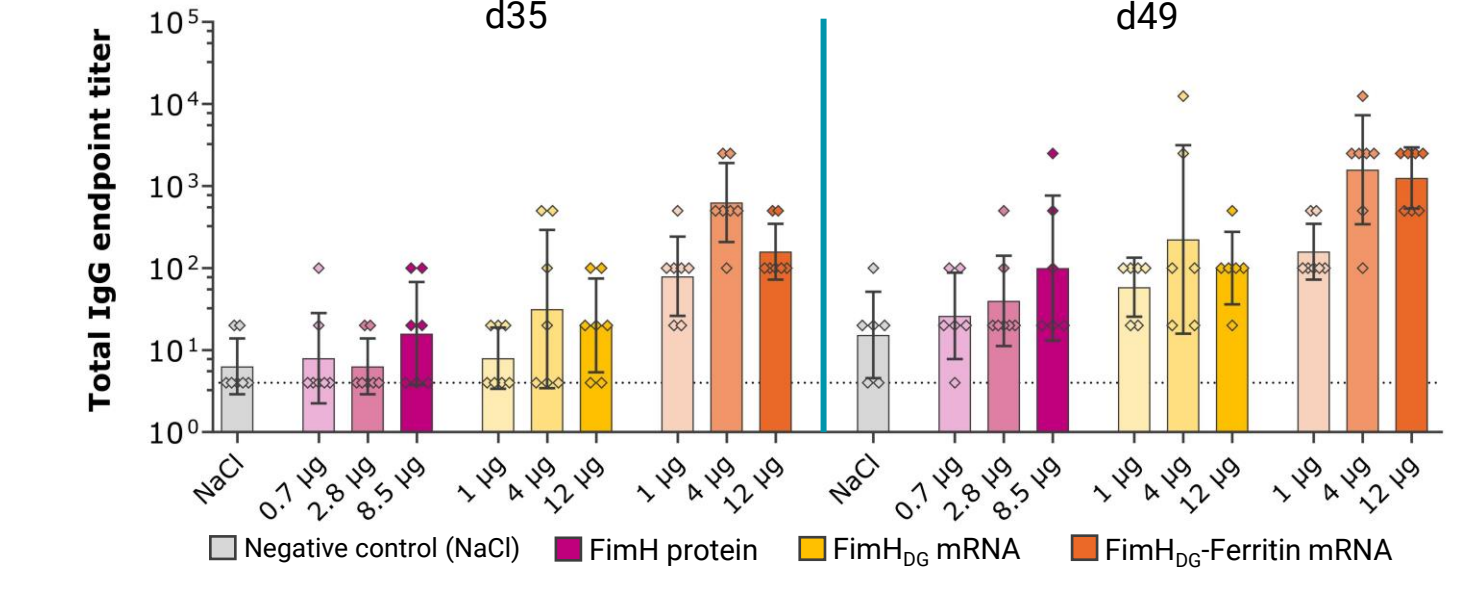


- Aim:** to compare the immunogenicity of FimH<sub>D6</sub> and FimH<sub>D6</sub>-Ferritin with an adjuvanted FimH protein subunit vaccine in another rodent model

- Figure 5.** Immunization with FimH<sub>D6</sub>-Ferritin mRNA vaccine showed the highest levels of FimH<sub>L</sub>-specific total IgG binding antibodies in serum

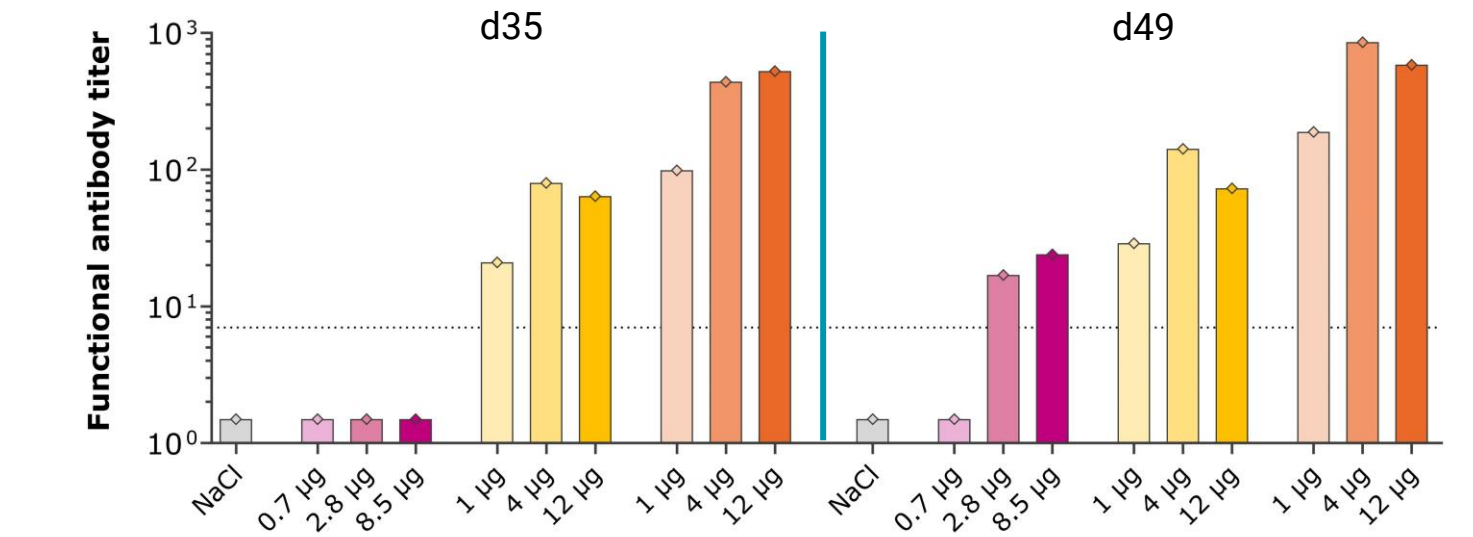


- Figure 6.** Immunization with FimH<sub>D6</sub>-Ferritin mRNA vaccine showed the highest levels of FimH<sub>L</sub>-specific total IgG binding antibodies in urine



GMs with geo SDs are shown in Figure 5 & 6

- Figure 7.** The groups with the highest antibody titers in serum and urine induced the highest serum functional antibody levels after 2 and 3 doses



Results from BAI assay on pooled samples are shown in Figure 7

- These results demonstrated that multimerization of FimH antigen via ferritin enhanced the immunogenicity compared with a monomeric antigen design

## CONCLUSIONS

- FimH<sub>D6</sub>-Ferritin mRNA candidate vaccine encoding FimH stabilized in a pre-binding conformation linked to a ferritin PNP-forming domain induced the highest levels of FimH-specific binding and functional antibody responses in serum and/or urine in mice and rats
- The introduction of modified nucleosides led to reduced IFN $\alpha$  levels in hPBMCs, without compromising the immunogenicity profile, which is expected to translate to a wider range of tolerable vaccine doses in humans

## KEY TAKEAWAYS



- FimH<sub>D6</sub> and FimH<sub>D6</sub>-Ferritin mRNA-based candidate vaccines induced higher levels of functional antibody and T cell responses compared to a protein subunit vaccine



- The multimeric FimH<sub>D6</sub>-Ferritin mRNA-based candidate vaccine was more immunogenic compared to the monomeric FimH<sub>D6</sub> vaccine



- Nucleoside modification (N1-methyl-pseudouridine) in the FimH<sub>D6</sub>-Ferritin mRNA-based candidate vaccine improved functional antibody responses



- FimH<sub>D6</sub>-Ferritin mRNA-based candidate containing modified nucleosides is a promising UPEC vaccine for further development

### References

- Sarshar, M. et al. FimH and anti-adhesive therapeutics: a disarming strategy against uropathogens. *Antibiotics*. 2020;9(7):397. doi: 10.3390/antibiotics9070397.
- Hospenthal, M. K. & Waksman, G. The remarkable biomechanical properties of the type 1 chaperone-usher pilus: a structural and molecular perspective. *Microbiol. Spectr.* 2019;7(1). doi: 10.1128/microbiolspec.PSIB-0010-2018.
- Waksman, G. & Hultgren, S. J. Structural biology of the chaperone-usher pathway of pilus biogenesis. *Nat. Rev. Microbiol.* 2009;7(11):765-74. doi: 10.1038/nrmicro2220.
- Andersen TE et al. Escherichia coli uropathogenesis in vitro: invasion, cellular escape, and secondary infection analyzed in a human bladder cell infection model. *Infect Immun.* 2012 May;80(5):1858-67. doi: 10.1128/IAI.06075-11.

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