A novel SARS-CoV-2 modified live vaccine with an optimized safety profile induces sterile immunity in Syrian hamsters

Angele Breithaupt[1], Tobias Britzke[1], Jacob Schön[2], Güliz Tuba Barut[3,4], Nico Joël Halwe[2], Lorenz Ulrich[2], Nadine Ebert[3,4], Bettina Trüeb[3,4], Melanie Brügger[3,4], Volker Thiel[3,4,5], Martin Beer[2] and Donata Hoffmann[2]

[1]Department of Experimental Animal Facilities and Biorisk Management, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany
[2]Institute of Virology and Immunology, Bern and Mittelhäusern, Switzerland
[3]Department of Infectious Diseases and Pathobiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland
[4]Institute of Diagnostic Virology, Friedrich Loeffler Institute, Greifswald -Insel Riems, Germany
[5]Multidisciplinary Center for Infectious Diseases (MCID), University of Bern, Bern, Switzerland

Introduction

Rationale: mRNA vaccines *vs.* live attenuated vaccines (LAV)

mRNA		LAV
✔✔✔	protection against severe disease	✔✔✔
✔ ✗ ✗	reduced transmission	✔✔✔
✔ ✗ ✗	viral protein spectrum	✔✔✔
✔✔✔	humoral, systemic, IgG based immune response	✔✔✔
✗	local, mucosal immune response, mucosa-bound IgA	✔✔✔
✔ ✗ ✗	cellular immune response	✔✔✔
i.m.	administration	i.n.
✗	simulate a natural infection	✔✔✔

Fig. 1: Comparison of characteristics of systemic mRNA-based SARS-CoV-2 vaccines and mucosal LAV [1]. LAV are expected to be more effective in reducing viral shedding and inducing much stronger and longer-lasting immunity.

Strategy: one-to-stop (=OTS) concept

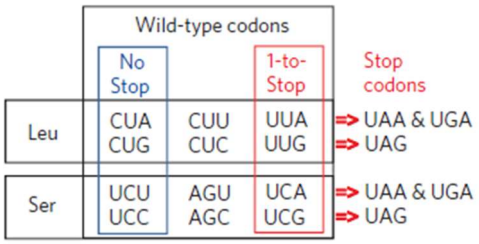


Fig. 2: OTS strategy: Introduction of synonymous codon changes using serine and leucin. Only one mutation more is needed to turn into a stop-codon with expected impact on viral fitness [2].

Final OTS candidate

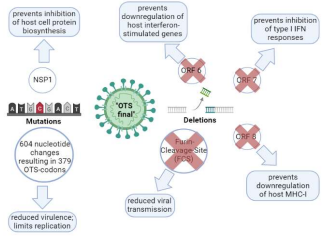


Fig. 3: OTS228 was attenuated by 379 OTS-codons (Orf1ab), 4 deletions (Orf6-7, polybasic cleavage site), and 2 point mutations (NSP1).

- **We evaluated a LAV based on the OTS genome recoding attenuation method [2] in Syrian hamsters.**
- **We asked for the level of attenuation, protective potential, including the ability of inducing sterile immunity.**

Materials and Methods

21-day safety study design

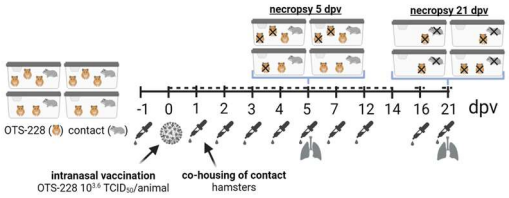


Fig. 4: Safety study in Syrian hamsters intranasally inoculated with $10^{3.6}$ $TCID_{50}$ of OTS228. Naive contact animals served as transmission controls.

14-day efficacy study design

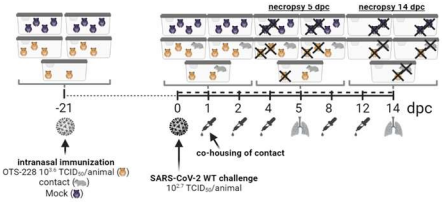
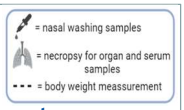


Fig. 5: Efficacy study in Syrian hamsters: 21 days after single OTS228 vaccination, we challenged with homologous (=WT $10^{2.7}$ $TCID_{50}$) SARS-CoV-2, or Omicron BA.2 ($10^{3.7}$ $TCID_{50}$) or BA.5 ($10^{3.9}$ $TCID_{50}$).

Evaluation



- **survival data**
- **body weight measurement**
- **tissue virus RNA load (RT-qPCR)**
- **virus shedding (nasal wash, RT-qPCR)**
- **5 dpi: pulmonary atelectasis (H&E) using a 500 × 500 µm grid, yielding percentage of affected lung fields**
- **5 dpi: virus antigen detection (IHC, Rockland #200-401-A50)**

Results

Safety study

i.n. OTS228 vaccination led to
- 100 % survival
- no body weight loss
- OTS228 genome detectable up to 7 days in nasal washes
- lack of pneumonia-associated atelectasis BUT
- vaccine virus antigen detection within slightly expanded pulmonary interstitium by mainly macrophages (in 2/5 hamsters) and focal perivascular infiltrates (1/5)
- no transmission to contact hamsters, confirmed by tissue-PCR and serology

Efficacy study: WT, BA.2, or BA.5 challenge

Virus challenge, 21 days after single OTS228 inoculation led to
- 100 % survival
- no / minimal transient body weight loss
- significantly reduced challenge virus loads in nasal washes & tissues by 5 dpi
- no challenge virus genome in lungs at 14 dpi
- no transmission after WT challenge confirmed by RT-qPCR, serology
- transmission to 1/3 (BA.2), 2/3 (BA.5) contact hamsters

Histopathology & virus antigen detection of OTS-228 vaccinated and mock vaccinated (control) hamsters, and after WT, BA.2, or BA.5 challenge, 5dpi

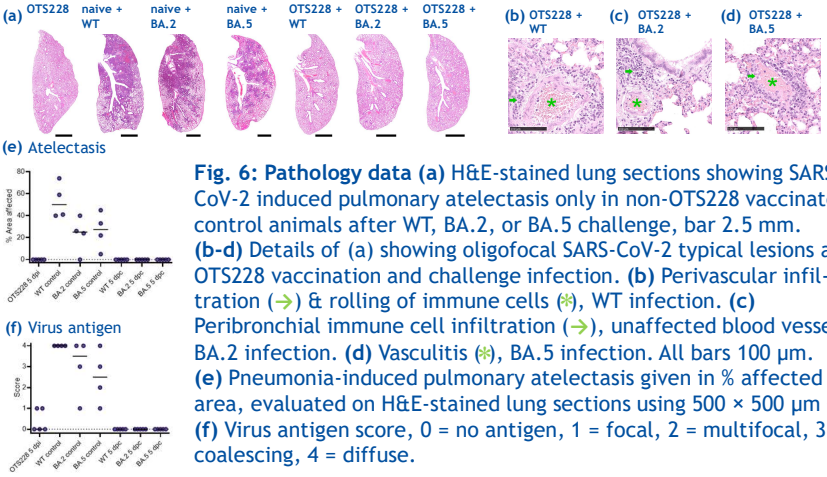


Fig. 6: Pathology data (a) H&E-stained lung sections showing SARS-CoV-2 induced pulmonary atelectasis only in non-OTS228 vaccinated control animals after WT, BA.2, or BA.5 challenge, bar 2.5 mm. **(b-d)** Details of (a) showing oligofocal SARS-CoV-2 typical lesions after OTS228 vaccination and challenge infection. **(b)** Perivascular infiltration (→) & rolling of immune cells (*), WT infection. **(c)** Peribronchial immune cell infiltration (→), unaffected blood vessel (*), BA.2 infection. **(d)** Vasculitis (*), BA.5 infection. All bars 100 µm. **(e)** Pneumonia-induced pulmonary atelectasis given in % affected area, evaluated on H&E-stained lung sections using 500 × 500 µm grids **(f)** Virus antigen score, 0 = no antigen, 1 = focal, 2 = multifocal, 3= coalescing, 4 = diffuse.

Conclusions

- ✔ Full attenuation and block of transmission of OTS228 in Syrian hamster super spreader model
- ✔ Full protection and a sterile immunity after single dosage intranasal vaccination against homologous SARS-CoV-2 challenge
- ✔ Clinical protection and significantly reduced shedding after single dosage intranasal vaccination against Omicron BA.2 and BA.5 challenge

References: [1] Lund et Randall 2021, Science; [2] Moratorio et al. 2017, Nature Microbiology

Abbreviations, not introduced: Orf, open-reading-frame; NSP-1, Non-structural protein 1; TCID, tissue culture infectious dose; dpi, day post infection; RT-qPCR, quantitative reverse transcription polymerase chain reaction- , H&E, hematoxylin-Eosin staing; IHC, immunohistochemistry