A chimeric vaccine based on a single fusion protein (W-PreS-O) shows induction of broadly neutralizing antibodies and protective effect against Omicron infection in *in vivo* models

Pia Gattinger [a], *Luibov I. Kozlovskaya* [b], *Bernhard Kratzer* [c], *Anna Ohradanova-Repic* [d], *Al Nasar Ahmed Sehgal* [c], *Laura Gebetsberger* [d], *Hannes Stockinger* [d], *Ilya V. Gordeychuk* [b,e], *Alexander V. Karaulov* [f,g], *Aydar A. Ishmukhametov* [b,e], *Winfried F. Pickl* [c] *and Rudolf Valenta* [a,f,g,h]

[a] Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Department of Pathophysiology and Allergy Research, Vienna, Austria
[b] Chumakov Federal Scientific Center for Research and Development of Immune-and-Biological Products of Russian Academy of Sciences (Institute of Poliomyelitis), Moscow, Russia
[c] Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute of Immunology, Vienna, Austria
[d] Medical University of Vienna, Center for Pathophysiology, Infectiology and Immunology, Institute for Hygiene and Applied Immunology, Vienna, Austria
[e] Sechenov First Moscow State Medical University, Institute for Translational Medicine and Biotechnology, Moscow, Russia
[f] Sechenov First Moscow State Medical University, Laboratory for Immunopathology, Department of Clinical Immunology and Allergology, 119048 Moscow, Russia
[g] Life Improvement by Future Technologies (LIFT) Center, Moscow, Russia.
[h] Center for Molecular Allergology, Karl Landsteiner University of Health Sciences, 3500 Krems, Austria

Background: Safe and effective vaccines for Omicron and eventually new upcoming SARS-CoV-2 variants in the future are needed. **Objectives:** To develop a flexible platform for recombinant SARS-CoV-2 subunit vaccines. Strain-specific (wild-type: W-PreS-W; Omicron: O-PreS-O), bivalent (mix of W-PreS-W and O-PreS-O) and chimeric (i.e., W-PreS-O) SARS-CoV-2 protein subunit vaccines based on hepatitis virus B (HBV)-derived PreS as carrier protein fused to two receptor binding domains (RBD) were produced and characterized, to identify the subunit vaccine which protects best against Omicron. **Methods:** *In vitro* characterization of protein subunit vaccines by protein chemical, immunological methods and immunization of BALB/c mice to study specific antibody, cytokine responses, safety and cross-protective virus neutralization. Immunization of Syrian hamsters and infection with Omicron BA.1. Determination of neutralizing antibody titres, weight, lung symptoms and viral loads in the upper and lower respiratory tracts. In addition, infectious virus titers from the lungs were measured using a plaque-forming assay. **Results:** All mentioned subunit vaccines could be easily produced, were stable at 4°C and safe. RBD-specific IgG levels induced by the different vaccines were comparable but W-PreS-O-induced virus neutralization titres against Omicron were 2-7-fold higher than the strain-specific and 6-fold higher than the bivalent vaccine. Syrian hamsters vaccinated with W-PreS-O developed

robust nABs against Omicron, showed almost no symptoms of pneumonia, and had significantly reduced infectious virus titres in the lungs. Importantly, the viral loads in the nasal cavities of W-PreS-O-vaccinated hamsters were close to or above the PCR cycle threshold considered to be non-infectious. **Conclusion:** Our data demonstrate that the chimeric W-PreS-O vaccine protects against Omicron in the Syrian hamster *in vivo* infection model. W-PreS-O is a highly promising candidate vaccine for Omicron infections and should be further evaluated in clinical studies. The fusion protein-based vaccine platform can be rapidly adapted to new upcoming virus variants.

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