A DNA COVID-19 vaccine candidate targeting antigen-presenting cells and adapted to RBD of emerging SARS-CoV-2 variants induced strong cross-neutralizing antibodies and protective immune responses in mice

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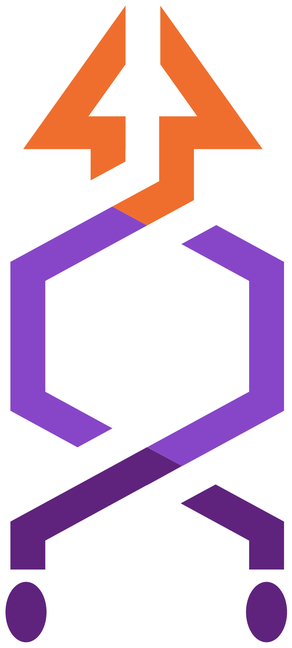
INTRODUCTION

Throughout the COVID-19 pandemic, several variants of concern (VoCs) of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have evolved affecting the efficacy of the approved COVID-19 vaccines. To address the need for vaccines that can induce strong and long-lasting cross-neutralizing antibody responses against circulating VOCs, and cross-reactive T cell responses that are less susceptible for immune escape than the antibody based protection, we developed a prophylactic SARS-CoV-2 vaccine candidate, referred here to as VB10.2129, based on our easily adaptable DNA plasmid vaccine platform.

The plasmid encodes a protein homodimer consisting of a targeting unit that binds chemokine receptors on antigen-presenting cells (human LD78β/CCL3L1), a dimerization unit (derived from the hinge and CH3 exons of human IgG3), and an antigenic unit consisting of the receptor binding domain (RBD) from the lineage B.1.351 Beta of SARS-CoV-2. Beta B.1.351 is a VoC with reduced susceptibility against antibodies induced by prototype Spike-based vaccines. B.1.351 RBD was selected as a heterologous booster to generate a broad protective immunity against SARS CoV-2 variants evading vaccine-induced immunity.

The VB10.2129 vaccine candidate was evaluated for the protein secretion, and further subjected to immunogenicity studies in BALB/c mice. Cross-neutralizing activity of induced antibodies against VoC was evaluated in pseudovirus and live virus neutralization assays using sera from vaccinated mice.

VB10.2129 VACCINE CANDIDATE

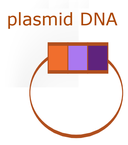


TARGETING UNIT
CCL3L1 chemokine that binds receptors on antigen presenting cells (APCs) and attracts immune cells

DIMERIZATION UNIT
Dimerization unit for cross-linking targeted receptors on APCs

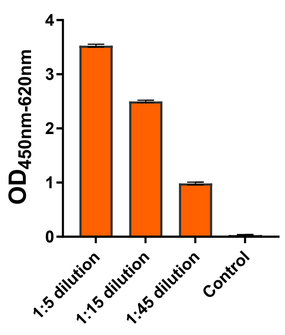
ANTIGENIC UNIT
RBD (319-542 aa) antigen of B.1.351 Beta variant of concern

PROTEIN SECRETION *IN VITRO*

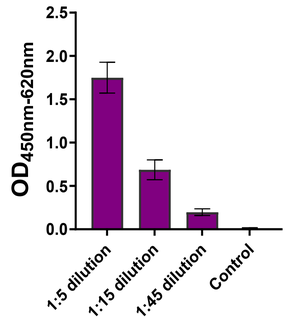


METHOD:
- Expi293 cells were transiently transfected with plasmid DNA
- Supernatant harvested at day 3 post transfection
- Sanwich ELISA using antibodies detecting the human IgG CH3 domain (coating antibody), human CCL3L1 and SARS-CoV-2 Wuhan-Hu-1 RBD

CCL3L1 detection



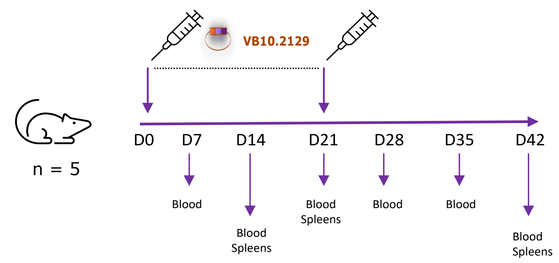
RBD detection



Vaccine protein is expressed and secreted; all three domains of the vaccine protein are detected

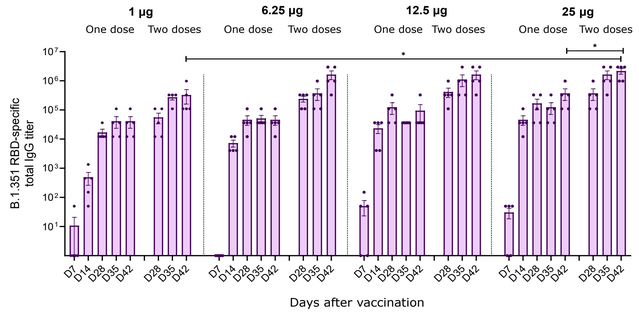
IMMUNOGENICITY OF VB10.2129 IN BALB/c MICE

Vaccine dose:
1 µg
6,25 µg
12,5 µg
25 µg



IMMUNOGENICTY EVALUATION:
- RBD-specific antibody levels were measured in sera from vaccinated mice by IgG ELISA and Mesoscale assays
- RBD-specific T cell responses were measured in splenocytes from vaccinated mice by IFN-γ ELISpot
- Neutralization activity of induced antibodies was estimated using pseudovirus and live virus neutralization assays

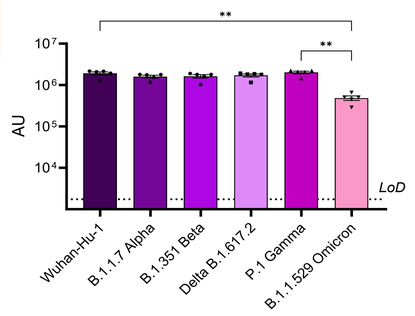
B.1.351 RBD specific antibody



Rapid (day 7), strong (>10^6 endpoint titer) and persistant (day 42) B.1.351 RBD-specific antibody responses

Mesoscale anti-RBD IgG assay

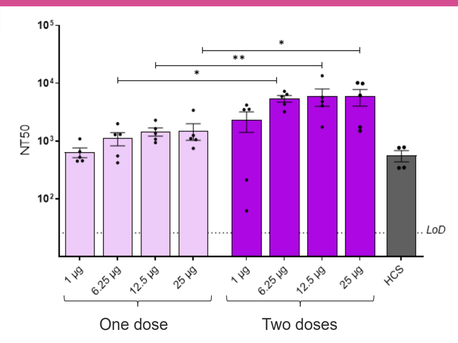
2 x 25 µg
Day 42



High titers of cross-reactive antibodies against five tested variants of concern, including Omicron B.1.1.529

B.1.351 pseudovirus neutralization assay

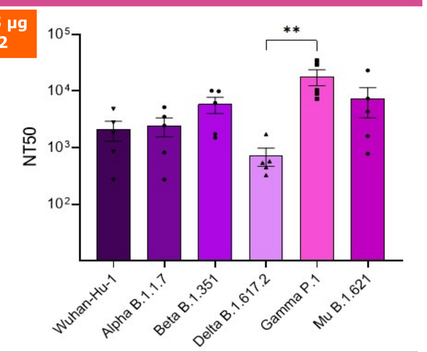
Day 42



Potent and dose-dependent neutralizing antibody responses against homologous Beta B.1.351 strain

VoCs pseudovirus neutralization assay

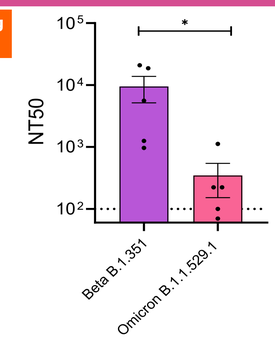
2 x 25 µg
Day 42



High titers of cross-neutralizing antibody responses across five tested variants of concern

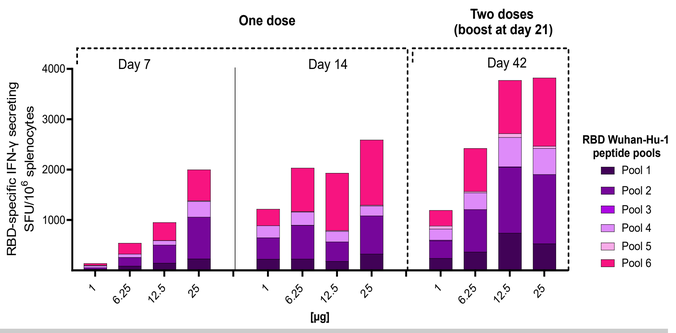
Live virus neutralization assay

2 x 25 µg
Day 42



Neutralizing activity of induced antibodies against live Beta B.1.351 and Omicron B.1.1.592 viruses

RBD-specific T cell immunity



Strong, dose-dependent and persistent T cell responses against RBD epitopes

CONCLUSIONS:

- Nykode has developed a COVID-19 vaccine candidate, VB10.2129, encoding the receptor binding domain (RBD) derived from the B.1.351 (Beta) variant of concern
- VB10.2129 induced strong cross-neutralizing antibodies against several VoCs accompanied by strong T cell responses in mice
- VB10.2129 serves as a potential booster vaccine for inducing antibody and T cell based protection against current and future SARS-CoV-2 VoCs

Statistical analysis:
Statistically significant differences were determined using two-tailed Mann-Whitney test for comparison of two groups or Kruskal-Wallis test for comparison of three or more groups and are indicated as follows: *, $p < 0.05$; **, $p < 0.01$, ****, $p < 0.0001$

References:
Fredriksen, et al. DNA Vaccines Increase Immunogenicity of Idiotypic Tumor Antigen by Targeting Novel Fusion Proteins to Antigen-Presenting Cells. Mol Ther, 2006
Ruffini, et al. Human chemokine MIP1α increases efficiency of targeted DNA fusion vaccines. Vaccine, 2010
Norheim, et al. Single dose immunization with a COVID-19 DNA vaccine encoding a chimeric homodimeric protein targeting receptor binding domain (RBD) to antigen-presenting cells induces rapid, strong and long-lasting neutralizing IgG, Th1 dominated CD4+ T cells and strong CD8+ T cell responses in mice, Biorxiv, 2020

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Abbreviations:
LoD = Level of detection; signal for PBS vaccinated mice
AU = Arbitrary unit
NT50 = Neutralization titers that achieved 50% neutralization
VoC = Variant of Concern
DPI = Day Post Infection
TCID50 = Tissue Culture Infectious Dose 50 assay, concentration at which 50% of the infected cells display cytopathic effect

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