Breadth of Vaccinated Cancer Patient Humoral Response to SARS-CoV-2 Spike Protein and 4RBD Variants

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Introduction

- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), the highly contagious, zoonotic virus responsible for the COVID-19 pandemic causes a wide range of potentially chronic symptoms.
- Since the original Wuhan Strain, several SARS-CoV-2 variants have emerged, such as the B.1.351 SARS-CoV-2 variant which first emerged in South Africa
- The Receptor Binding Domain (RBD), located within the Spike protein of SARS-CoV-2 serves as a highly immunogenic epitope for potent neutralizing antibodies.
- Current mRNA vaccines (Moderna and Pfizer-BioNTech) encode for the Spike protein of SARS-CoV-2, which allows the body to build antigen-specific antibodies.
- To manage the COVID-19 pandemic, it is essential to develop serological assays which measure protective antibody titers upon infection or vaccination.
- Serological assays which indicate presence and level of antibody protection against SARS-CoV-2 can be used as a platform for variant screening.
- RBD-foldon 2.2 is a novel antigen produced by fusing RBD with the trimerization domain Fibritin from Bacteriophage T4. Its amino acid sequence is based on the original Wuhan Strand that was sequenced in Washington. (Breckenridge, 2021).
- B.1.351 RBD-foldon 2.2 antigen is identical to the RBD-foldon 2.2 antigen except it uses the B.1.351 variant RBD sequence.
- Using cancer patient sera samples from the University of Louisville Co-Immunity study, the breadth and robustness of response was examined in comparison to patients that indicated “no chronic conditions”.

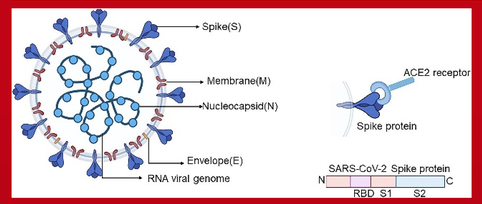


Figure 1. General Structure of SARS-CoV-2 particle. Includes four structural proteins: the envelope protein (E), nucleocapsid protein (N), spike protein (S), and membrane protein (M). (Min & Sun)

Preliminary Data

RBD-foldon 2.2 Plasmid Design: Foldon nucleotide sequence was added to RBD nucleotide sequence using a Proline-rich linker (previously used Glycine-Serine linker). RBD-foldon 2.2 also differed from the initial RBD-foldon construct by a modified signal peptide sequence. Fused sequence was inserted into Geneware® plasmid expression vector which encodes DNA for Tobacco Mosaic Virus (TMV), enabling protein expression in plants. **Plasmid Amplification:** The plasmid was transformed in E. Coli bacteria, then DNA was isolated (OMEGA Bio-Tek E.Z.N.A Plasmid DNA Midi Prep Kit). Amplified & prepped DNA was transcribed into RNA. Infectious RNA was mixed with plant inoculation buffer for Nicotiana benthamiana inoculation. **Virion Extraction: V**irion particles were extracted by Polyethylene Glycol (PEG) precipitation. Samples were loaded onto an SDS-PAGE to confirm virion had been isolated and TMV was expressed. **RBD-foldon 2.2 Extraction and Purification:** Plant extract was purified with an Immobilized Metal Affinity Chromatography (IMAC) gravity-flow column. Final elution sample was buffer exchanged using Ultrafiltration/Diafiltration (UF/DF) from imidazole containing buffer to 1x PBS so the concentration of the RBD-foldon could be determined using a spectrophotometer. (Breckenridge, 2021)

Methods

- **Sample Selection:** 36 cancer patients were age/sex matched to 36 individuals with no underlying health conditions, that received the same mRNA vaccine within two weeks of each other.
- **ELISA End-point Titers:** Sera samples were diluted 1:100 on an antigen-coated plate, then diluted three-fold down each row of a 96-well plate. ELISAs included detection of IgG and IgA antibodies against Spike, RBD-foldon, RBD-foldon 2.2, and RBD-foldon resembling the B.1.351 variant. Signal was detected at 450 nm. Values were blank subtracted, and the endpoint titer was determined with a cutoff of average negative values. (Bushau, 2021).
- **Statistical Analysis:** Student’s t-test was used to compare the mean value of end-point titers with a confidence interval of 95% between cancer patient group and patient group with no chronic conditions/control.

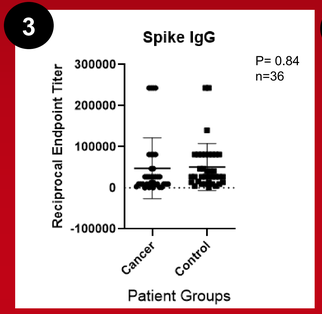
Hypothesis

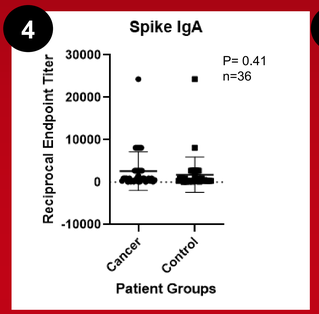
We hypothesize there will be a difference in humoral response to RBD-variant antigens in COVID-19 vaccinated cancer patients currently undergoing treatment versus patients with no chronic conditions.

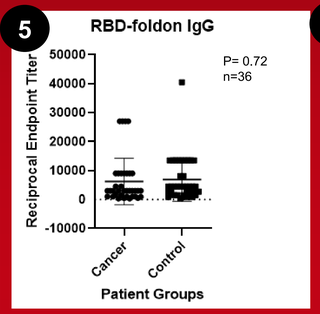
Significance

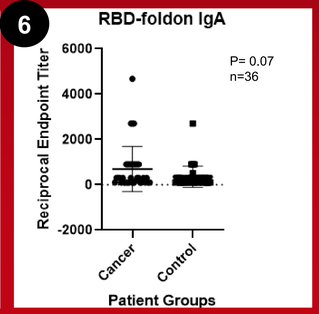
- Study will inform level of protection in immunocompromised groups which may have a suppressed response after vaccination.
- Variant screening essential to understand level of antibody protection produced by Pfizer and Moderna vaccines.

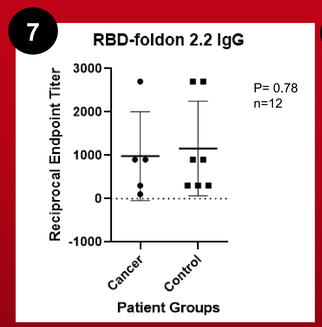
Results

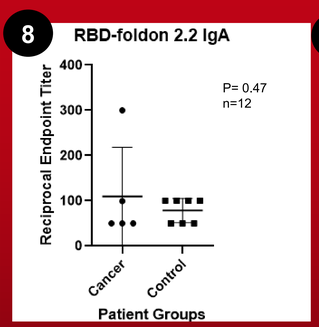


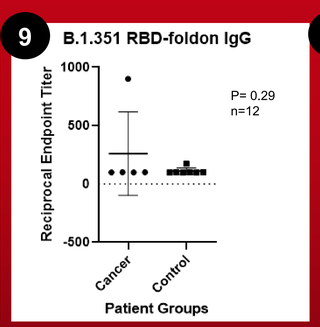












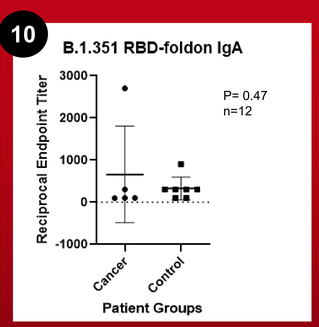


Figure 3. Spike IgG. Mean and standard deviation of reciprocal endpoint titers with spike antigen and IgG antibody. **Figure 4.** Spike IgA. Mean and standard deviation of reciprocal endpoint titers with Spike antigen and IgA antibody. **Figure 5.** RBD-foldon IgG. Mean and standard deviation of reciprocal endpoint titers with RBD-foldon antigen and IgG antibody. **Figure 6.** RBD-foldon IgA. Mean and standard deviation of reciprocal endpoint titers with RBD-foldon antigen and IgA antibody. **Figure 7.** RBD-foldon 2.2 IgG. Mean and standard deviation of reciprocal endpoint titers with RBD-foldon 2.2 antigen and IgG antibody. **Figure 8.** RBD-foldon 2.2 IgA. Mean and standard deviation of reciprocal endpoint titers with RBD-foldon 2.2 antigen and IgA antibody. **Figure 9.** B.1.351 RBD-foldon IgG. Standard deviation of reciprocal endpoint titers with B.1.351 RBD-foldon antigen and IgG antibody. **Figure 10.** B.1.351 RBD-foldon IgA. Standard deviation of reciprocal endpoint titers with B.1.351 RBD-foldon antigen and IgA antibody.

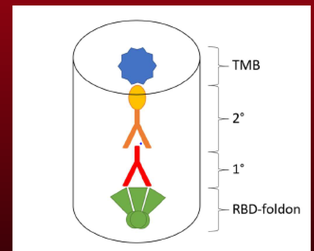


Fig. 2. RBD-foldon ELISA Layout (1° Antibody: Human Sera anti-RBD, 2 ° Antibody: Rabbit anti-Human HRP Conjugate, TMB: Reporter Enzyme) (Joey Breckenridge Jr., M.S.)

Conclusions

- There is no significant difference between cancer patients and age matched controls for Spike IgG and IgA or RBD-foldon IgG and IgA.
- There is no significant difference between cancer patients and patients with no chronic conditions in response for variant B.1.351 RBD-foldon IgG and IgA.
- RBD-foldon 2.2 elicits a lower humoral response than RBD-foldon, indicating the edited linker affects how the antigen presents
- B.1.351 RBD-foldon appears to elicit a lower response than RBD-foldon 2.2.

References

1. COVID-19 Serological Diagnostic Development Using a SARS-CoV-2 RBD-Foldon Fusion. (Thesis by Joey Breckenridge Jr., 2021) **2.** Serological Assessment of SARS-CoV-2 Infection During the First Wave of the Pandemic in Louisville, Kentucky. (Hamorsky et al.,2021) **3.** Stability of plasmid and viral Virion banks supporting the cGMP manufacture of Q-Griffithsin from a TMV-based viral vector (Corman et al., 2020) **4**. Antibodies and Vaccines Target RBD of SARS-CoV-2 (Min Long & Sun Qui, 2021)

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