

Exosomes engineered with SARS-CoV-2 spike and nucleocapsid proteins induce strong immune response against multiple variants of concern (VOCs)

Justin B Nice, Mafalda Cacciottolo, Yujia Li, Michael J. LeClaire, Ryan Twaddle, Ciana L. Mora, Stephanie Y. Adachi, Esther R. Chin, Meredith Young, Jenna Angeles, Kristi Elliott, Minghao Sun
Capricor Therapeutics, San Diego, CA

Abstract

Here we designed an exosome-based, bivalent vaccine with the goal of producing broader immunity against SARS-CoV-2 using Capricor's StealthX™ technology. Spike, in its naturally folded structure presented on the surface of exosomes, was designed to protect against newer spike mutations. In addition, nucleocapsid is a more conserved SARS-CoV-2 protein than spike. Both cell lines produced exosomes highly enriched in the desired protein, and when combined in a *in vivo* model produced a strong immune response and T-cell memory with only nanogram levels of protein presented on the surface of exosome.

Cells were engineered using lentiviral transduction and expanded for exosome production and the resulting cell-free supernatant was concentrated using a tangential-flow filter (TFF), proteins were then removed using Sephacryl size exclusion chromatography, and the resulting exosome-containing fractions concentrated again using TFF. Exosome size and concentration was measured using Particle Metrix ZetaView®. Spike or nucleocapsid protein concentration was measured using an ELISA assay. Mice were injected with two rounds of spike-expressing and nucleocapsid-expressing exosomes (day 0 and 21) and immune response was measured by ELISAs for IgG against spike or nucleocapsid at days 14 and 35. Results showed that IgG production against spike increased up to 1,500-fold while IgG against nucleocapsid increased up to 7-fold. Neutralizing of different VOCs of SARS-CoV-2 was carried out by Retrovirox, Inc. The results showed that mouse plasma could neutralize delta variant at 100%, as well as partial protection against omicron BA.1 and BA.5.2.1 variants.

This study demonstrates that exosomes can be engineered with viral antigens to be used for rapid vaccine development with implications beyond COVID-19 and to other vaccines that combine the power of Stealth X targeting, multiplexing, low dosage, better safety profile and short turnaround time.

Introduction

The COVID-19 pandemic led to the rapid development of new mRNA-based vaccines. These vaccines were exclusively targeted to the spike protein located on the surface of the virus. However, rapid mutations in the SARS-CoV-2 virus led to new variants with many being classified as variants of concern (VOC). These variants led to significant changes in the sequence of the spike protein and a subsequent decrease in the effectiveness of natural and vaccine immunity.

The goal of this study was to develop an exosome-based vaccine which employed both spike and nucleocapsid proteins. Unlike the spike protein, the nucleocapsid protein has mutated at a slower rate, even when compared to SARS (2003).

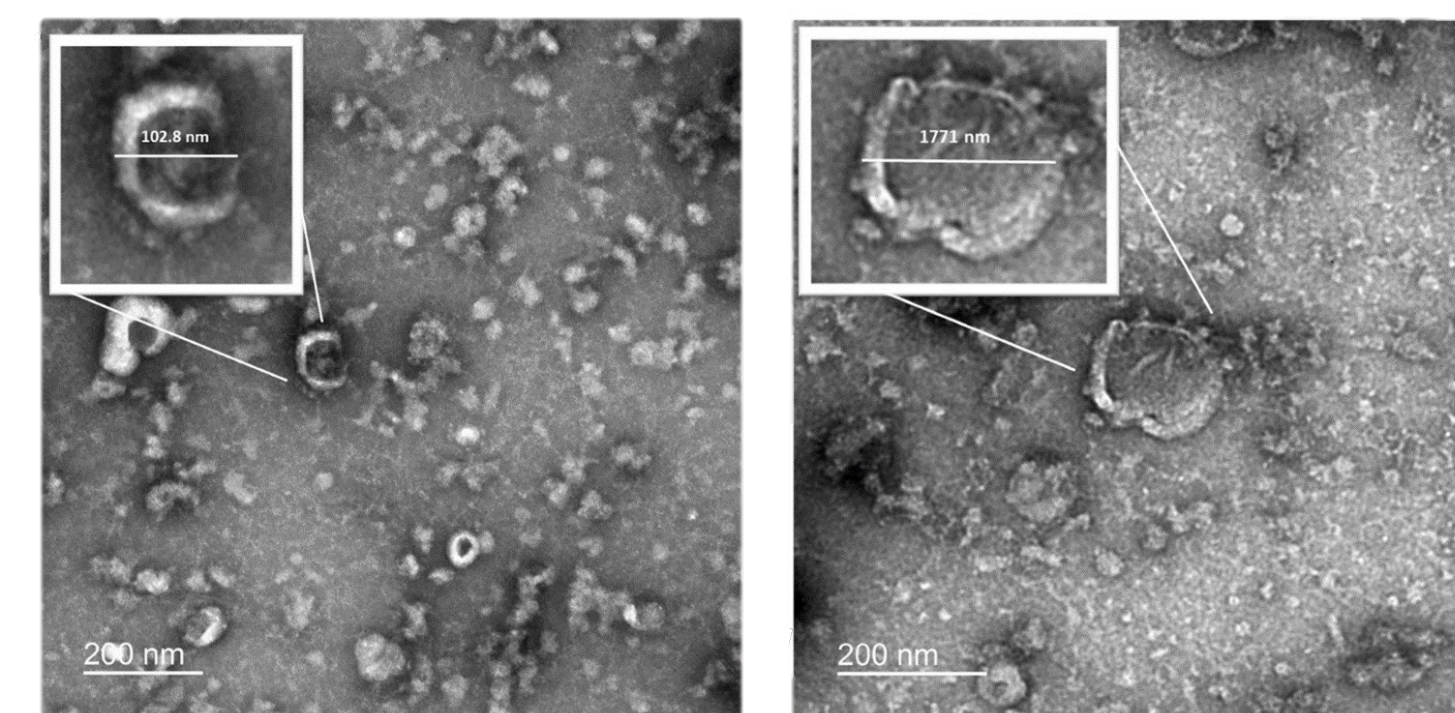
An exosome-based vaccine was chosen due to their demonstrated safety profile and the ability to quickly engineer the host cell line. The 293F cell line that was chosen for our StealthX™ (STX) vaccine has several advantages of stem cell lines commonly used for exosome-based therapeutics. 293F cells can be 1) easily transduced with high efficiency, 2) grown in suspension culture, leading to high volumes of cell culture supernatant for exosome processing.

Capricor's StealthX™ technology was used in the development of the engineered cell lines for both spike and nucleocapsid producing exosomes. StealthX™ is designed to fuse the protein of interest to a protein enriched in exosomes leading to high trafficking of the protein of interest to exosomes. In the case of the STX-S+N vaccine, both constructs were designed to present the protein on the surface of the exosome, leading to enhanced recognition by immune cells. However, the STX system allows for the protein to be contained within the lumen of exosomes, leading to the potential development of other exosome-based therapeutics further down Capricor's pipeline.

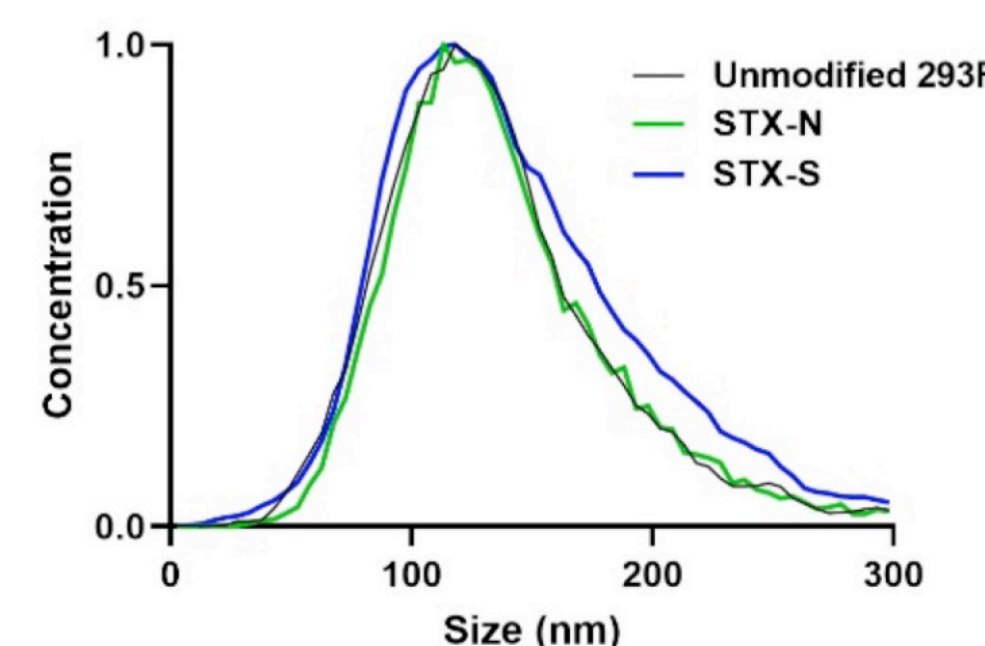


Results

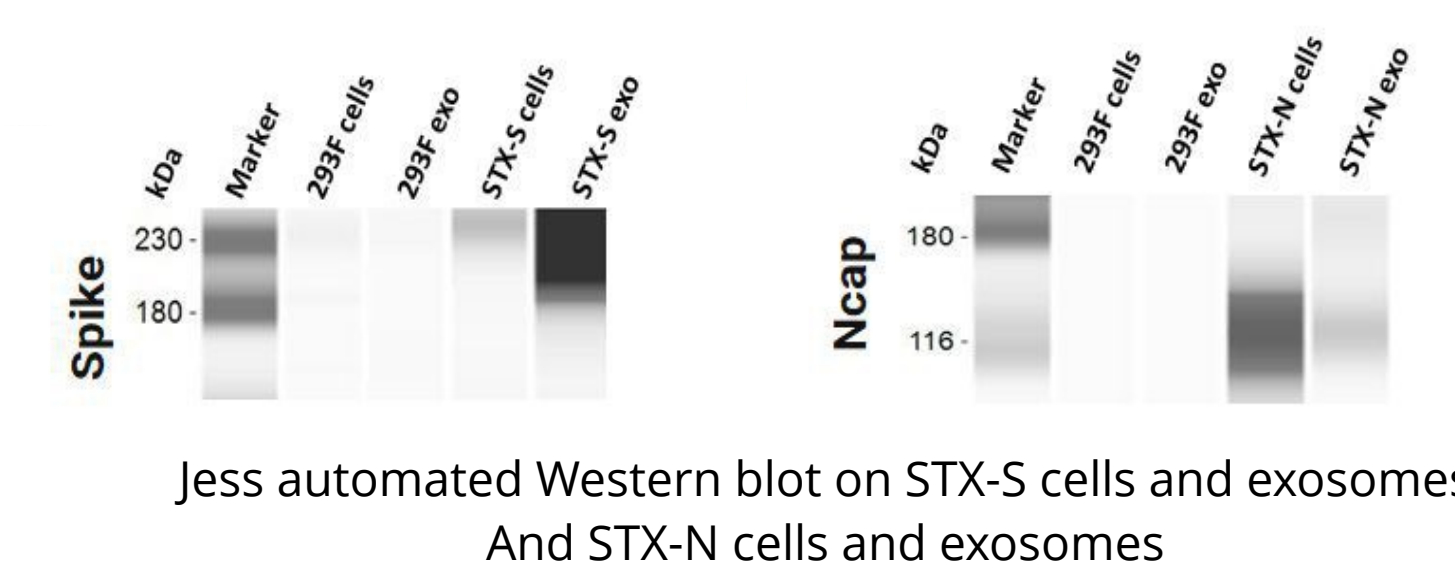
Exosome Characterization



Negative-stain TEM of STX-S and STX-N exosomes



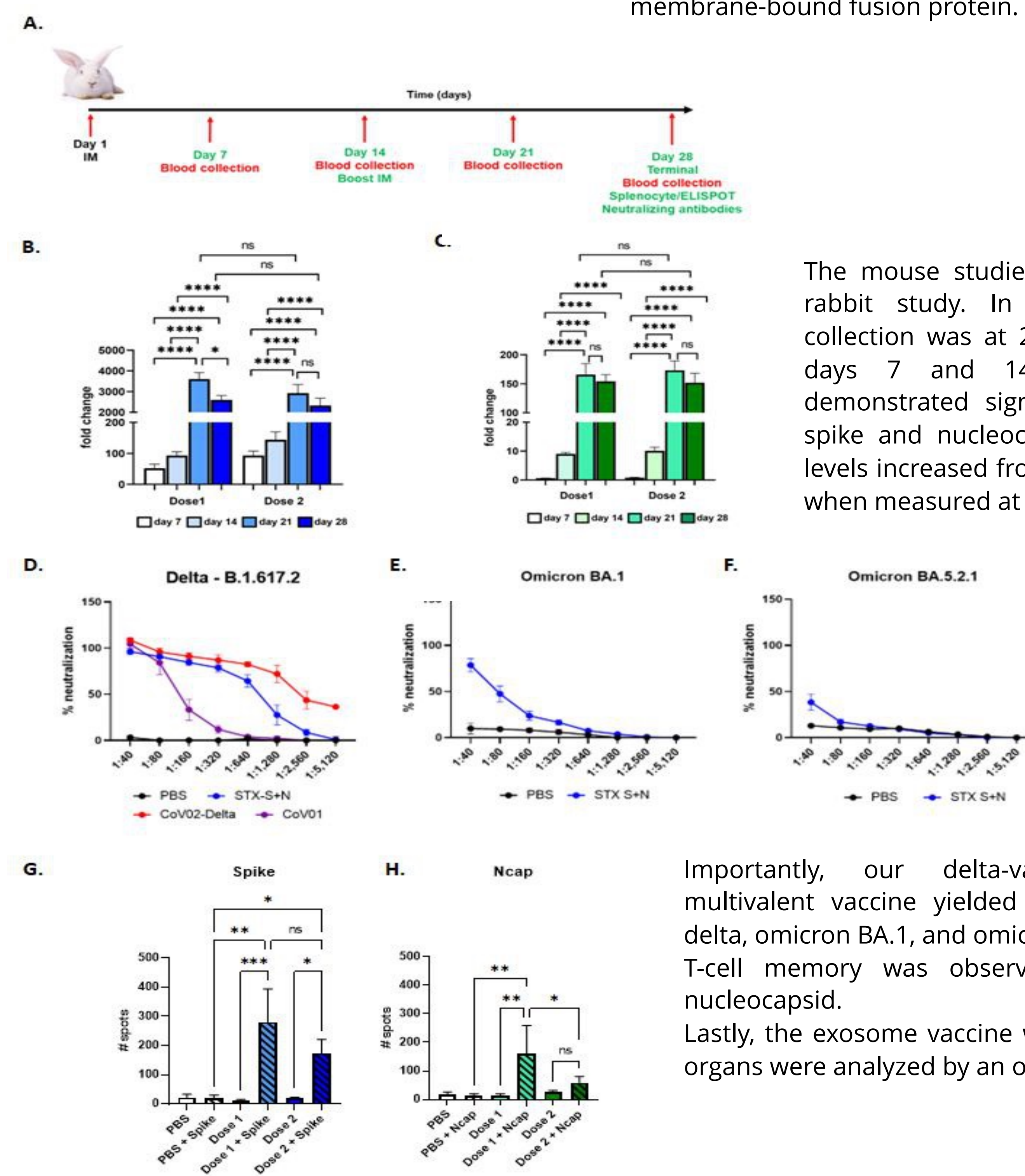
ZetaView NTA on STX and unmodified exosomes



Jess automated Western blot on STX-S cells and exosomes
And STX-N cells and exosomes

Exosomes were visualized by TEM; the presence of a lipid bilayer was clearly visible on both STX-S and STX-N exosomes. In addition, spike protrusions were visible on STX-S exosomes. The size and concentration of unmodified 293F and StealthX™ exosomes were measured on a Particle Metrix ZetaView®. The size distribution of modified exosomes was the same as unmodified exosomes. In addition, modification did not alter the number of particles produced by 293F cells. Cells and the resulting exosomes from STX-S and STX-N were run on a Jess Western blot system using anti-spike or anti-nucleocapsid antibodies. Equal concentrations of protein were loaded in the cartridge. STX-S exosomes were enriched in spike protein compared with their parental cells. STX-N exosomes contained a lower level of nucleocapsid than cells. In the future we aim to increase the trafficking of soluble proteins to exosomes by optimizing the design of the membrane-bound fusion protein.

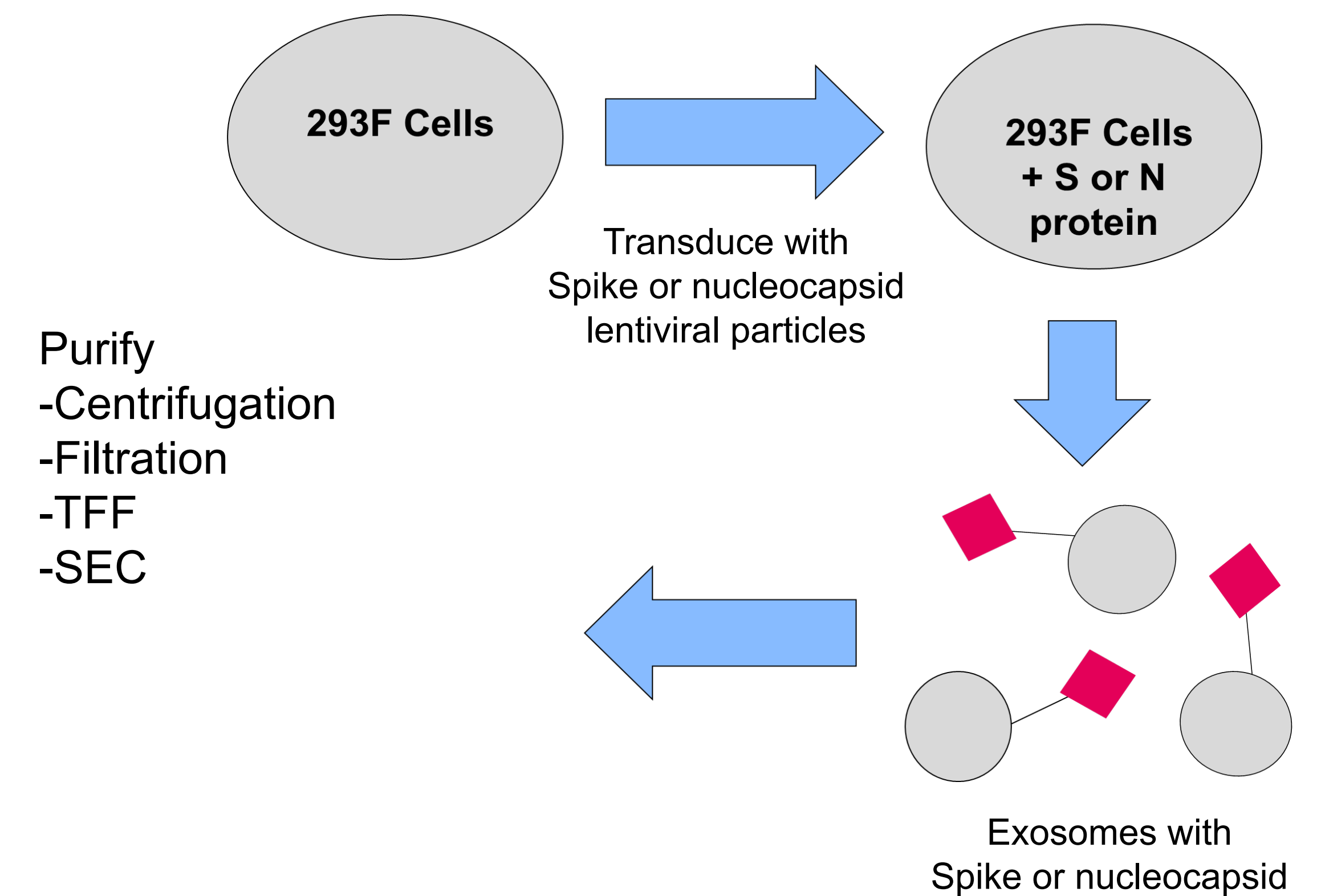
In vivo Studies



The mouse studies were also repeated in a rabbit study. In this study the terminal collection was at 28 days. Blood collected at days 7 and 14 (prior to the boost) demonstrated significant IgG levels to both spike and nucleocapsid. At 21 days the IgG levels increased from day 14 with a small drop when measured at 28 days.

Importantly, our delta-variant spike/nucleocapsid multivalent vaccine yielded neutralizing antibodies to delta, omicron BA.1, and omicron BA.5.2.1 variants. T-cell memory was observed with both spike and nucleocapsid. Lastly, the exosome vaccine was not immunogenic after organs were analyzed by an outside pathology lab.

Methodology



293T cells were used in the production of spike and nucleocapsid lentiviral particles. Particles were collected and used to transduce 293F cells. After sorting or selection the cells were expanded into suspension (Freestyle™ 293 Media). Following the three-day growth period, the cell culture supernatant was centrifuged and filtered. The resulting supernatant was concentrated using a TFF filter and the subsequent concentrate was run by SEC to remove free-proteins. A final TFF concentration step was performed on the exosome-containing fractions.

Exosome size and concentration was measured using ParticleMetrix ZetaView. The presence of a lipid bilayer and surface-associated spike was confirmed by TEM. Spike or nucleocapsid protein concentration was measured using an ELISA assay. In addition, the expected size of the spike/nucleocapsid -fusion proteins was verified using a Jess automated Western blot system.

Mice were injected with two rounds of spike-expressing and nucleocapsid-expressing exosomes (day 0 and 21) and immune response was measured by ELISAs for IgG against spike or nucleocapsid at days 14 and 35. T-cell memory was measured using an ELISpot assay on unstimulated splenocytes or stimulated with spike/nucleocapsid protein. Mouse organs were sent to Reveal Biosciences for pathology. Mouse plasma was sent to Retrovirox Inc for neutralizing antibody assays.

Conclusion

This study demonstrated the ability to rapidly develop a protein-exosome-based vaccine for the SARS-Cov2. The multivalent vaccine led to a robust immune response in both mice and rabbits without any safety issues. Due to the low levels of protein need in this vaccine other multivalent vaccines such as the yearly flu vaccine or the recent RSV outbreaks. In addition, the efficient trafficking of the protein of interest to the exosome can be used in many protein-replacement therapeutics.

References

Exosome-Based Multivalent Vaccine: Achieving Potent Immunization, Broadened Reactivity, and Strong T-Cell Responses with Nanograms of Proteins. Cacciottolo M, Nice JB, et al. Microbiol Spectr. 2023 doi: 10.1128/spectrum.00503-23

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