Maximizing the immunogenicity of adenoviral (Ad)-based vaccines encoding stabilized RSV pre-fusion F

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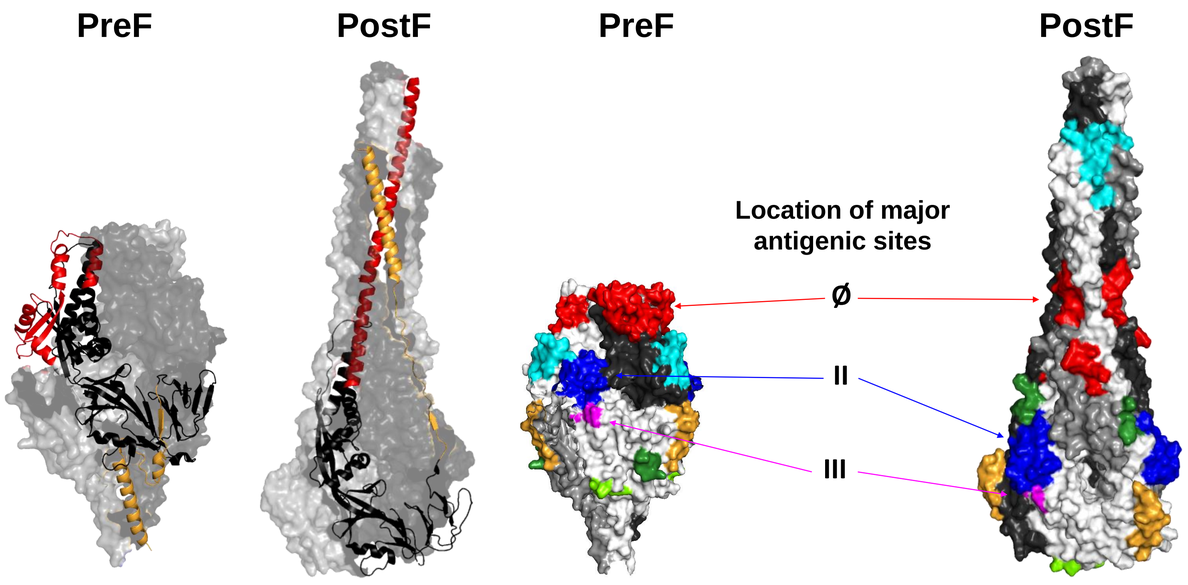
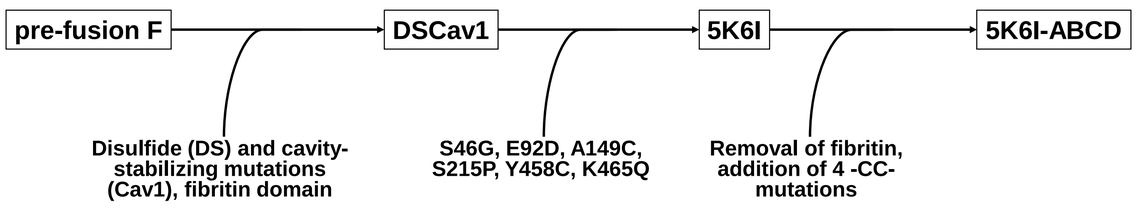
1. INTRODUCTION

STABILIZED RSV PREFUSION (F) AS VACCINE ANTIGEN



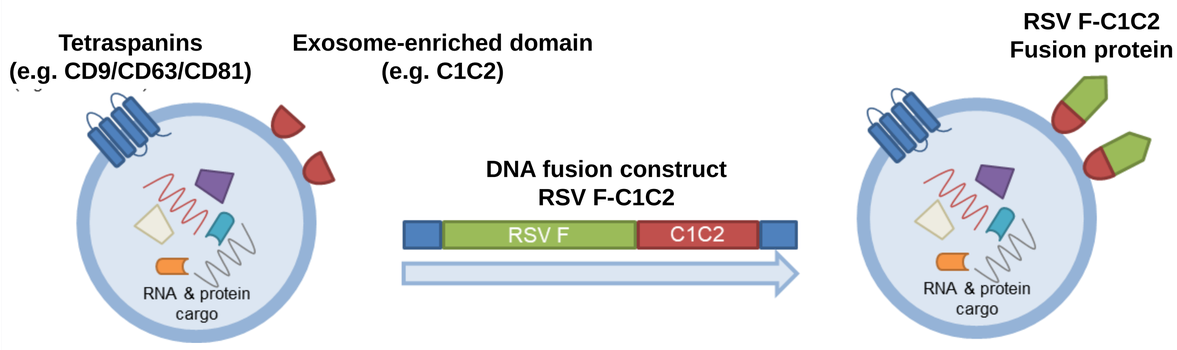
- PreF harbors the most potent neutralizing epitopes, so efforts have been made to stabilize the preF conformation for vaccine purposes.



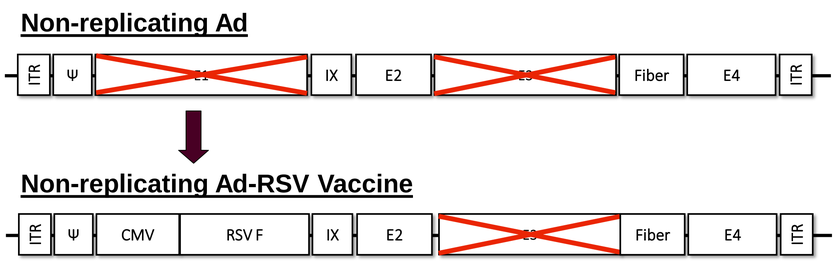
2. INTRODUCTION

OPTIMIZING ANTIGEN DELIVERY AND IMMUNOGENICITY

- Exosomes play crucial roles in regulating immunity.
- By fusing the C1C2 domain of lactadherin to preF we can tether preF to the surface of exosomes, effectively creating a molecular adjuvant.



- We engineered RSV PreF and C1C2 fusion antigen constructs into an immunogenic non-replicating Ad-based vaccine for *in vivo* testing.



3. METHODS

Antigen validation:

- PreF was expressed using Expi293F cells, purified with Ni-NTA affinity chromatography, and validated using ELISA.

Antigen optimization:

- Cells were transfected with plasmids encoding PreF_C1C2.
- Exosomes were purified from supernatant by ultra-centrifugation and size exclusion chromatography, and verified with ELISA.

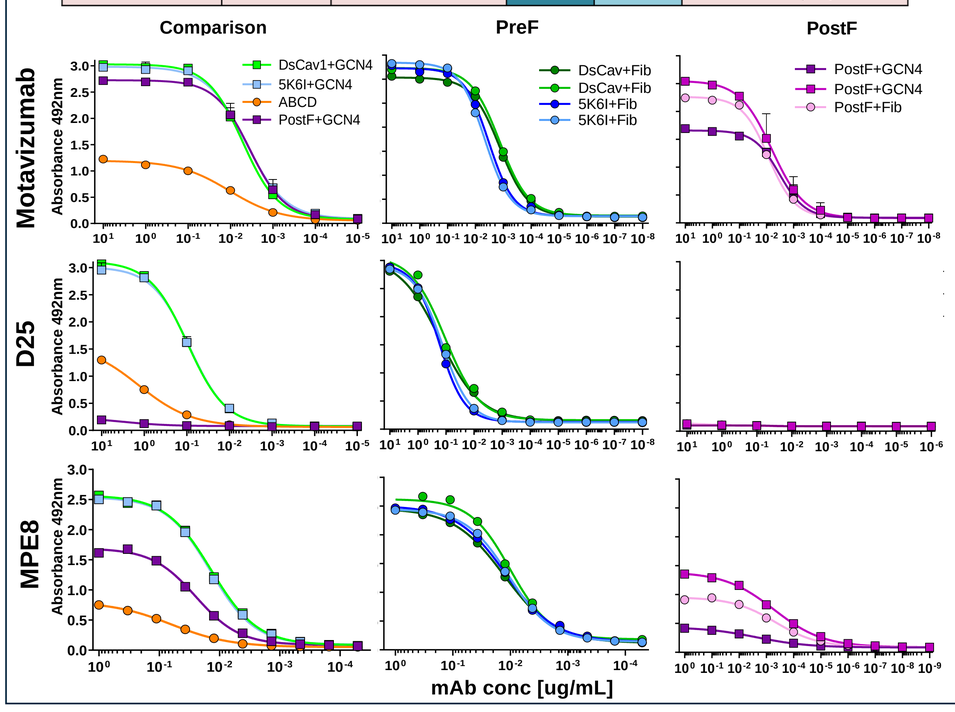
Vaccine construction:

- Antigen cassettes were genetically engineered into Ad5 vectors.
- Vaccines were amplified in T-Rex-293 cells, purified by ultracentrifugation, validated with SDS-PAGE and silver stain, and titered using microBCA and $TCID_{50}$.

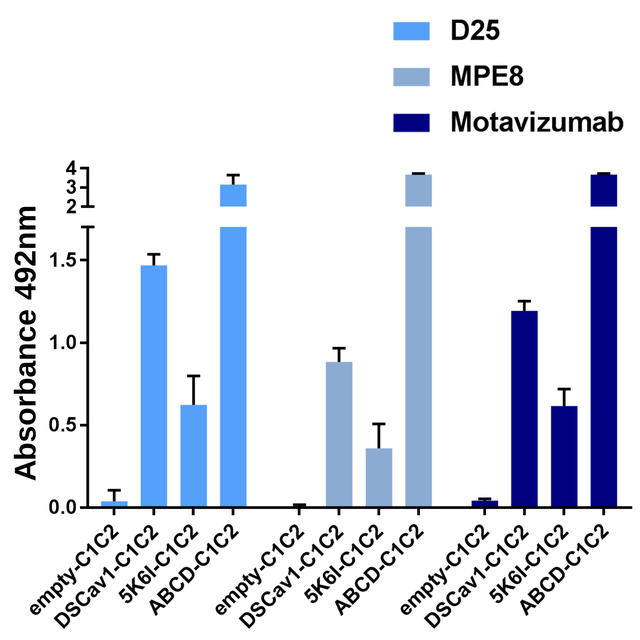
4. RESULTS

ANTIGENIC SITES Ø, II, AND III ARE INTACT

Antibody	Species	Antigenic site	PreF	PostF	Other
Motavizumab	Human	II			FDA declined drug
D25	Human	Ø			Quaternary specific
MPE8	Human	III			Quaternary specific



PRE F PRESENT ON SURFACE OF EXOSOMES



- ELISA on host-derived exosomes reveals the presence of, and correct conformation of preF on the surface of exosomes.

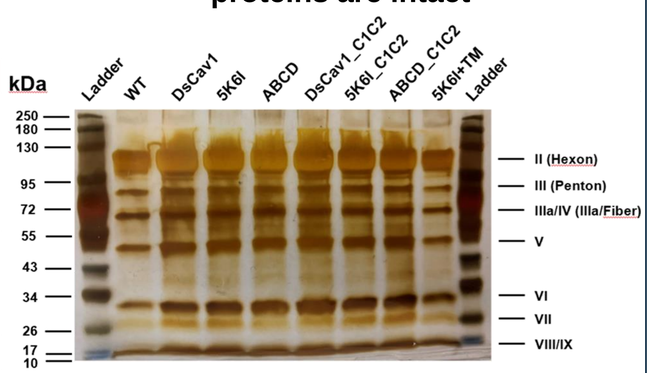
5. RESULTS

SUCCESSFUL ENGINEERING OF NON-REPLICATING AD5 VACCINES ENCODING OPTIMIZED PRE F ANTIGENS

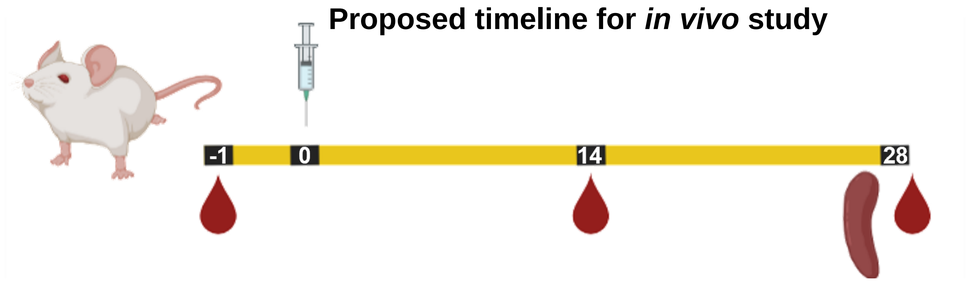
Viral vector titers are validated for *in vivo* experiments.

Construct	VP/mL	PFU/mL
Ad5_RSV-F_WT	2.43×10^{12}	2.19×10^{11}
Ad5_RSV-F_DsCav1	6.86×10^{12}	1.20×10^{11}
Ad5_RSV-F_5K6I	7.53×10^{12}	2.20×10^{11}
Ad5_RSV-F_ABCD	1.70×10^{12}	3.30×10^{11}
Ad5_RSV-F_DsCav1+C1C2	3.69×10^{12}	3.46×10^{11}
Ad5_RSV-F_5K6I+C1C2	2.98×10^{12}	4.29×10^{11}
Ad5_RSV-F_ABCD+C1C2	3.79×10^{12}	4.69×10^{11}
Ad5_RSV-F_5K6I+TM	3.85×10^{12}	4.08×10^{11}

Ad5 major and minor strucutral proteins are intact



Proposed timeline for *in vivo* study



6. SUMMARY & CONCLUSIONS

SUMMARY

- An RSV vaccine capable of eliciting sufficient NAb titers for maternal immunizations is needed.
- Exosome display is a molecular adjuvanting approach which, when combined with novel stabilized preF, could increase the magnitude of NAb titers.
- We confirmed that we can successfully target RSV-F_C1C2 to the surface of exosomes, and important antigenic sites are intact.
- We have successfully constructed a panel of Ad5-based vaccines encoding variants of the RSV preF antigen and have performed quality control of the vaccine stocks in preparation for use in a comparative immunogenicity study in mice.

Exosome Display, combined with stabilized preF antigen, delivered through an Ad5 vector, could provide a vaccine able to curb RSV-related infant mortality.

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