Vaccine Development For Emerging Infectious Diseases

Andrea Gambotto M.D.

SARS-CoV

RESEARCH ARTICLES

Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Paul A. Rota,[1]* M. Steven Oberste,[1] Stephan S. Monroe,[1] W. Allan Nix,[1] Ray Campagnoli,[1] Joseph P. Icenogle,[1] Silvia Peñaranda,[1] Bettina Bankamp,[1] Kaija Maher,[1] Min-hsin Chen,[1] Suxiong Tong,[1] Azaibi Tamin,[1] Luis Lowe,[1] Michael Frace,[1] Joseph L. DeRisi,[2] Qi Chen,[1] David Wang,[2] Dean D. Erdman,[1] Teresa C. T. Peret,[1] Cara Burns,[1] Thomas G. Ksiazek,[1] Pierre E. Rollin,[1] Anthony Sanchez,[1] Stephanie Liffick,[1] Brian Holloway,[1] Josef Limor,[1] Karen McCaustland,[1] Melissa Olsen-Rasmussen,[1] Ron Fouchier,[3] Stephan Günther,[4] Albert D. M. E. Osterhaus,[3] Christian Drosten,[4] Mark A. Pallansch,[1] Larry J. Anderson,[1] William J. Bellini[1]

The coronaviruses (order *Nidovirales*, family *Coronaviridae*, genus *Coronavirus*) are a diverse group of large, enveloped, positive-stranded RNA viruses that cause respiratory and enteric diseases in humans and other animals. At ~30,000 nucleotides (nt), their genome is the largest found in any of the RNA viruses. There are three groups of coronaviruses; groups 1 and 2 contain mammalian viruses, whereas group 3 contains only avian viruses. Within each group, coronaviruses are classified into distinct species by host range, antigenic relationships, and genomic organization. Coronaviruses typically have narrow host ranges and are fastidious in cell culture. The viruses can cause severe disease in many animals; and several viruses, including infectious bronchitis virus, feline infectious peritonitis virus, and transmissible gastroenteritis virus, are important veterinary pathogens. Human coronaviruses (HCoVs) are found in both group 1 (HCoV-229E) and group 2 (HCoV-OC43) and are responsible for ~30% of mild upper respiratory tract illnesses (*8–10*).

Structure of Coronaviruses

Spike (S) glycoprotein
Receptor binding and fusion to cell
Most antigenic
Trimeric

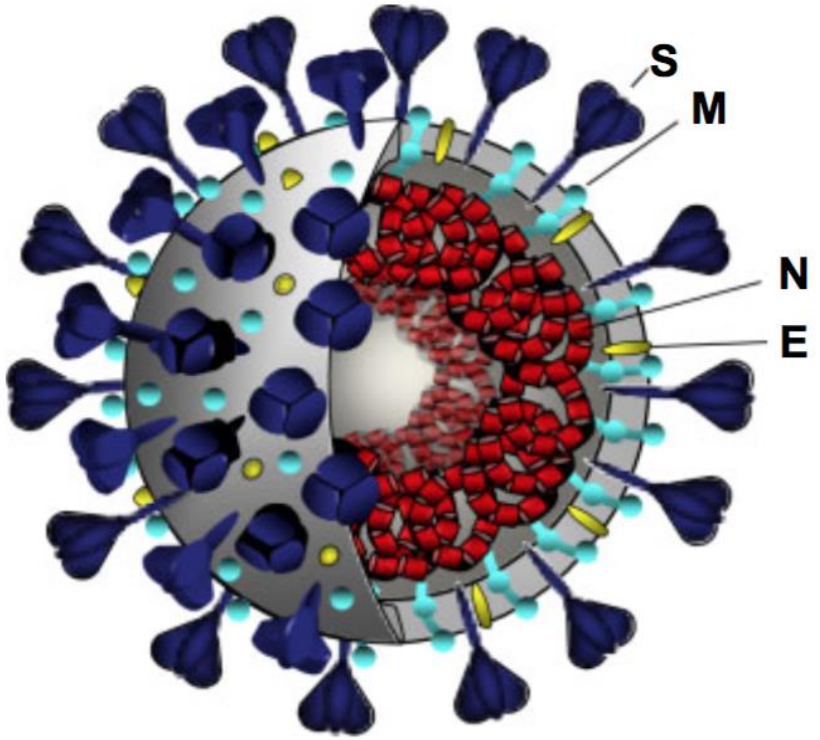
Nucleocapsid (N) phosphoprotein
RNA-binding, synthesis, translation
IFN I antagonist

Membrane (M) glycoprotein
Triple membrane spanning

Small Envelope (E) glycoprotein
Ion channel
Pentameric

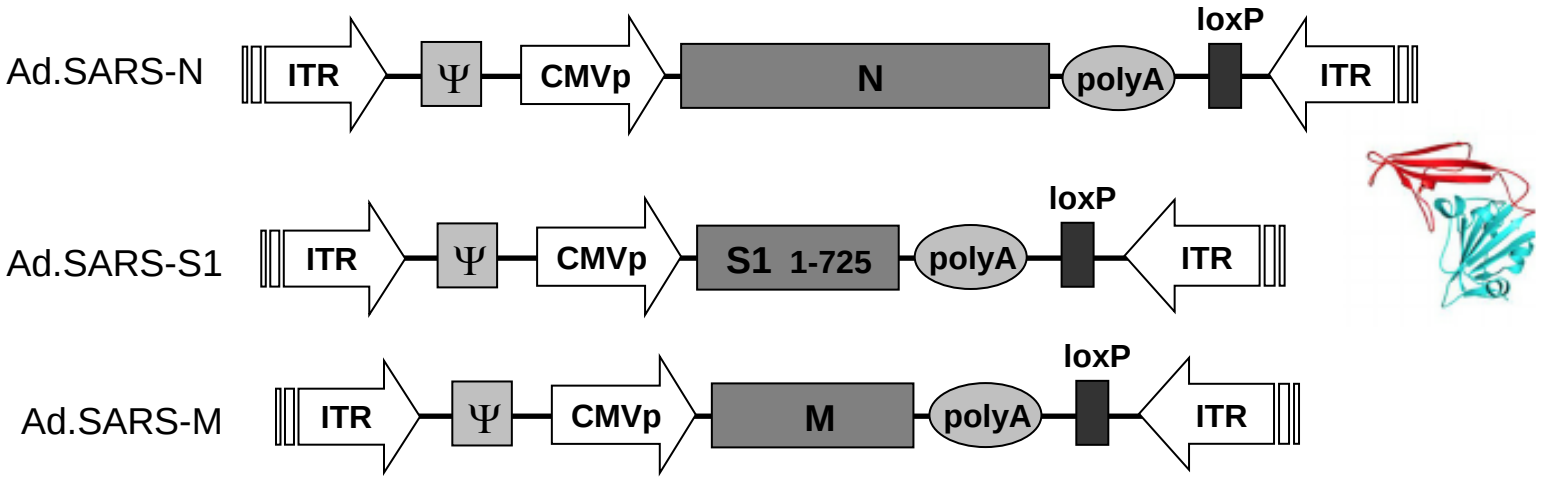
RNA
(+) ssRNA

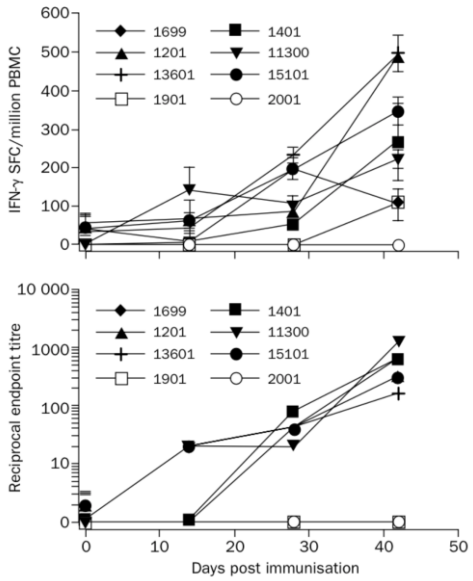
Envelope
Bilipid, host-derived membrane

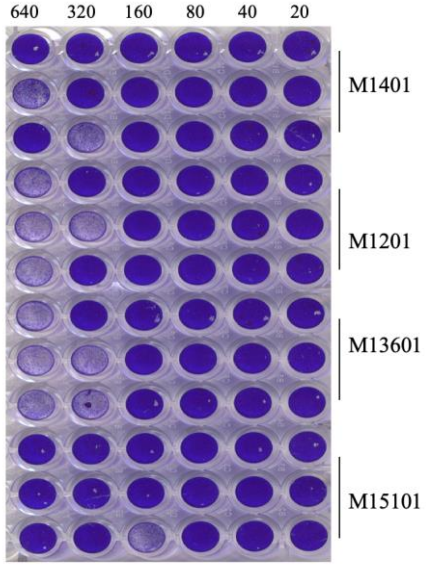


Kayvon Modjarrad, Journal of Virus Eradication 2016; 2: 1–4

SARS-CoV Vaccine Development







Research letters

THE LANCET • Vol 362 • December 6, 2003 • www.thelancet.com

Effects of a SARS-associated coronavirus vaccine in monkeys

Wentao Gao, Azaibi Tamin, Adam Soloff, Leonardo D'Aiuto, Edward Nwanegbo, Paul D Robbins, William J Bellini, Simon Barratt-Boyes, Andrea Gambotto

The causative agent of severe acute respiratory syndrome (SARS) has been identified as a new type of coronavirus. Here, we have investigated the ability of adenoviral delivery of codon-optimised SARS-CoV strain Urbani structural antigens spike protein S1 fragment, membrane protein, and nucleocapsid protein to induce virus-specific broad immunity in rhesus macaques. We immunised rhesus macaques intramuscularly with a combination of the three Ad5-SARS-CoV vectors or a control vector and gave a booster vaccination on day 28. The vaccinated animals all had antibody responses against spike

and antibody responses. The rhesus macaque was chosen for these studies because it is a highly relevant translational model for people. Immunological assays including the ELISPOT assay have been well characterised and validated in this model.

We did western blot analysis directed toward the spike protein S1 fragment—the most likely to elicit neutralising responses—on serum samples from immunised animals. We transfected HEK293 cells with expression plasmids encoding the S1 fragment or a control empty plasmid, and

MERS-CoV

BRIEF REPORT

Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia

Ali Moh Zaki, M.D., Ph.D., Sander van Boheemen, M.Sc.
Albert D.M.E. Osterhaus, D.V.M., Ph.D., and Ron

RAPID COMMUNICATIONS

Severe respiratory illness caused by a novel coronavirus, in a patient transferred to the United Kingdom from the Middle East, September 2012

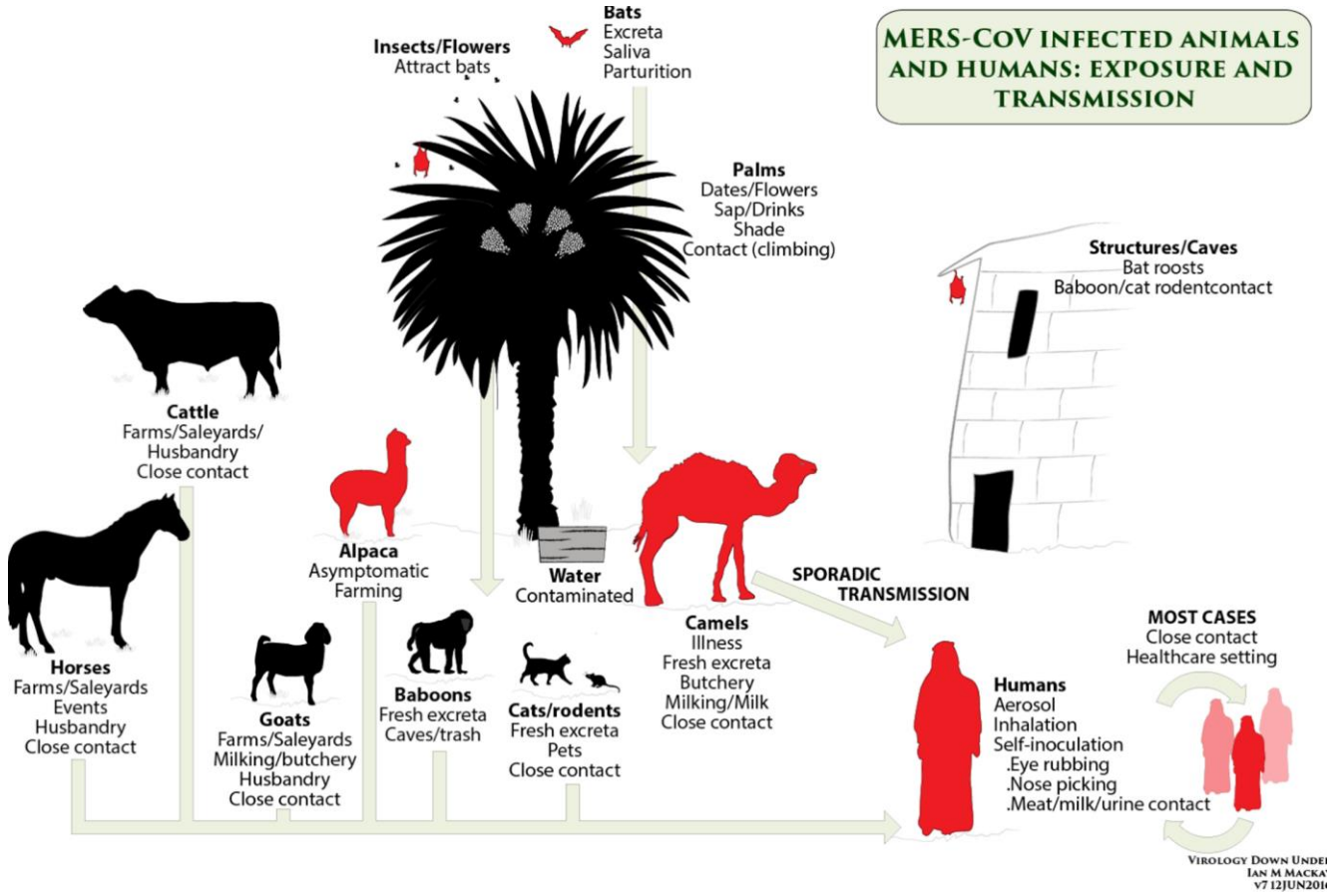
A Bermingham[1], M A Chand (meera.chand@hpa.org.uk)[1], C S Brown[1,2], E Aarons[3], C Tong[3], C Langrish[3], K Hoschler[1], K Brown[1], M Galiano[1], R Myers[1], R G Pebody[1], H K Green[1], N L Boddington[1], R Gopal[1], N Price[3], W Newsholme[3], C Drosten[4], R A Fouchier[5], M Zambon[1]

1. Health Protection Agency (HPA), London, United Kingdom
2. Centre for Clinical Infection and Diagnostics Research, King's College London, London, England
3. Guy's and St Thomas' NHS Foundation Trust and King's Health Partners, London, United Kingdom
4. Institute of Virology, University of Bonn Medical Centre, Bonn, Germany
5. Department of Virology, Erasmus Medical Centre, Rotterdam, the Netherlands

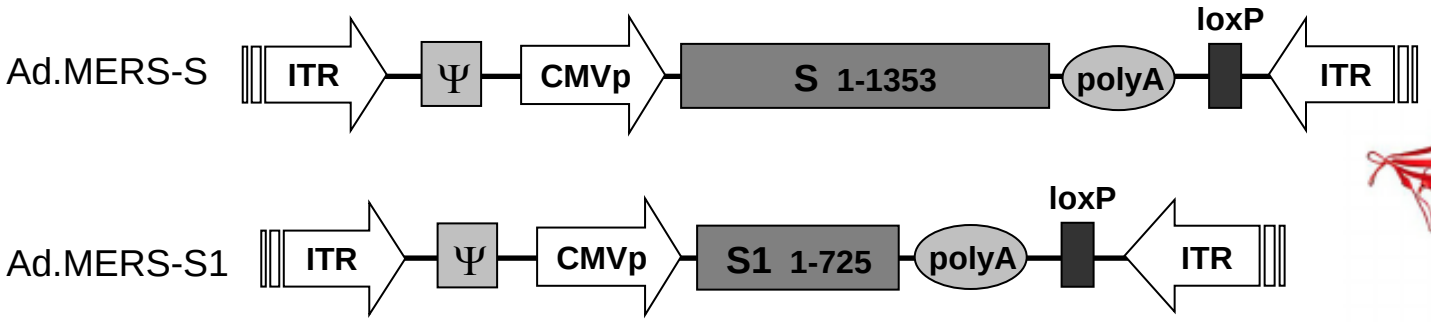
- Viruses from cases 1 and 2 99.5% identical

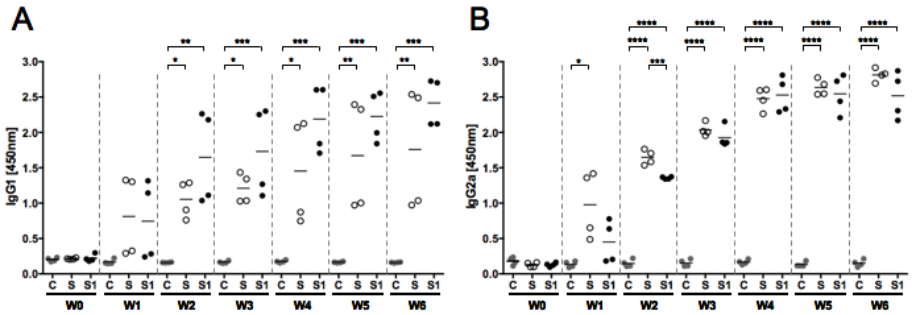
MERS-CoV is a Zoonotic Virus That is Transmitted From Animals to Humans

The origins of the virus are not fully understood but, according to the analysis of different virus genomes, it is believed that it originated in bats and was transmitted to camels sometime in the distant past.



MERS-CoV Vaccine Development

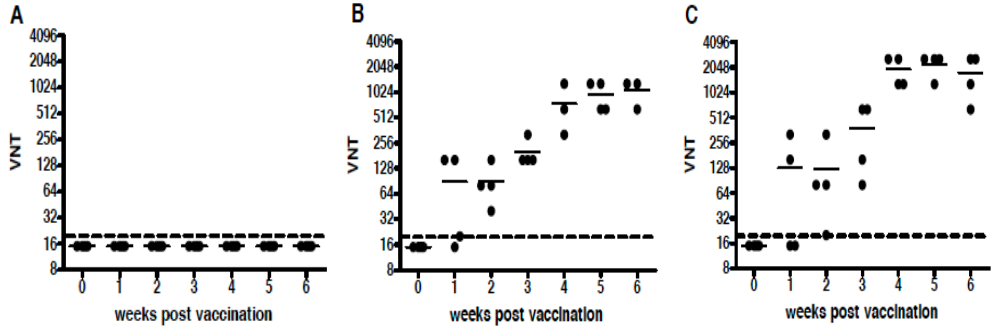




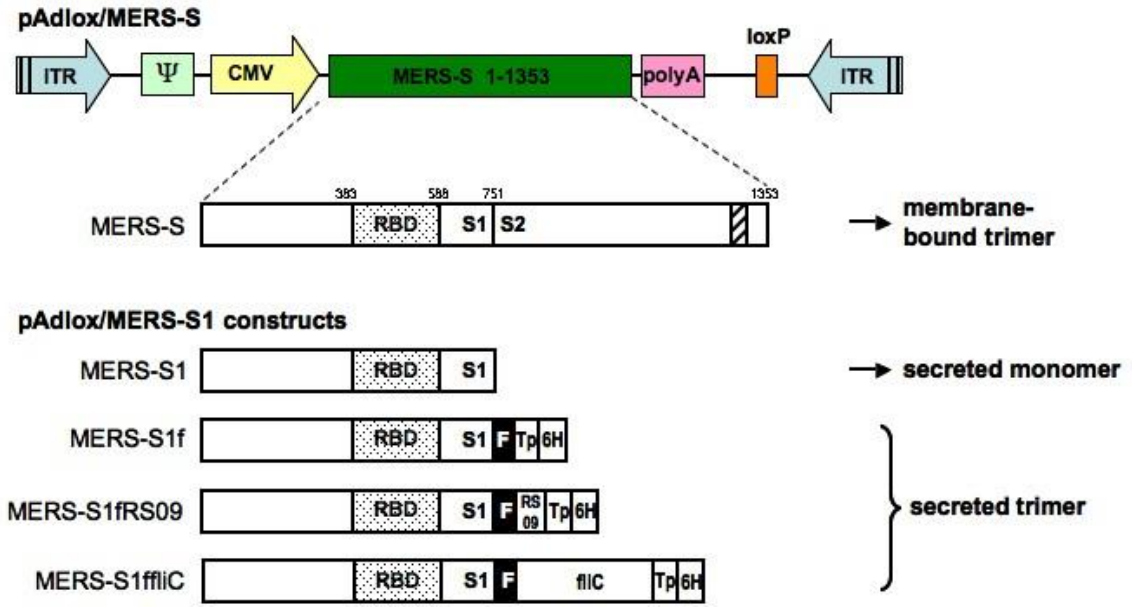
Vaccine
Volume 32, Issue 45, 14 October 2014, Pages 5975–5982

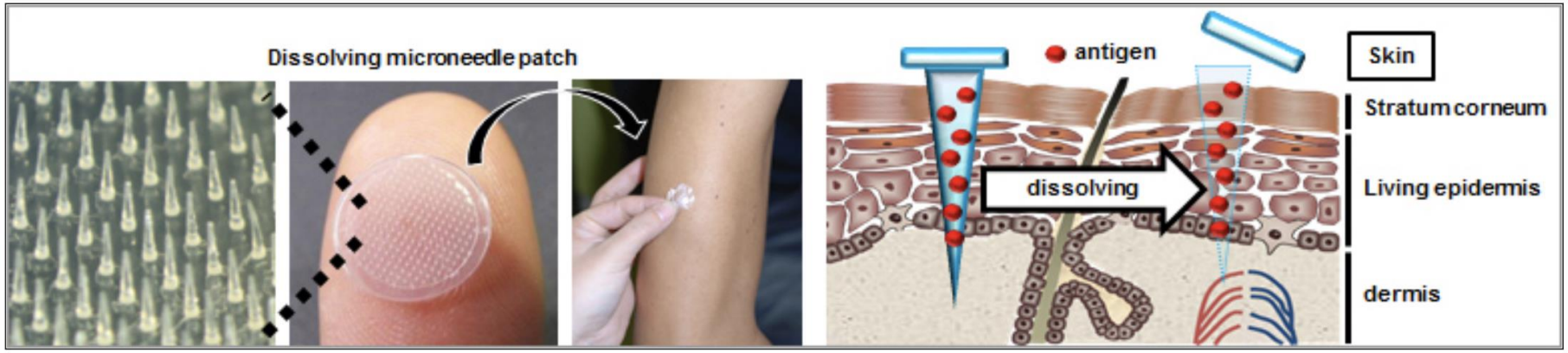
Immunogenicity of an adenoviral-based Middle East Respiratory Syndrome coronavirus vaccine in BALB/c mice

Eun Kim[a], Kaori Okada[a], Tom Kenniston[a], V. Stalin Raj[b], Mohd M. AlHajri[c], Elmoubasher A.B.A. Farag[c], Farhoud AlHajri[d], Albert D.M.E. Osterhaus[b], Bart L. Haagmans[b], Andrea Gambotto[a],

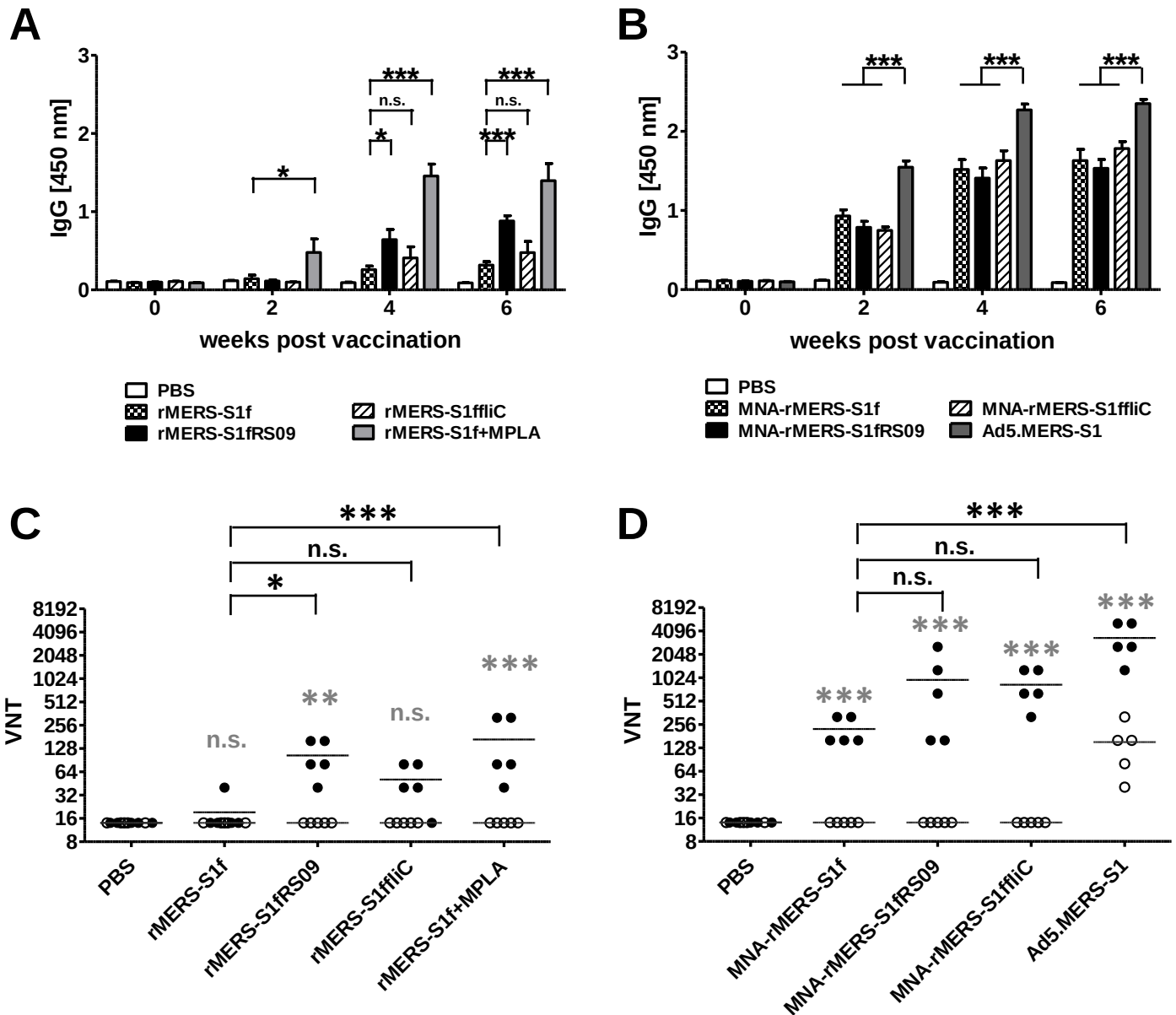


Carboxymethylcellulose-Based Microneedle Patches for Subunit Vaccine Delivery

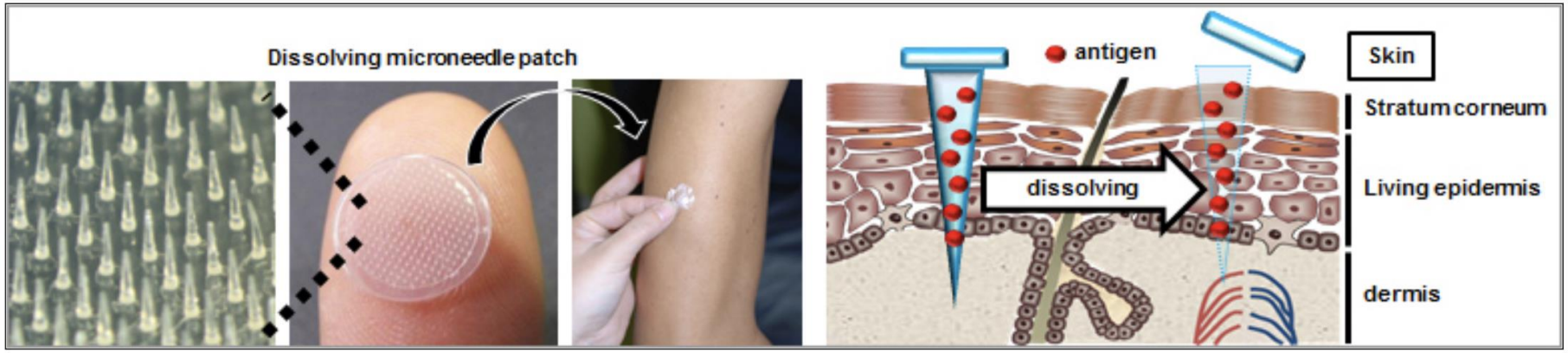


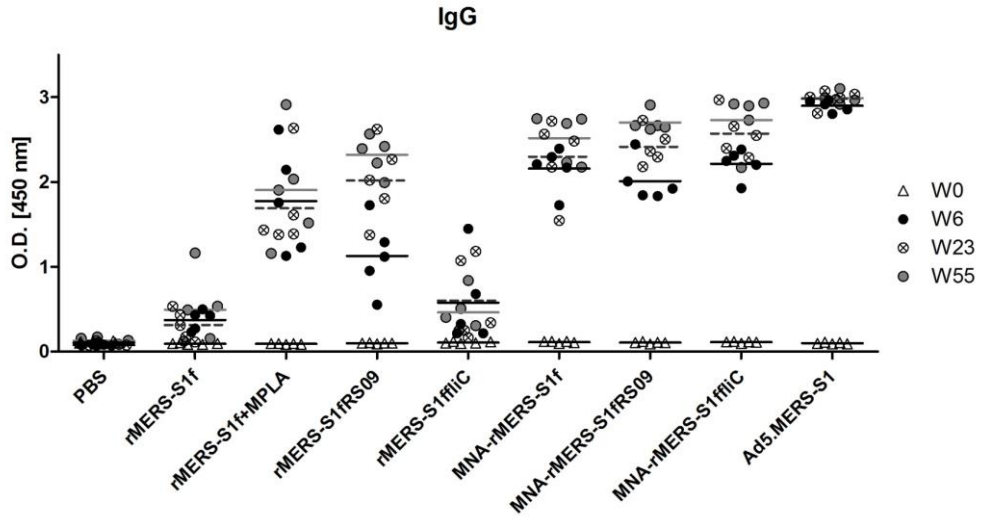


MERS-CoV Vaccine Development



Carboxymethylcellulose-Based Microneedle Patches for Subunit Vaccine Delivery





SARS-CoV-2 (COVID-19)

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

A Novel Coronavirus from Patients with Pneumonia in China, 2019

Na Zhu, Ph.D., Dingyu Zhang, M.D., Wenling Wang, Ph.D., Xingwang Li, M.D., Bo Yang, M.S., Jingdong Song, Ph.D., Xiang Zhao, Ph.D., Baoying Huang, Ph.D., Weifeng Shi, Ph.D., Roujian Lu, M.D., Peihua Niu, Ph.D., Faxian Zhan, Ph.D., Xuejun Ma, Ph.D., Dayan Wang, Ph.D., Wenbo Xu, M.D., Guizhen Wu, M.D., George F. Gao, D.Phil., and Wenjie Tan, M.D., Ph.D., for the China Novel Coronavirus Investigating and Research Team

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SARS-CoV-2 (COVID-19)

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journal homepage: www.elsevier.com/locate/ebiom

Research paper

Microneedle array delivered recombinant coronavirus vaccines: Immunogenicity and rapid translational development

Eun Kim[a], Geza Erdos[b], Shaohua Huang[a], Thomas W. Kenniston[a], Stephen C. Balmert[b], Cara Donahue Carey[b], V. Stalin Raj[e,1], Michael W. Epperly[c], William B. Klimstra[d], Bart L. Haagmans[e], Emrullah Korkmaz[b,f], Louis D. Falo Jr.[b,f,g,h,*], Andrea Gambotto[a,**]

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[e] Department of Viroscience, Erasmus Medical Center Rotterdam, Rotterdam, the Netherlands
[f] Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, Pittsburgh, PA 15231, USA
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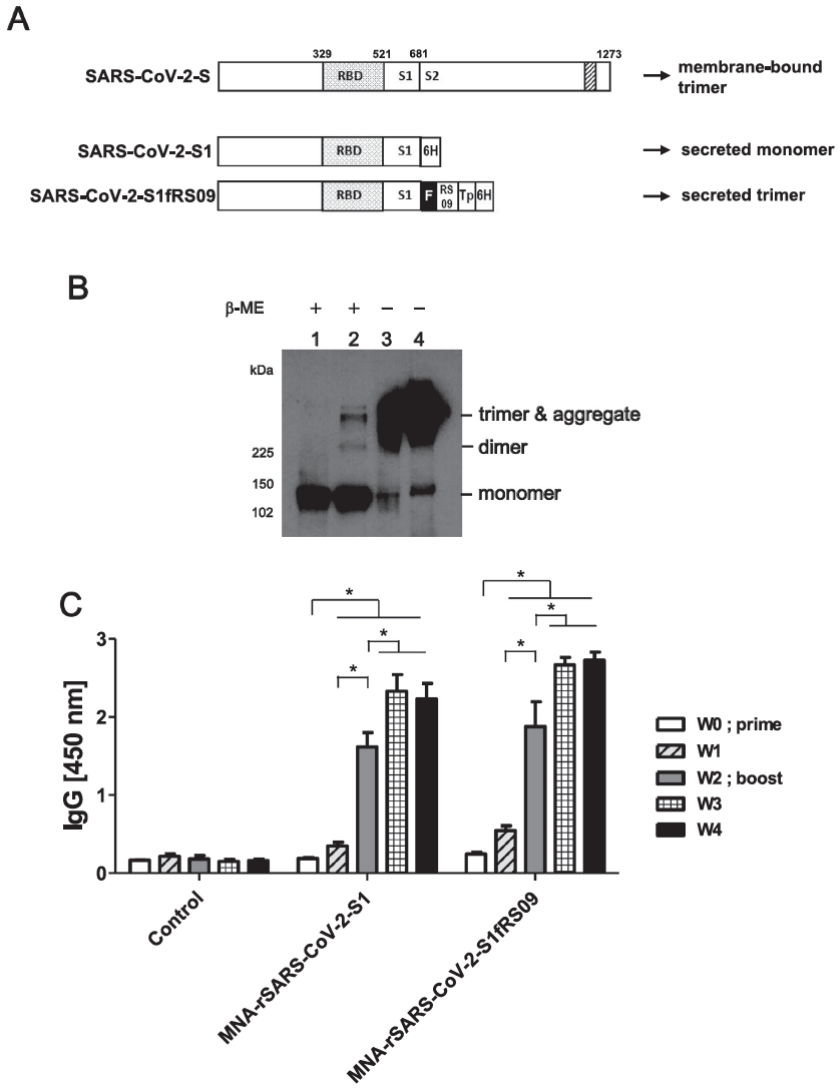
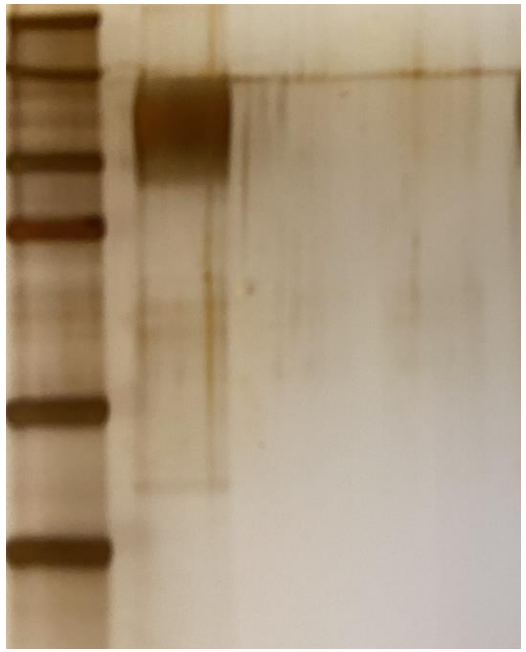
[h] The McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA

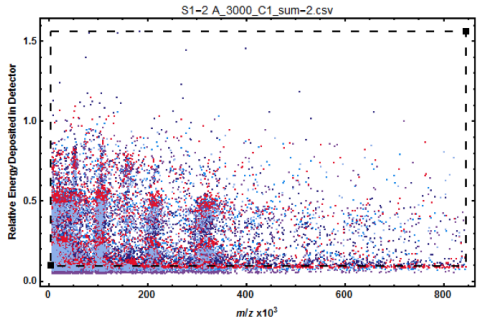
SARS-CoV-2 Vaccine Development



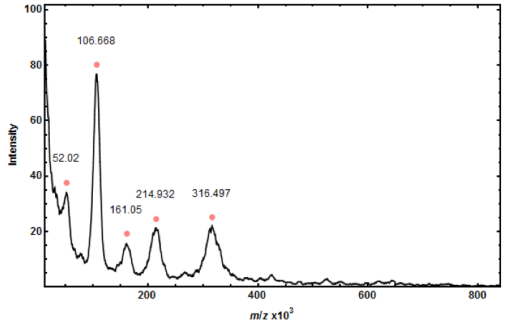
SARS-CoV-2 Vaccine Development

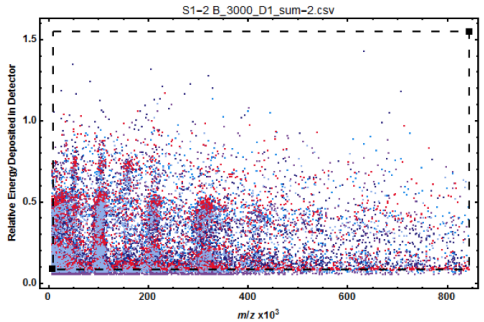
rSARS-CoV-2-S1 (*PittCoVacc*) S1 1-661 fRS09



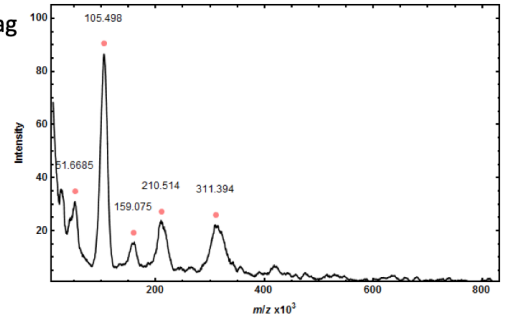


A with His-tag

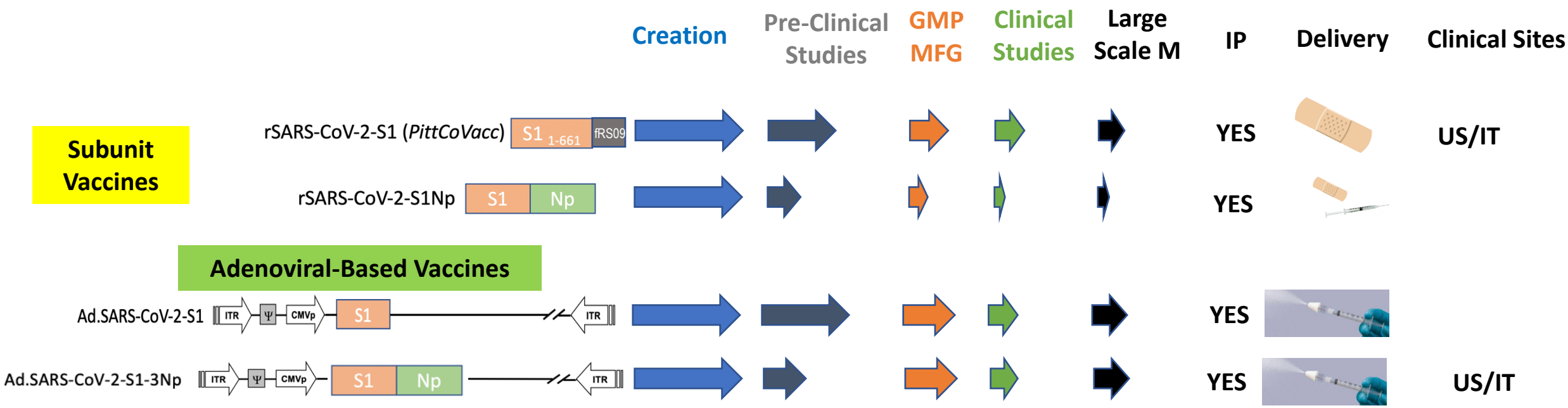




B without His-tag

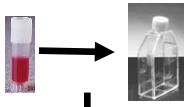


SARS-CoV-2 Vaccines Pipeline:



Manufacturing of MASTER VIRAL BANK

STEP 1 Thaw HEK293 Master Cell Bank into a T75 flask



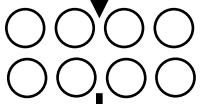
Split T75 Flask into 3 T150 Dish **STEP 2**

~ 4 days

STEP 3 Split 3 T150 dishes into 8 T150 dishes

~ 4 days

STEP 4 Seed 8 T150 dishes into 1 cell factory



~ 4 days

STEP 5 Split 1 cell factory into 3 cell factories

~ 4 days

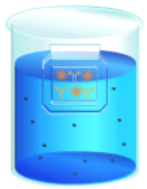
STEP 6
Change the media and inoculate Seed Virus Stock at 5 MOI in each cell factory

~ 3 days

~ 3 days

Virus is harvested and purified using two CsCl gradient ultracentrifugation **STEP 7**

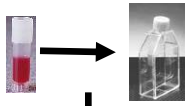
STEP 8
Virus is dialyzed against 5% threalose storage buffer.



STEP 9
Final virus product is aliquoted at 1x10^12 vp/ml in 500ul and aliquots are stored at -80°F.

Manufacturing of DRUG SUBSTANCE

STEP 1 Thaw HEK293 Master Cell Bank into a T75 flask



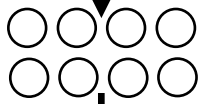
STEP 2 Split T75 Flask into 3 T150 Dish

~ 4 days

STEP 3 Split 3 T150 dishes into 8 T150 dishes

~ 4 days

STEP 4 Seed 8 T150 dishes into 1 cell factory



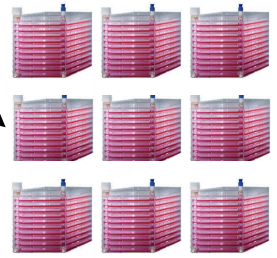
~ 4 days

STEP 5 Split 1 cell factory into 3 cell factories

~ 4 days

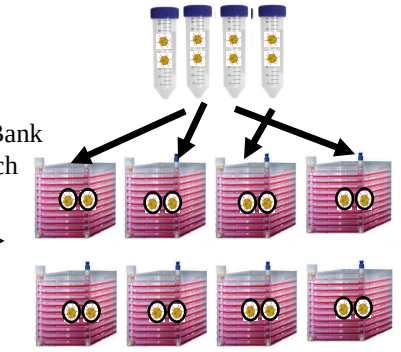
STEP 6 Split 3 cell factories into 9 cell factories

~ 4 days

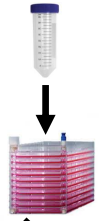


STEP 7 Change the media and inoculate Master Viral Bank at 5 MOI or Mock in each cell factory

~ 3 days



~ 3 days

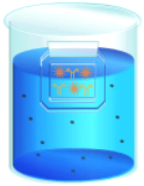


STEP 8 Virus or Mock is harvested and purified by one CsCl gradient ultracentrifugation

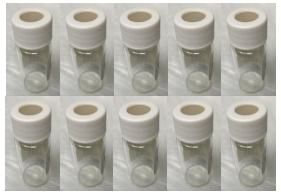
STEP 9 Purified Virus is treated with benzonase.

STEP 10 Benzonase treated Virus or Mock is harvested and purified by a second CsCl gradient ultracentrifugation

STEP 11 Virus is dialyzed against 5% threalose storage buffer.



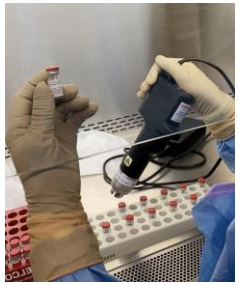
STEP 12 DRUG SUBSTANCE was aliquoted at 6.2x10^12 vp/ml in 19.2 ml aliquots are stored at -80°F.



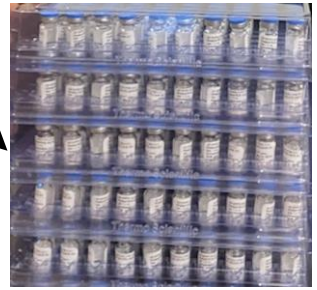
Manufacturing of DRUG PRODUCT

STEP 1 DRUG SUBSTANCE aliquots of 6.2x10^11 vp/ml are diluted to desired concentration to become DRUG PRODUCT

STEP 2 DRUG PRODUCT is aliquoted at 5x10^10 vp/ml in 1.2ml final volume

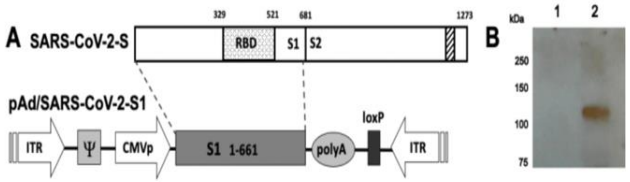


STEP 3 DRUG PRODUCT is vialed capped and labeled



STEP 3 DRUG PRODUCT is stored at -80°F until use.

Ad.SARS-CoV-2-S1 Clinical Trial



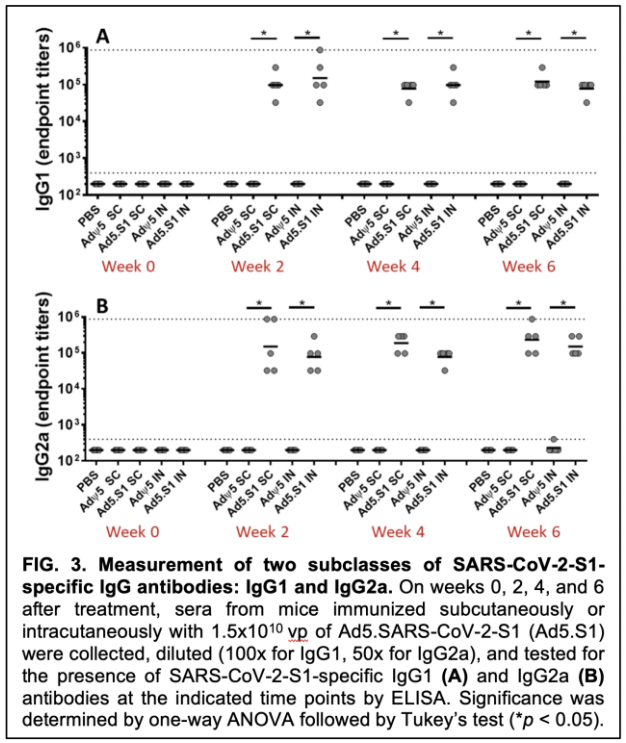
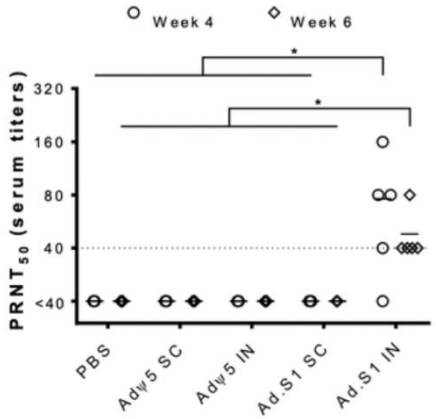
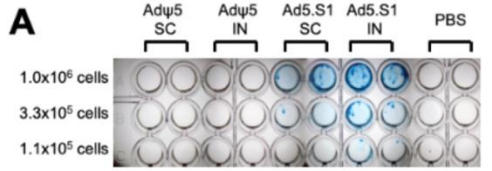
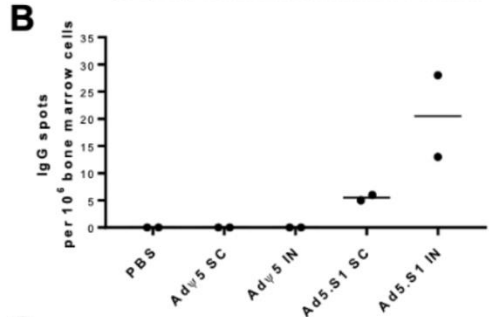
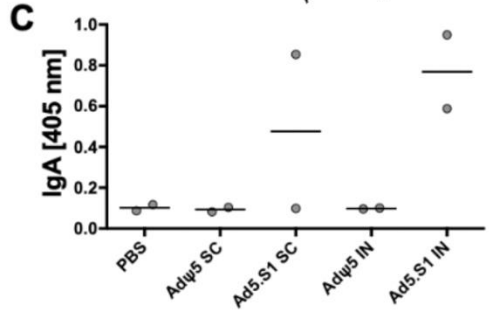


FIG. 3. Measurement of two subclasses of SARS-CoV-2-S1-specific IgG antibodies: IgG1 and IgG2a. On weeks 0, 2, 4, and 6 after treatment, sera from mice immunized subcutaneously or intracutaneously with 1.5x10[10] vp of Ad5.SARS-CoV-2-S1 (Ad5.S1) were collected, diluted (100x for IgG1, 50x for IgG2a), and tested for the presence of SARS-CoV-2-S1-specific IgG1 **(A)** and IgG2a **(B)** antibodies at the indicated time points by ELISA. Significance was determined by one-way ANOVA followed by Tukey's test (*$p < 0.05$).







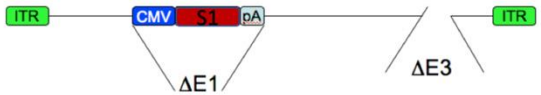


PROPOSTA STUDIO COVID-19

IDENTIFICAZIONE DELLA SPERIMENTAZIONE CLINICA
TITOLO STUDIO/STUDY TITLE: A Phase I/II Clinical Trial of Ad.SARS-CoV-2-S1 Vaccine in Healthy Volunteers for the prevention of COVID-19 disease.
SPONSOR/PROMOTORE: UPMC Italy
SPERIMENTATORE RESPONSABILE DELLO STUDIO/ STUDY RESPONSIBLE INVESTIGATOR: (richiedente) Nome e Cognome: Dr. Gennaro Daniele
CENTRO COORDINATORE/ COORDINATOR CENTER *(solo per studi multicentrici):* N.A.
CENTRI COINVOLTI NELLA SPERIMENTAZIONE/CENTERS INVOLVED IN THE STUDY: Fondazione Policlinico Universitario Agostino Gemelli IRCCS – Roma Istituto Mediterraneo per i Trapianti e Terapie ad Alta Specializzazione ISMETT IRCCS - Palermo

FARMACO/I O INTERVENTO TERAPEUTICO / DRUG/S OR THERAPEUTIC INTERVENTION:
breve descrizione del razionale, delle caratteristiche del/i farmaco/i e del meccanismo d'azione/funzionamento. Fornire dettagli sulla fornitura del farmaco.

The study product is a recombinant adenoviral vector encoding the spike 1 glycoprotein from SARS-CoV-2. Below is the schematic diagram of the Ad.SARS-CoV-2-S1 vaccine:

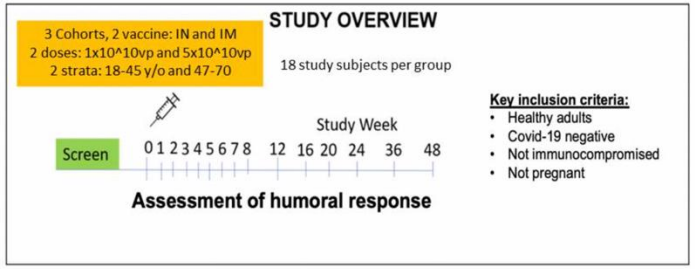


The SARS-CoV-2-S1 from BetaCoV/Wuhan/IPBCAMS-WH-05/2020 (GISAID accession id. EPI_ISL_403928 amino acids 1 to 661) gene was codon-optimized for optimal expression in mammalian cells by the UpGene codon optimization algorithm and synthesized by gene synthesis. We will evaluate Ad.SARS-CoV-2-S1 a liquid formulation supplied as single-use doses 1x10^10vp and 5x10^10vp for intranasal or intramuscular administration,. The intranasal delivery will be carried out using mucosal atomization device (MAD Nasal, Teleflex Medical, CE). Our considerable preliminary data supports intranasal adenoviral based vaccines delivery as an effective route of immunization.

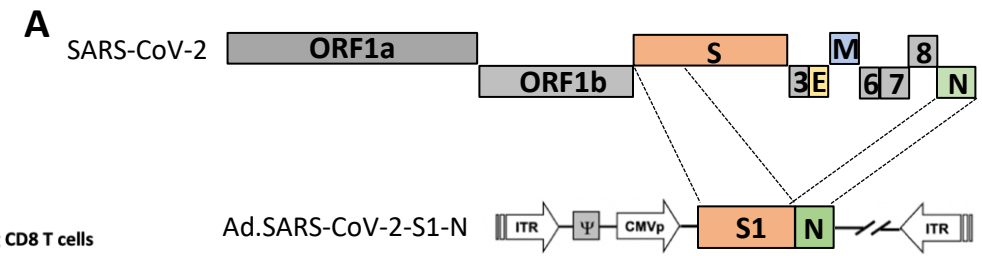
TIPO DI STUDIO /STUDY TYPE
Indicare se lo studio è osservazionale (prospettico o retrospettivo) o sperimentale. Nel caso si tratti di uno studio sperimentale indicarne brevemente le caratteristiche (se randomizzato, se in cieco ecc.), la fase (1-2-3-4), si dovrà poi indicare se si tratta di uno studio di superiorità, di equivalenza o di non inferiorità.

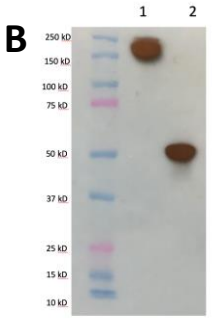
This study is a first in human, open label, phase I/II protocol that will evaluate the safety and immunogenicity of the Ad.SARS-CoV-2-S1 preventative SARS-CoV-2 vaccine.

On December 31, 2019, Chinese authorities reported a cluster of pneumonia cases in Wuhan, China, most of which included patients who reported exposure to a large seafood market selling many species of live animals. Emergence of another pathogenic zoonotic HCoV was suspected, and by January 10, 2020,

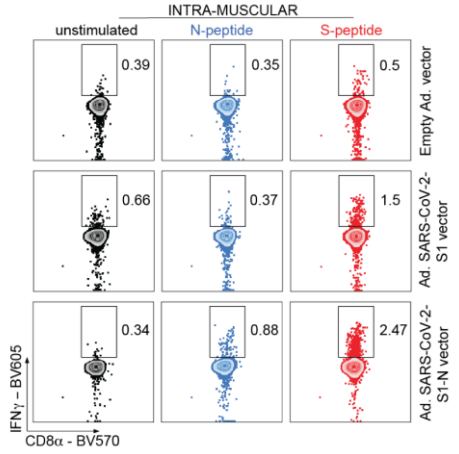


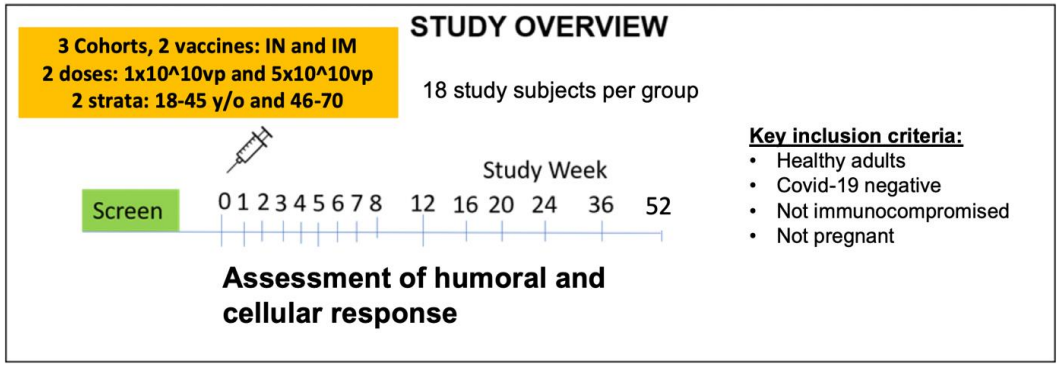
Ad.SARS-CoV-2-S1-N Clinical Trial





Frequency, Abs number of IFNg+ producing CD8 T cells and representative flow analysis





TITLE:
A Phase 1/2 Clinical Trial of Ad.SARS-CoV-2-S1-N Vaccine in Healthy Volunteers for the prevention of COVID-19 disease

PROTOCOL NUMBER: Ad.SARS.CoV-2-S1-N-AG0014

PHASE OF DEVELOPMENT: 1/2

STUDY CENTER: University of Pittsburgh School of Medicine

OBJECTIVES:

Phase 1

Safety: To evaluate the safety and tolerability of Ad.SARS-CoV-2-S1-N when administered intranasally (IN) or intramuscularly (IM) at 2 dose levels at Weeks 0 in healthy adults age 18-70.

Primary Immunogenicity: To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at Week 8 as measured by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.

Secondary Immunogenicity:

To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at Week 12 as measured by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.

To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at time points other than Week 8 and 12 by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.

Phase 2

Safety: To evaluate the safety and tolerability of Ad.SARS-CoV-2-S1-N as compared to placebo when administered at (TBD) dose levels at Weeks 0 in healthy adults age 18-70.

Primary Immunogenicity: To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at Week 8 as measured by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.

Secondary Immunogenicity:

To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at Week 12 as measured by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.

To evaluate the immunogenicity of Ad.SARS-CoV-2-S1-N at time points other than Week 8 and 12 by SARS-CoV-2 (spike) protein binding and neutralizing antibodies and SARS-CoV-2 (spike1 and nucleoprotein) CD8+ and CD4+ specific response.