To realize the unknowns about COVID-19 vaccines

- Infection mechanisms of SARS-CoV-2
- New nucleotide-based vaccines: characteristics
- Efficacy and adverse events

JICA Web seminar
2021.8.04
Kitasato University
Tetsuo Nakayama

Self introduction

Tetsuo Nakayama MD (Birthday: Nov. 7, 1950)
Project Professor, Laboratory of Viral Infection I, Kitasato Institute for Life Sciences

【Profile】

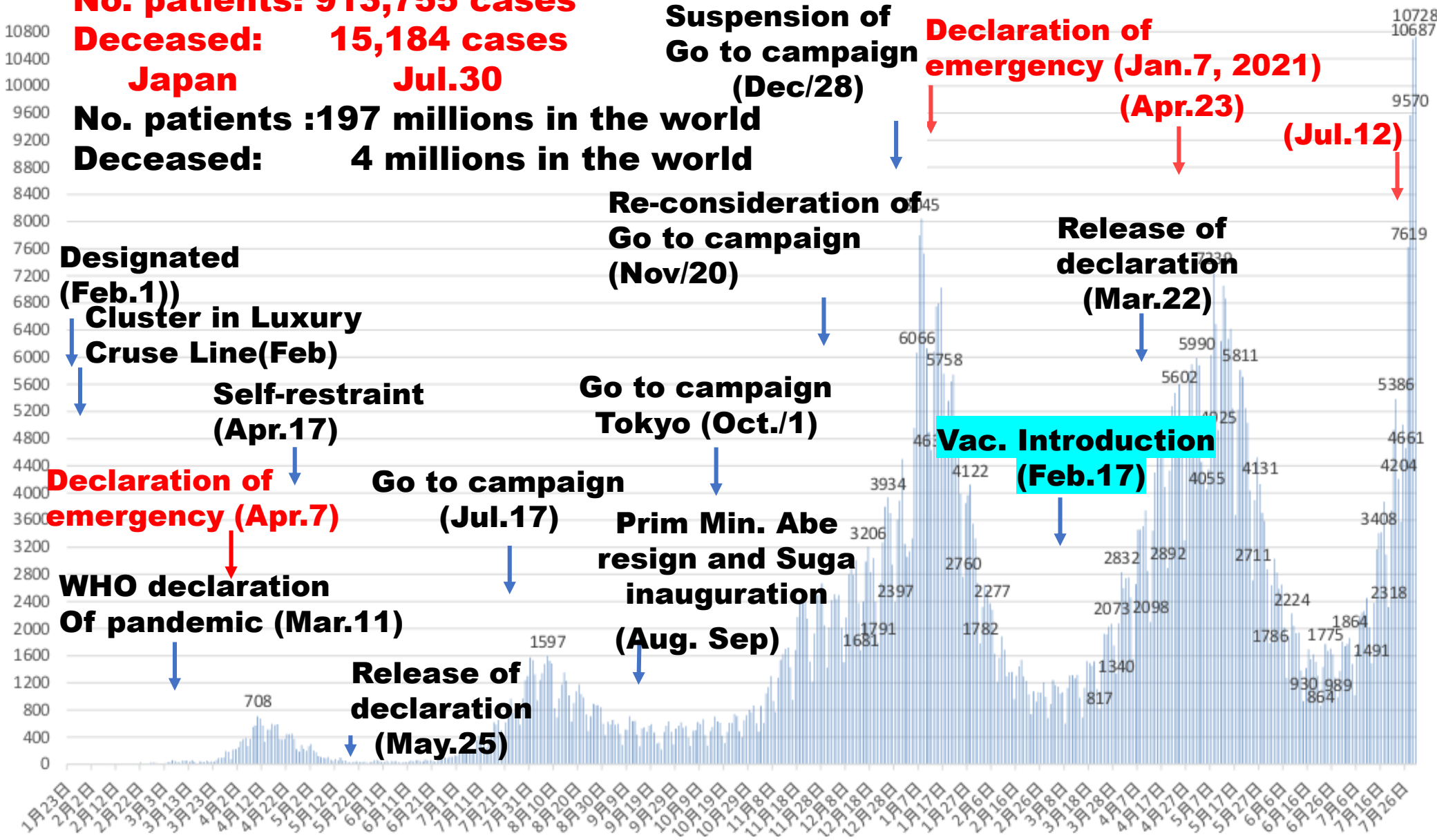
I graduated from School of Medicine, Keio University in 1976.
Thereafter took the clinical pediatric training course in Department of Pediatrics, Keio University for two years.
I worked as a general pediatrician in Saiseikai-Central Hospital, Tokyo, until 1992.
I moved to Department of Virology, Kitasato Institute for studying virology and vaccinology in 1992.
I worked as Director of Kitasato Institute for Life Science from 2009 to 2015 and retired in 2015.
Now, I am working as Project Professor.
My primary scientific interests are pediatric infectious diseases and vaccine immunology, and recently works on RSV, measles, mumps, and SARS-CoV-2 viruses.

Epidemiology of COVID-19 and control strategy

2021. Jul.30

No. patients: 913,755 cases
Deceased: 15,184 cases
Japan Jul.30

No. patients :197 millions in the world
Deceased: 4 millions in the world

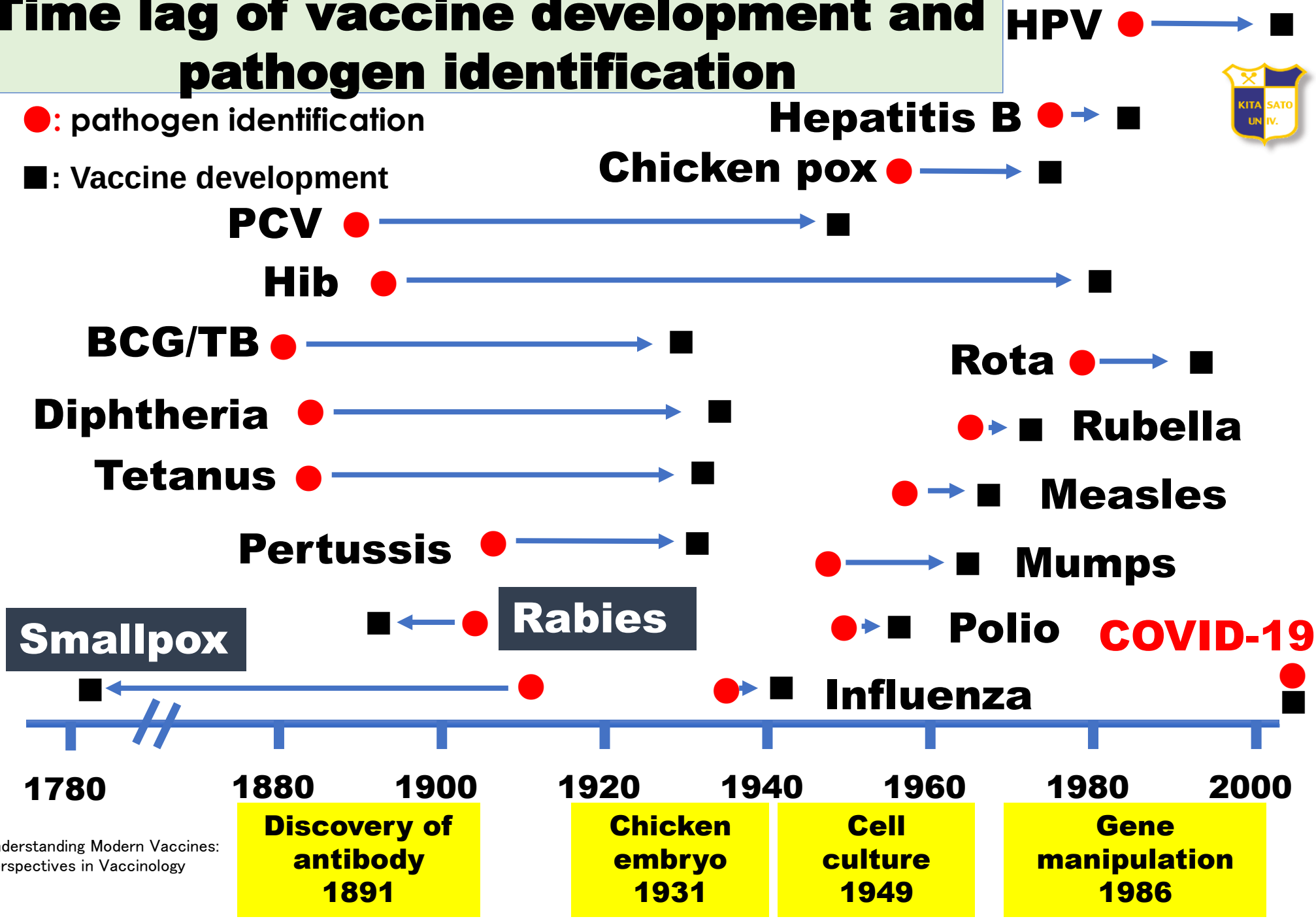


https://www.mhlw.go.jp/stf/covid-19/kokunainohasseijoukyou.html

Time lag of vaccine development and pathogen identification

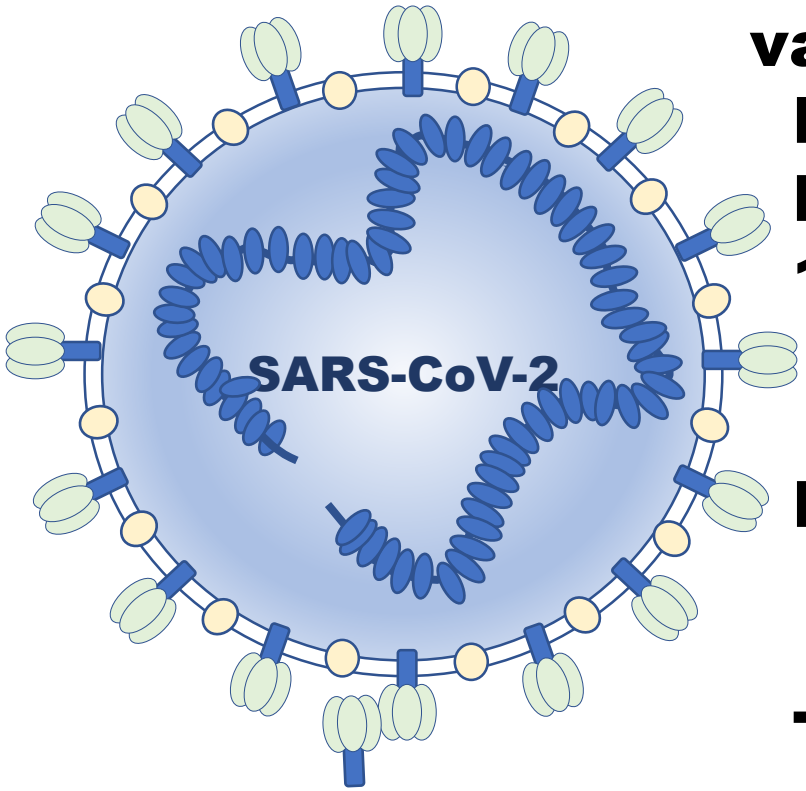
●: pathogen identification

■: Vaccine development



Understanding Modern Vaccines: Perspectives in Vaccinology

SARS-CoV-2 ?



Corona viruses were detected in various animals.

BC 8000 : origin of CoV

BC 3000 : α、β、γ、δ CoV

1964: identification of human CoV as pathogen of common cold

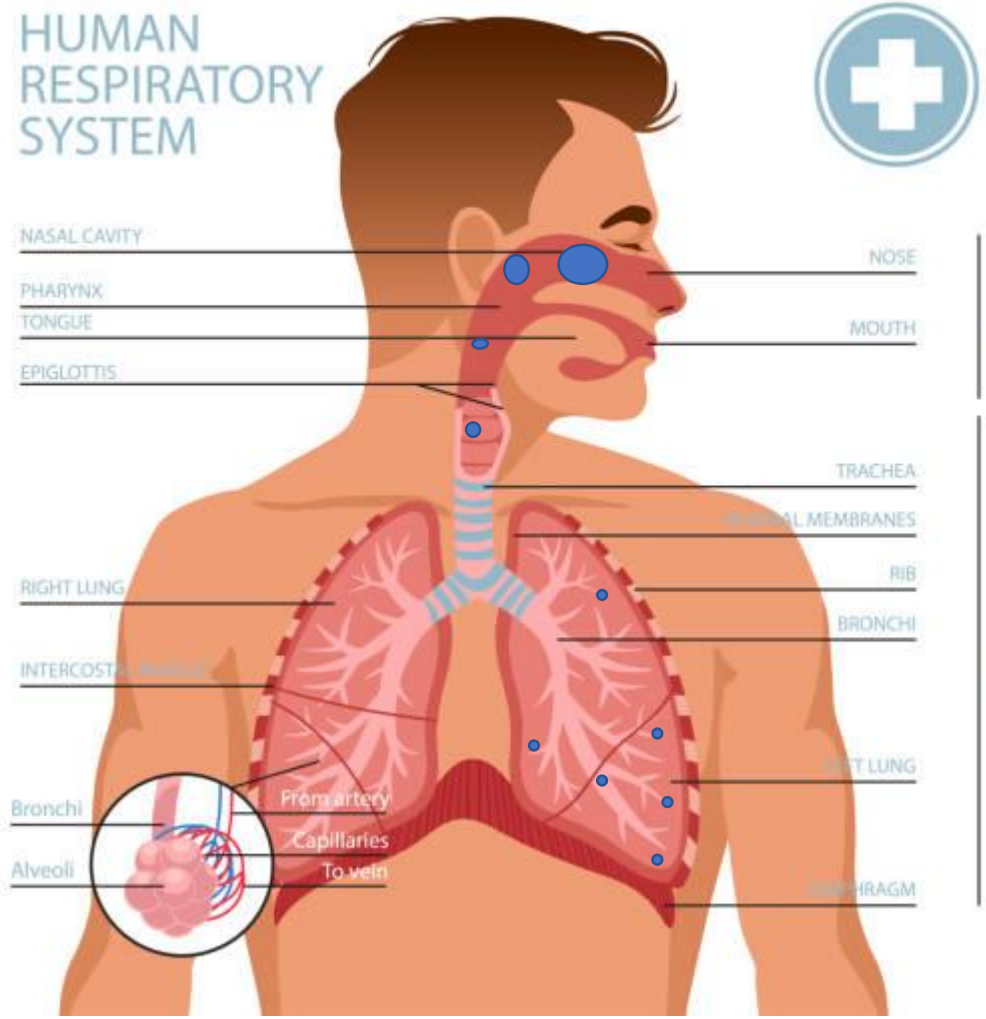
Feline infectious peritonitis

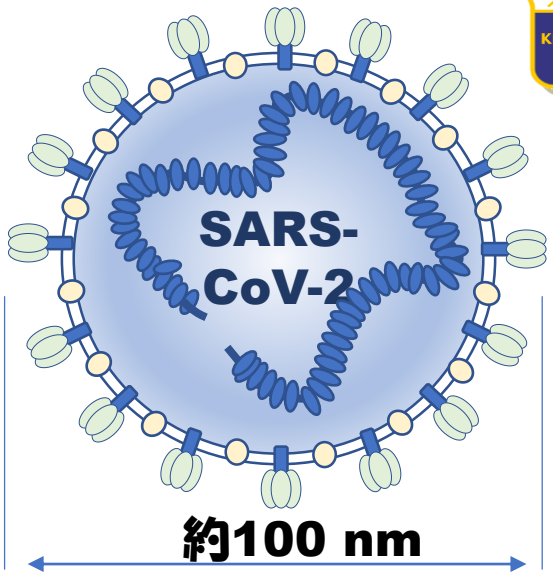
First place of cause of death for kitten（ADE)

Transmissible gastroenteritis of swine diarrhea

Spike antigens
look like a crown or sun corona.

COVID-19; airborne infection
aerosol infection





<5 μm

1 m
1 mm 1/1000 m
1 μm 1/1000 mm
1 nm 1/1000 μm

Vaccine induces IgG antibodies in blood stream not in respiratory tracts. Recently, it was reported that IgA antibodies were detected in respiratory tracts after vaccination with mRNA vaccine.

Platform for vaccine development

＊Live vaccines

1）Conventional vaccine; measles, rubella, mumps vaccines propagated through animal cells, establishing *ts* phenotype

2）Usage of animal pathogens other than human pathogen; BCG, Rota virus

3）Viral vectored vaccine

3-1) non-replicating vector（single round infectious vector）
Sendai virus、adenovirus、Lentivirus

3-2) replicating viral vector
conventional viral vector: vaccinia virus (MVA, LC16m8)、measles vaccine, parainfluenza virus

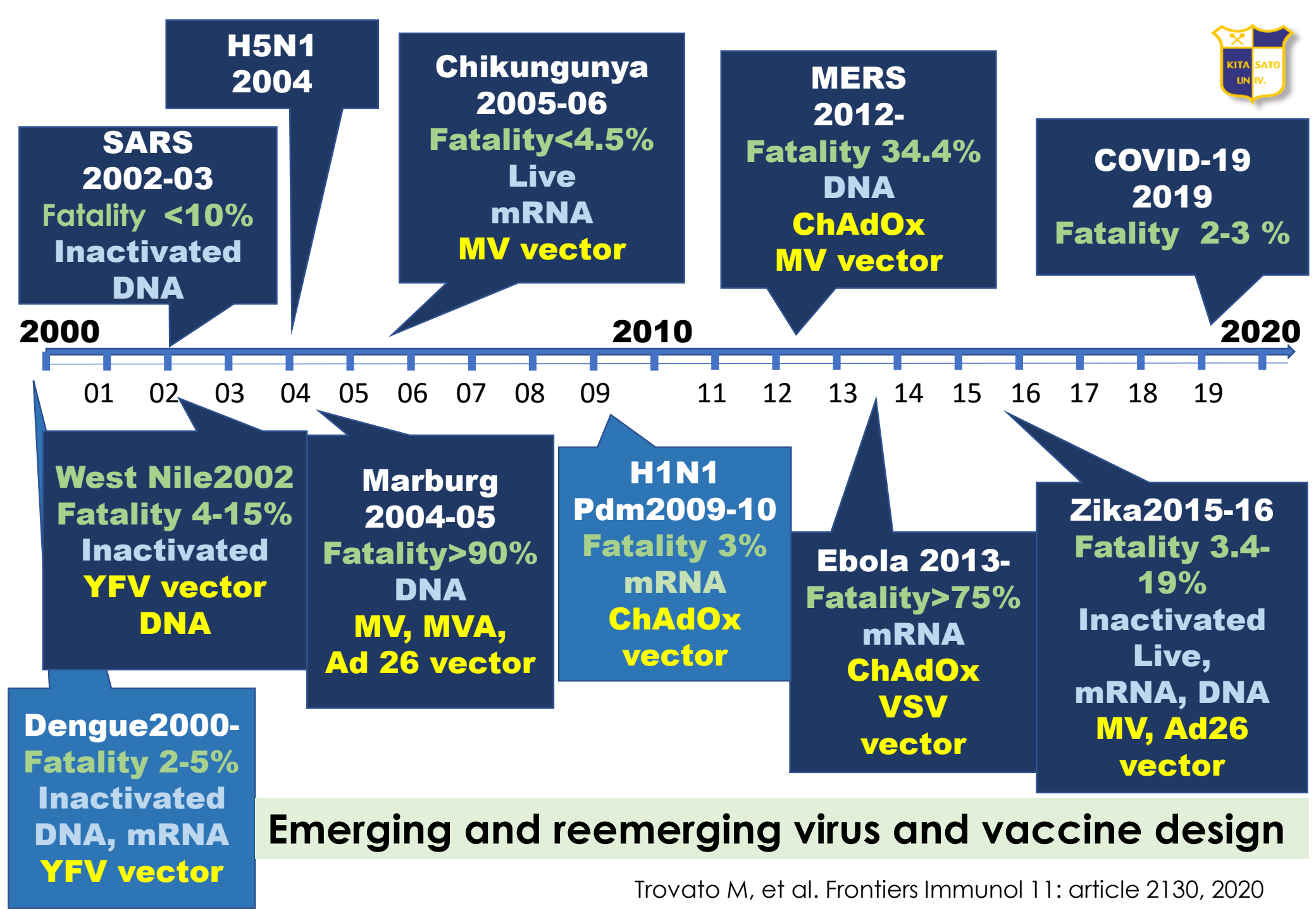
＊Inactivated vaccines: with or without adjuvant

1) whole virus inactivated vaccine: JEV, Hepatitis A

2) subunits: influenza

3) VLP, purified protein: Hepatitis B, HPV

4）new platform: DNA, mRNA vaccine (Lipid nano-particle)



Emerging and reemerging virus and vaccine design

Trovato M, et al. Frontiers Immunol 11: article 2130, 2020

Characteristics of vaccine platforms

	Live attenuated	Whole virus Inactivated	Purified protein	Genome-based
Merits	Long history of clinical usage			
	Cell-mediated immunity High antibody			Cell-mediated immunity High antibody
	Long duration of immunity	High antibody	High antibody response with adjuvants	Rapid development and production
Demerits	Adverse events	Short duration of immunity	Short duration of immunity	No experience of large-scale clinical usage
	Long time to develop	Multiple dosages	Multiple dosages	

Characteristics of gene-based vaccines

	mRNA	Virus vector	DNA
Characteristics	Rapid development Disappear within several days. Not enter into nucleus	Rapid development Once vector system is established. Large scale production	Rapid development Large scale production
Demerits	Fragile Thermostability Lower production scale	Integration into genome (lentivirus). Pre-exist antibody reduces immune response	Device to deliver DNA into cell nucleus
	Pfizer	AstraZeneca	Inovio
	Moderna	ChanSino	
		Janssen	
		Gamaleya	

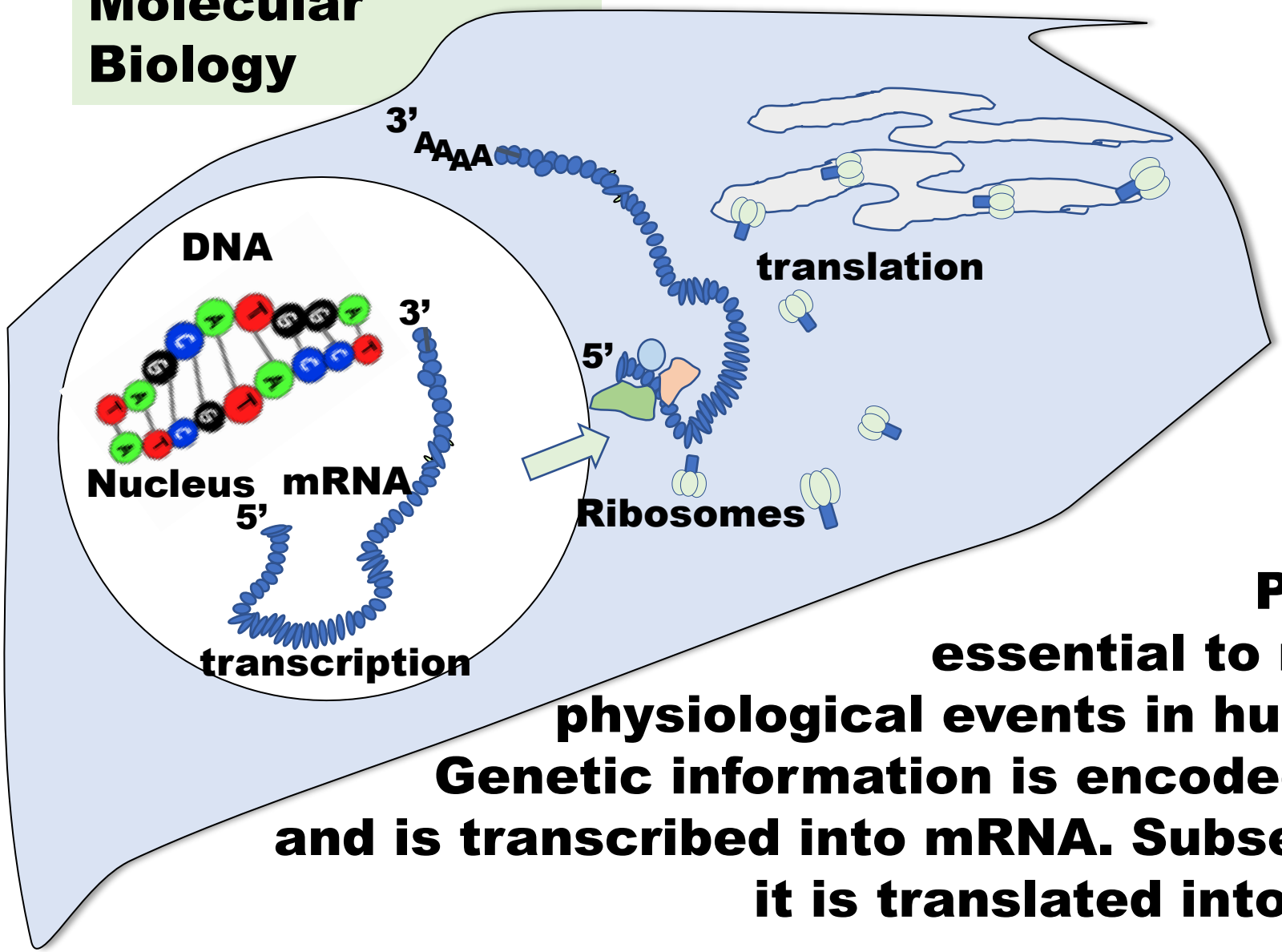
Domestic COVID-19 Vaccines in Japan Under investigation

Daiichi-Sankyo

Anges

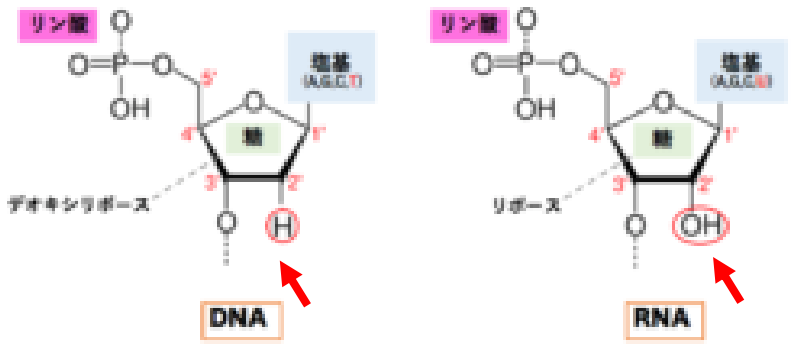
KM Biologics (Whole virion inactivated), Shionogi (Purified protein)

Central dogma of Molecular Biology



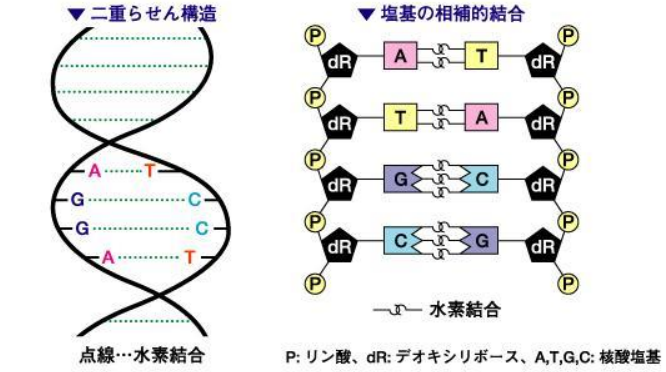
Protein is essential to maintain physiological events in human life. Genetic information is encoded in DNA and is transcribed into mRNA. Subsequently, it is translated into protein.

Viral genome
RNA virus: has ribonucleic acid（A, C, G, U)
DNA virus: has deoxy ribonucleic acid（A, C, G, T)



Hydrogen-bond: A-T, G-C nucleic acid bonds
Phosphate bond: elongation of nucleic acids

図2　DNAの二重らせん構造
DNA塩基の相補的結合–A（アデニン）とT（チミン）、C（シトシン）とG（グアニン）間でのみ起こり、一方の塩基配列が決まれば、相手の塩基配列が決まる。



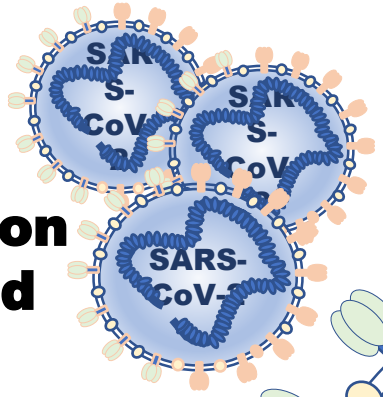
Triplet of A, C, G, T determines 20 essential amino acids and protein is synthesized along with the extension of amino acids.
ATG: start codon (initiation signal)
TAG, TAA: stop codon (termination signal)

DNA ⇒ Messenger RNA (mRNA) ⇒ Protein synthesis

Modified from Textbook of Biocgemistry

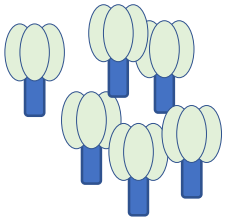
Schematic image of COVID-19 vaccines

Whole virion inactivated



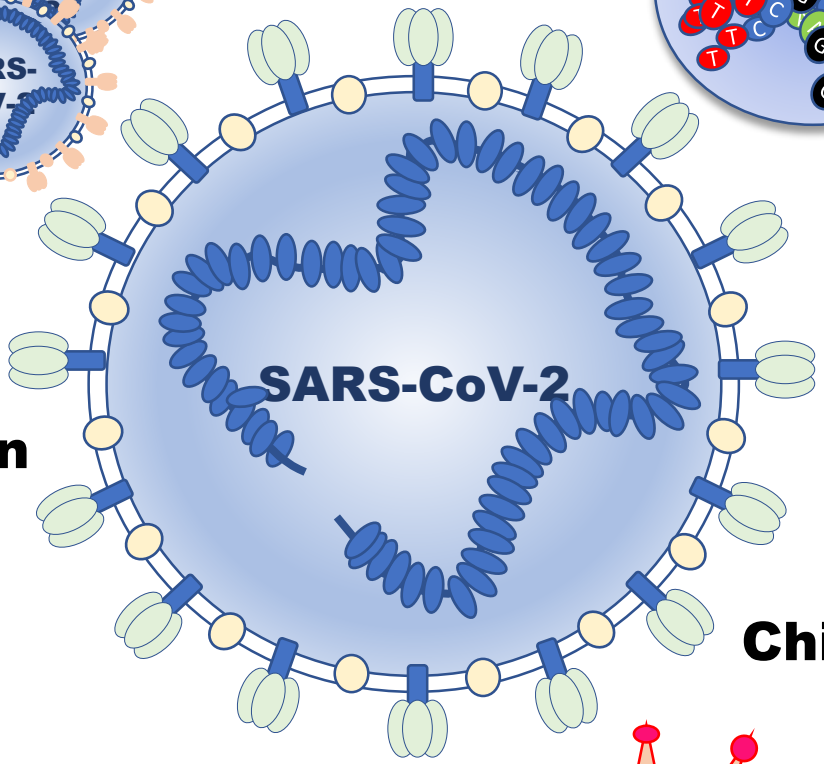
SinoVac (China)
73% efficacy

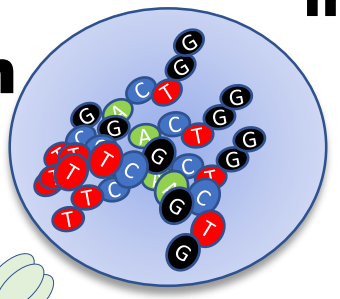
Purified Protein +Matrix M



Novavax (US)
89.3% efficacy

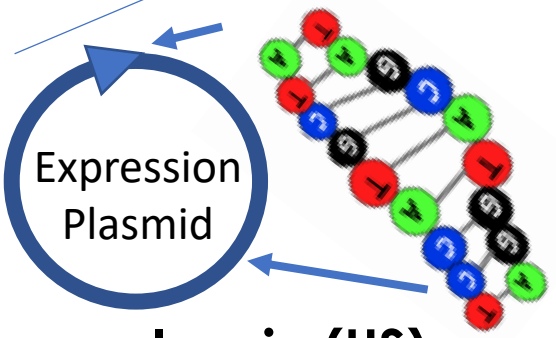
Spike protein



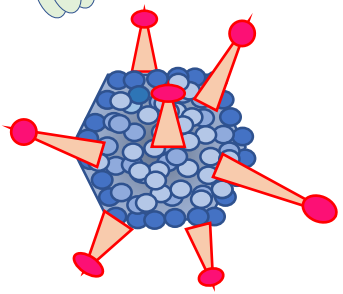


mRNA-LNP
Pfizer (US)
Moderna (US)
95% efficacy

DNA



Inovio (US)

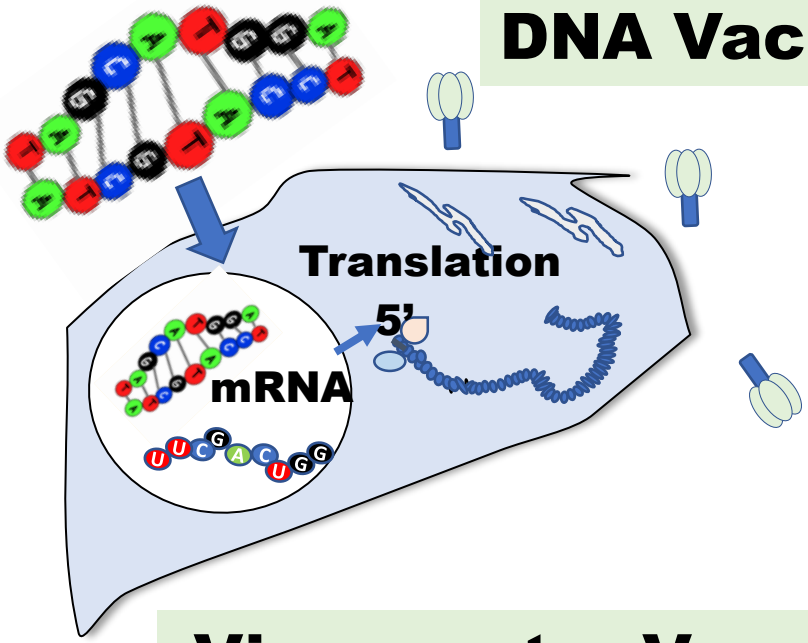


Chimpanzee Adenovirus Vector
AstraZeneca (Engl)
Janssen (US)
CanSino (China)
Gamaleya (Russia)
62 – 91% efficacy

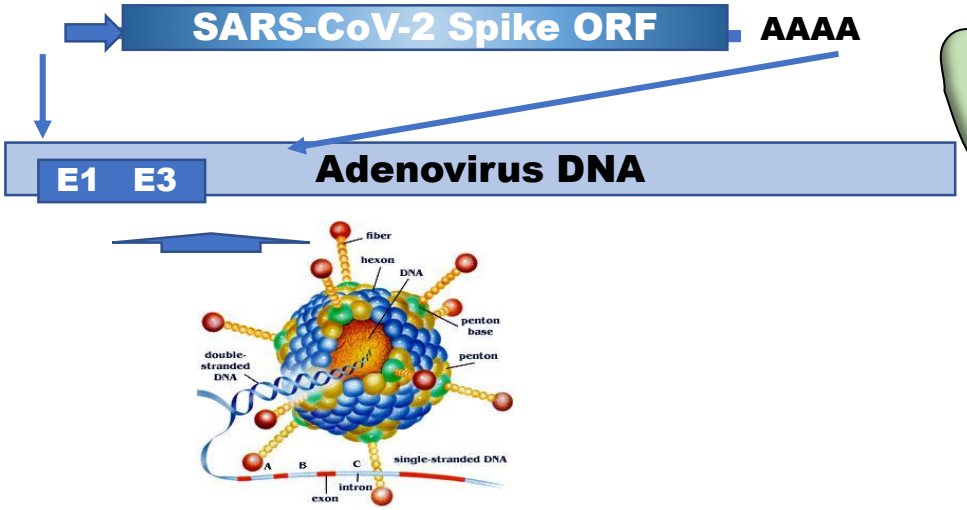
Red letters: vaccine efficacy

Immune response by new vaccines

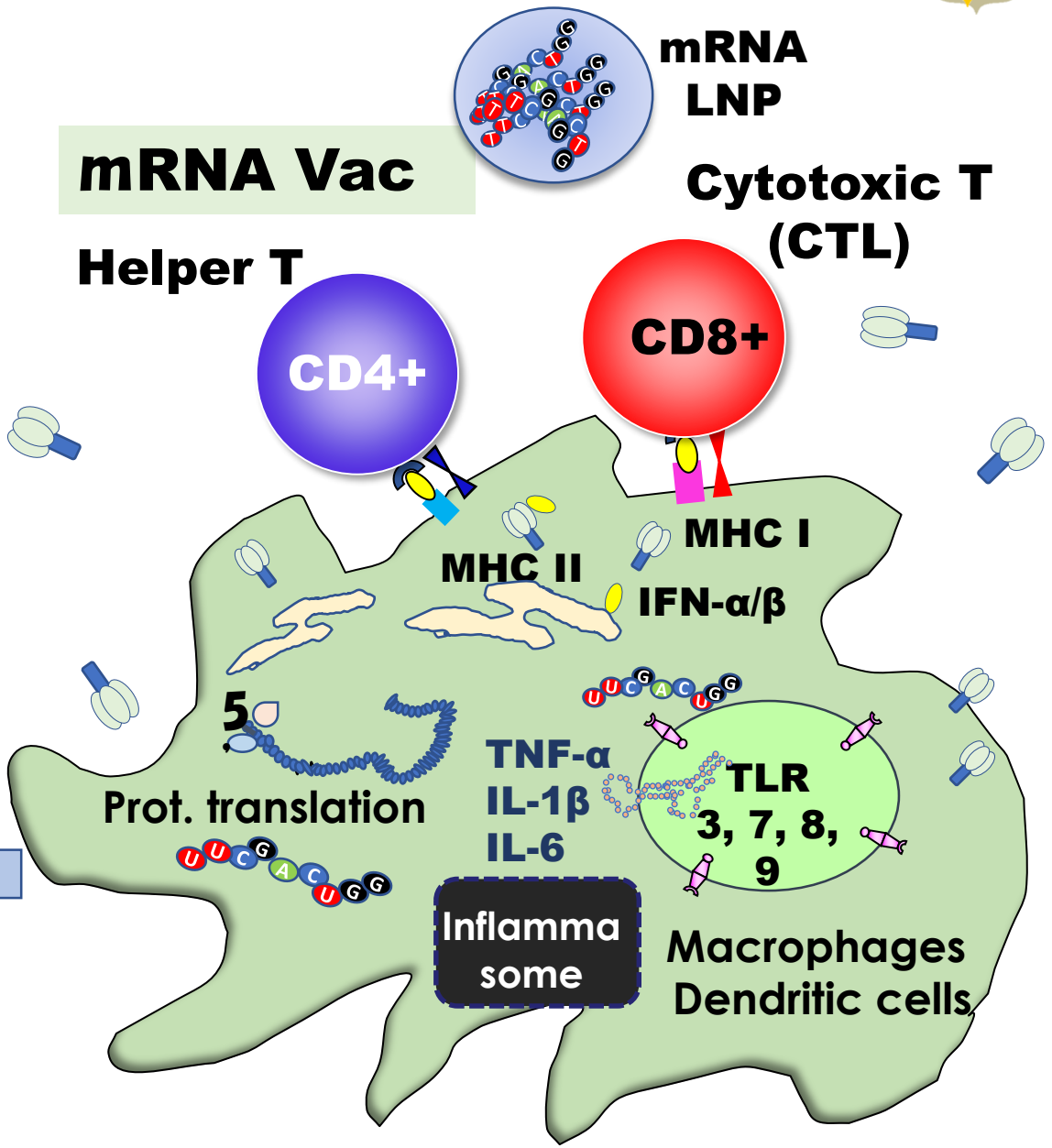
DNA Vac.



Virus vector Vac.



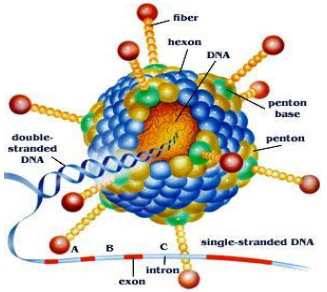
mRNA Vac



What is the difference among three vaccines?

DNA vaccine

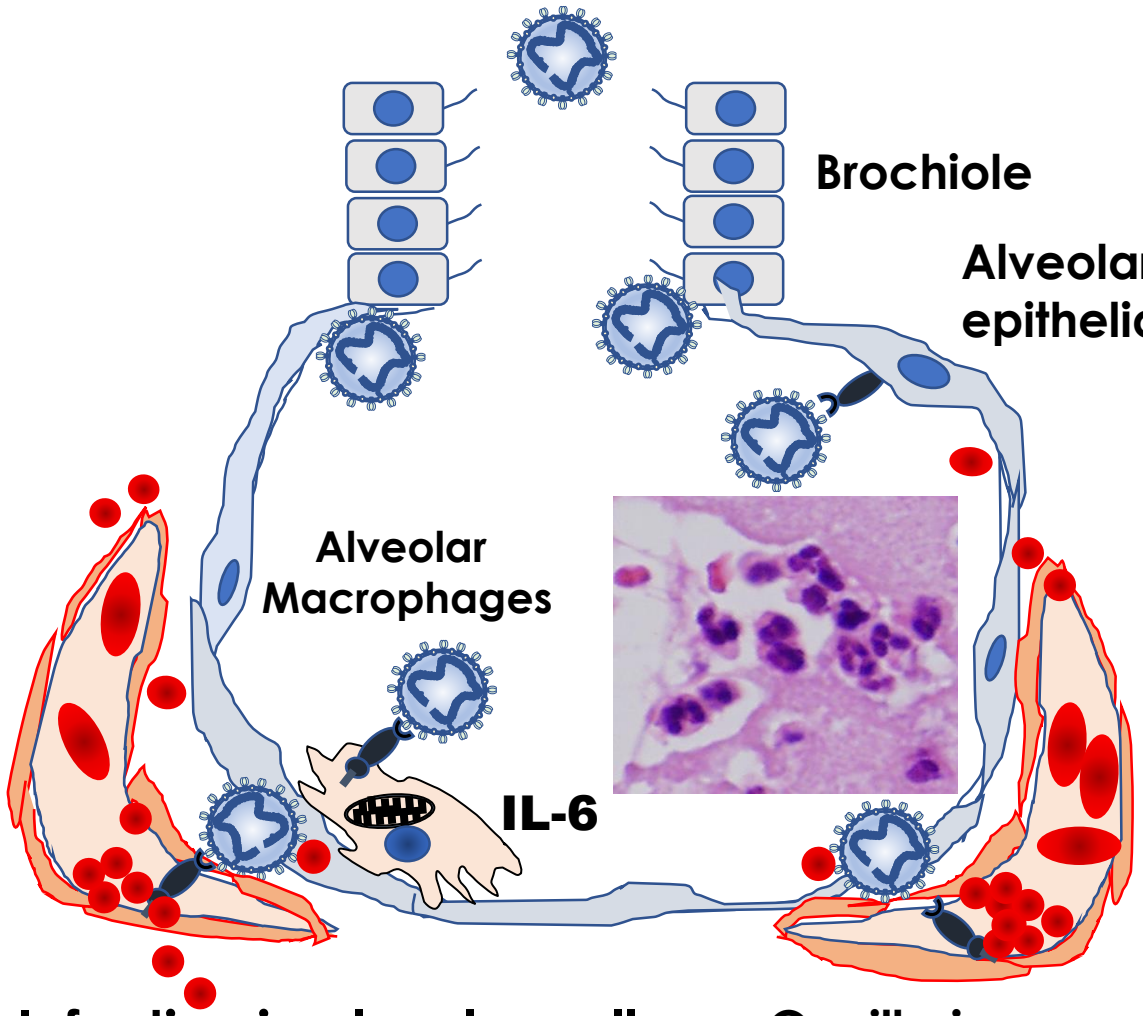
mRNA vaccine



mRNA:
Translation of protein
Activation of innate immunity

Adenoviral vector vaccine

Pathogenesis of serious pneumonia



SARS-CoV-2

ACE2

RBCs

Platelet

Infection in alveolar cells

Destruction of alveolar structure

Respiratory distress

Infection in alveolar macrophages

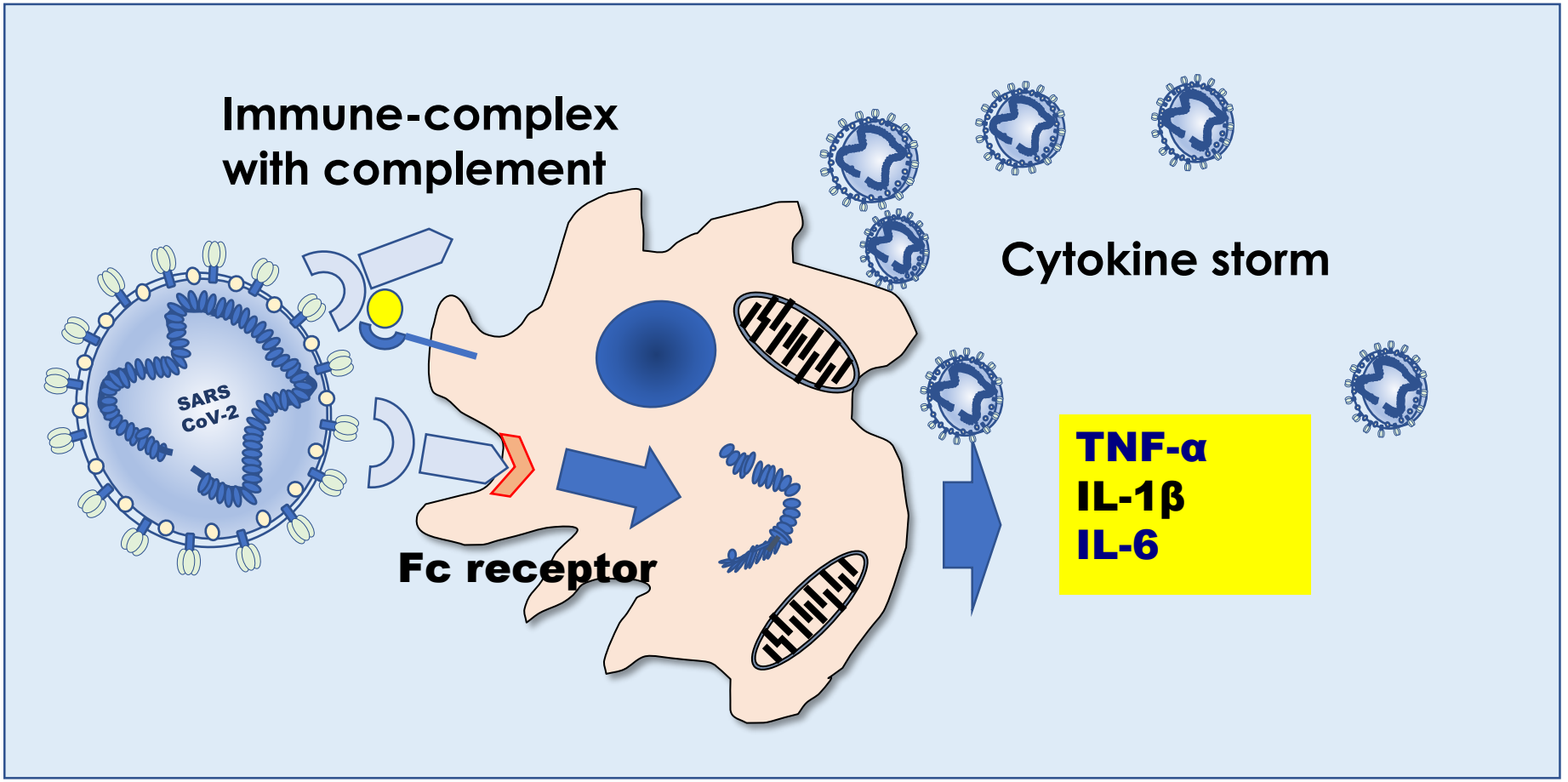
Production of inflammatory cytokines (IL-6, TNF-α, IL-1β)

Cytokine storm

Virus particle binds ACE2 expressed on epithelium

Thrombosis

Antibody dependent enhancement (ADE)



ADE : Binding antibodies without neutralizing activity binds with virus particle, internalizes into cells, and enhances virus growth, inducing inflammatory cytokines

Brief summary of laboratory examinations

Detection of antibodies:

EIA antibody: binding antibody against SARS-Co-V2

(Only binding, not reflecting protective neutralizing activity)

S, RBD antibodies: post infection and vaccination、

N antibody: post infection

Neutralizing antibody: protective antibody,

but protective level is not known.

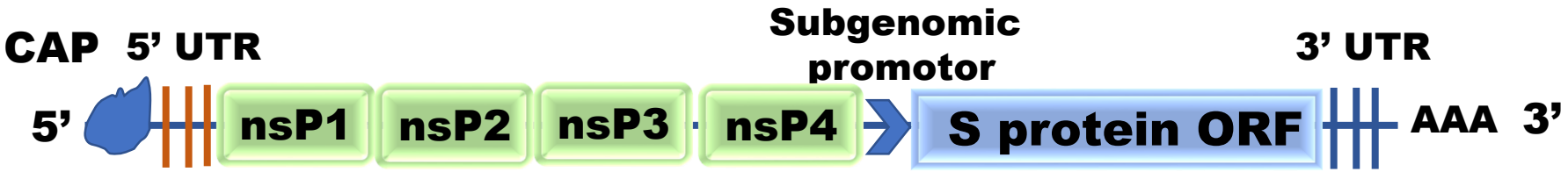
Detection of antigen:

becomes positive when >10 $^{3\text{-}4}$ virus (infectious level).

Negative does not mean free of virus particles.

PCR:

becomes positive when 10-100 copies are present, even dead virus debris.



Nano Lett. 2017 March 08; 17(3): 1326–1335.
doi:10.1021/acs.nanolett.6b03329.

30 years basic research is packed in mRNA vaccine

Optimization of mRNA vaccine

1. **Synthesis of CAP : transcription start**
2. **5', 3' UTR: stable mRNA**
3. **Poly A tail: stable mRNA**
4. **Codon optimi… translation**
5. **Purifi… RNA : contamination of … increases IFN production.**
6. **Modified uridine : reduction of TLR 7, 8 signal⇒reduced IFN-α/β ⇒safety**
7. **S protein mutation : AA mutation proline at 986, 987 positions : stable prefusion structure**
8. **Self-amplifying mRNA : increased protein translation**
9. **Ionizable cationic lipid : bind with mRNA**

mRNA basic research

1961 Discovery of mRNA
63 mRNA induces IFN
69 Protein expression of mRNA
1975 Discovery of mRNA CAP
78 Liposome-entrapped RNA
1983 Cap analogue commercialized
84 RNA polymerase commercialized
85 T7 RNA Polymerase commercialized
89 Cationic lipid mediated mRNA delivery
1990 Protein expression by mRNA in mouse
92 Vasopressin mRNA Injection in ra
95 Expression of cancer antigen
97 Anti tumor T cell response
99 dsRNA activates TLR3, inducing type I IFN
2001 Modified Uridine regulates innate immunity
2004 Ex vivo trial using DCs
05 ssRNA activates TLR7/8
09 Induction into iPS
2010 preclinical design of influenza, RSV, and CMV
12 Clinical trial against rabies
13

Nature Rev 13; 759-780, 2014

Moderna mRNA development

2019. 12. 31 SARS-CoV-2 Sequence open

2020. 1. 10 Vaccine design

2020. 1. 13

2020. 2. 02 Protein expression

2020. 2. 04

2020. 2.18 Protein 3D structure

2020. 2.19 Application of trial

2020. 2.20

2020.3. 02 Approved

Phase I 3.16
Phase II 5.29
Phase III 7.27

Nature 586; 567–571, 2020

Development of Virus Vector System

2020 MVA vector system

MVA-BN-Filo 2016, phase I

MVA/Flu H5N1/HA 2014 Phase I and II

2010

2000 ～

MVA/Malaria TRAP 2003 Phase I and II

Measles Vector

2000

Animal study - - - - - - - - -

MVA/Flu HA 1994 Mouse

1990

West Nile
Chikungunya
H5N1
SARS
MERS
TB

Proliferative vector

1980

1970

MVA 1968
Chicken embryo fibroblast

1960

CVA 1953

Leukemia in patients with ADA deficiency treated with Lentivirus vector

2003

Transfer defective gene

Gene therapy in patients with ornithine transcarbamylase deficiency

1999

1997

Try on patients with Cystic fibrosis

1995

Sendai virus vector

1990

ADA deficiency
Ex vivo Gene therapy

Retro virus vector

Adenovirus vector

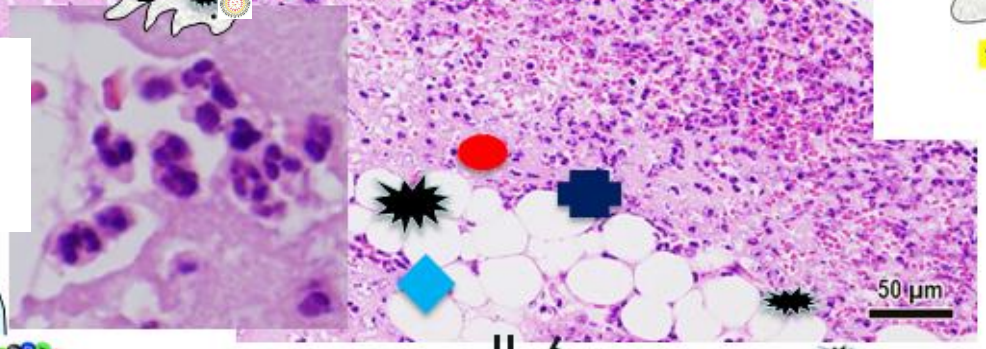
Non-proliferative vector

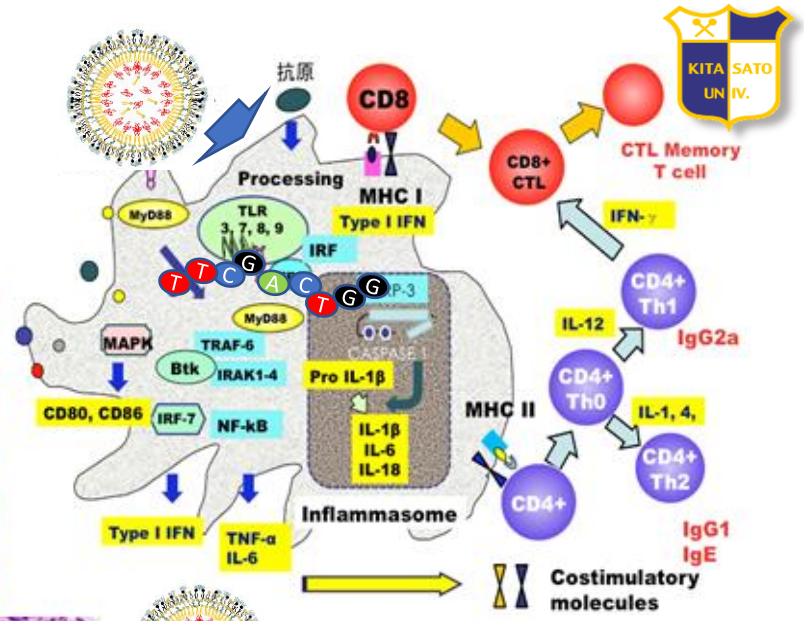
Retrovirus, Adenovirus, Lentivirus, Sendai virus were developed as virus vector to transfer the genome

Nemerow G, et al. Febs Letters 593: 3395- 3418, 2019
Wilson JM. Mol Gen Metab 96: 151-157, 2009

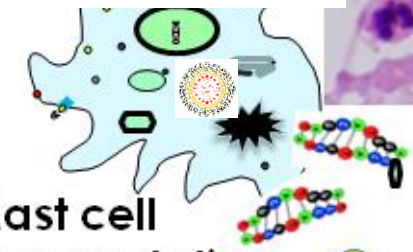
How about following vaccination?

Accumulation of neutrophils and NETs





Regional lymph nodes



Mast cell degranulation

TNF-α

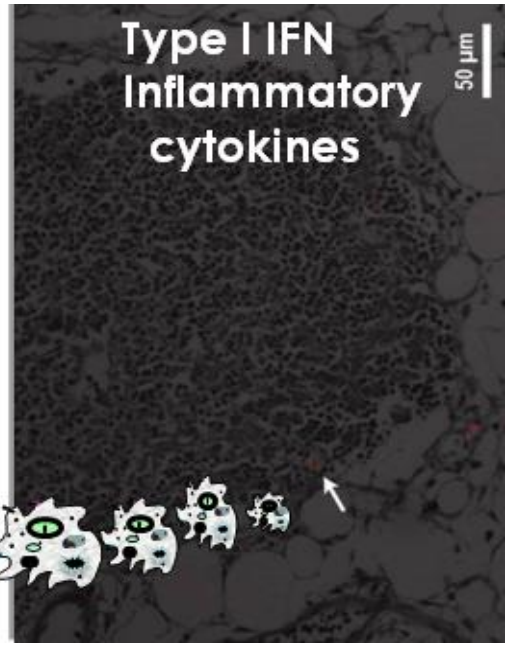
IL-6

IL-1β

Inflammatory cytokines

Cytokine receptors

Sensory nerve end



Unknowns of COVID-19 vaccine

1. Persistence of immunity
2. Vaccine prevents serious diseases, not infection.
3. Application for pregnant women and school-aged children
4. Mutated strains
   Are vaccines effective?
5. How did the clinical trial perform?
6. Vaccine adverse events
   Anaphylaxis, Thrombosis, Cardiomyopathy, ADE, Death
7. Groundless rumors
   mRNA integrates into human genome.
   Unknown serious diseases will develop several years later.
   mRNA moves to gonadal tissues, causing infertility.
   COVID-19 is not serious among young adults, and no serious complication even if infected.
   Vaccines increase the number of death.

COVID-19 is serious in pregnant women, but no influence on fetus.

Among 58 cases of COVID-19 in pregnant women, 40 were infected before the 2nd trimester.

3 (7.5%) had oxygen therapy.

Remaining 18 were infected during the 3rd trimester.

7 (38.9%) had oxygen therapy,

and 1 assisted with respirator passed away.

There was a tendency of increased number of premature birth but no influence on fetus.

Immunization for pregnant women

Immunization is not recommended because of the paucity of clinical data. Medical Association of Obstetrics and Gynecology announces.

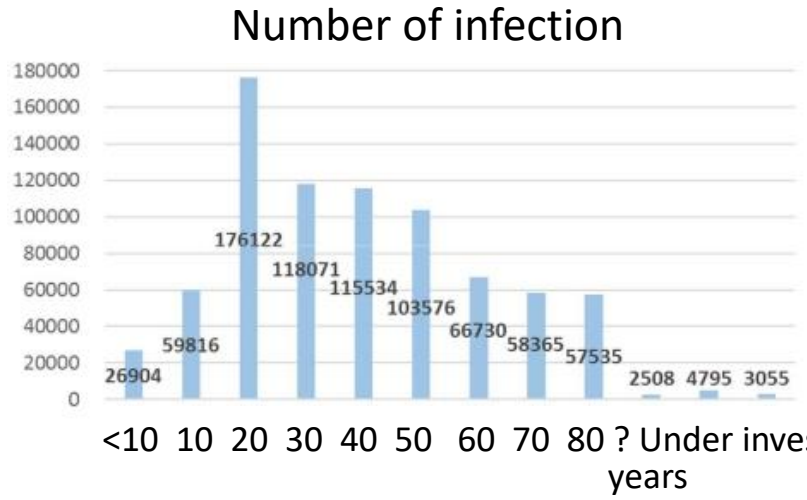
「Pregnant women are not excluded from vaccination. When immunized, please explain the long-term safety issue because of paucity of evidence and obtain the consent. And refrain from immunization until 12 weeks of pregnancy. Please check fetal heartbeat.」

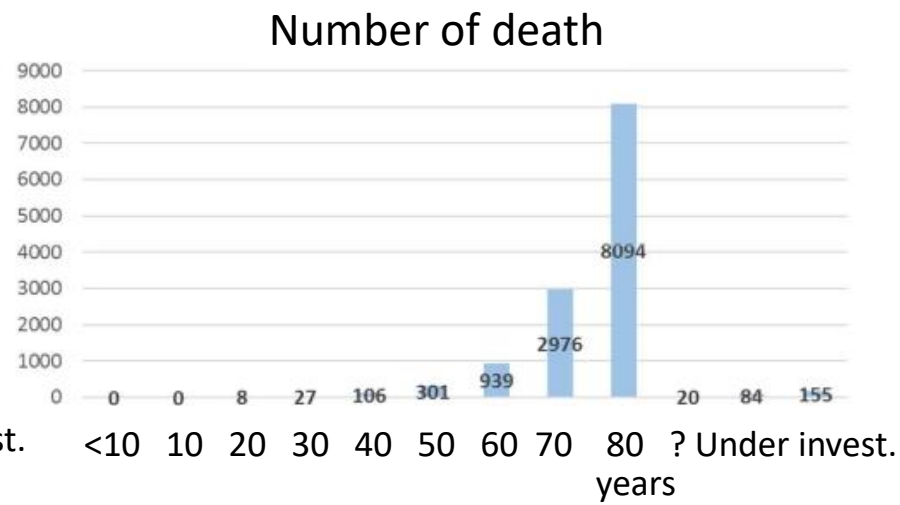
In 827 immunization in the US, no increase in the incidence of abortion, low birth weight, congenital malformation, and fetus death was noted.

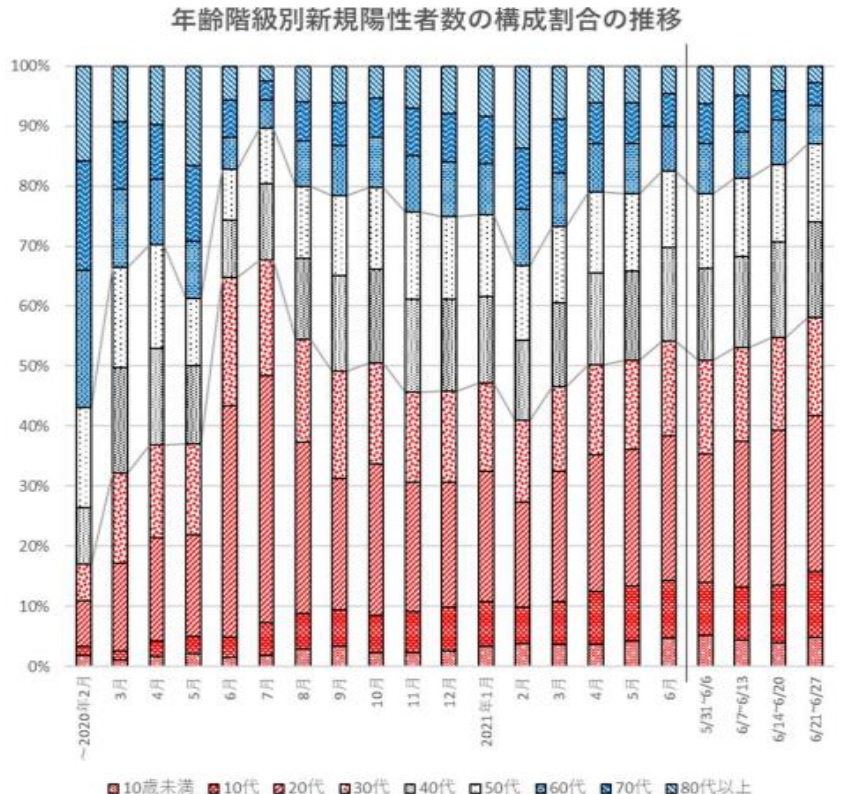
Immunological feature during pregnancy

- Protect pregnant women themselves and fetus from infectious diseases.
- During pregnancy, decreased level of cell-mediated immunity and neutrophil functions during the 1st trimester. No influence on the antibody response.
- Rare case of serious adverse events are observed, but not increasing compared with healthy subjects.
- Live vaccines are prohibited but not inactivated vaccines.

Number of COVID-19 infection and death







60-70 Y
50-60 Y
40-50 Y
30-40 Y
20-30 Y
10-20 Y
<10 Y

Most of the infection is 20-40 years of age. There are few cases of serious diseases, but proportionally, the number of serious cases increases. Several cases with sensory loss of smell and taste, prolonged cough, respiratory distress, fatigue, and alopecia are noted as late complication.

To cope with immunization for children

Number of COVID-19 infection and death in different age groups

Age	Population (thousands)	COVID Infection	Infection /million	COVID Death	Death /million	Death after vaccination/million
<10	9859	26904	2729	0	-	Overall incidence is 17.3/million
10-19	11171	59816	5355	0	-	
20-29	12628	176122	13947	8	0.63	
30-39	14303	118071	8255	27	1.68	
40-49	18520	115534	6238	106	5.72	
50-59	16278	103576	6363	301	18.80	
60-69	16227	66730	4112	939	57.87	
70-79	15927	58365	3665	2976	186.85	
>80	11249	57535	5115	8094	719.45	

Infection in Vaccine recipients

5,186/157 million 33/million

Clinical trial of Pfizer mRNA vaccine in 12-15 years, compared with 16-25 years

Pfizer	1st dose		2nd dose	
	12～15Y（n=1131)	16～25Y (n=537)	12～15Y（n=1131)	16～25Y (n=537)
Local pain	86% (cont:23%)	83% (cont: 16%)	79% (cont: 18%)	78% (cont: 12%)
Fever	10% (cont: 1%)	7% (cont: 1%)	20% (cont: 1%)	17% (cont: 0%)
Fatigue	60% (cont: 41%)	60% (cont: 39%)	66% (cont: 25%)	66% (cont:23%)
Headache	55% (cont: 35%)	54% (cont: 37%)	65% (cont: 24%)	61% (cont:24%)
Muscle pain	24% (cont: 13%)	27% (cont: 14%)	32% (cont: 8%)	41% (cont:10%)

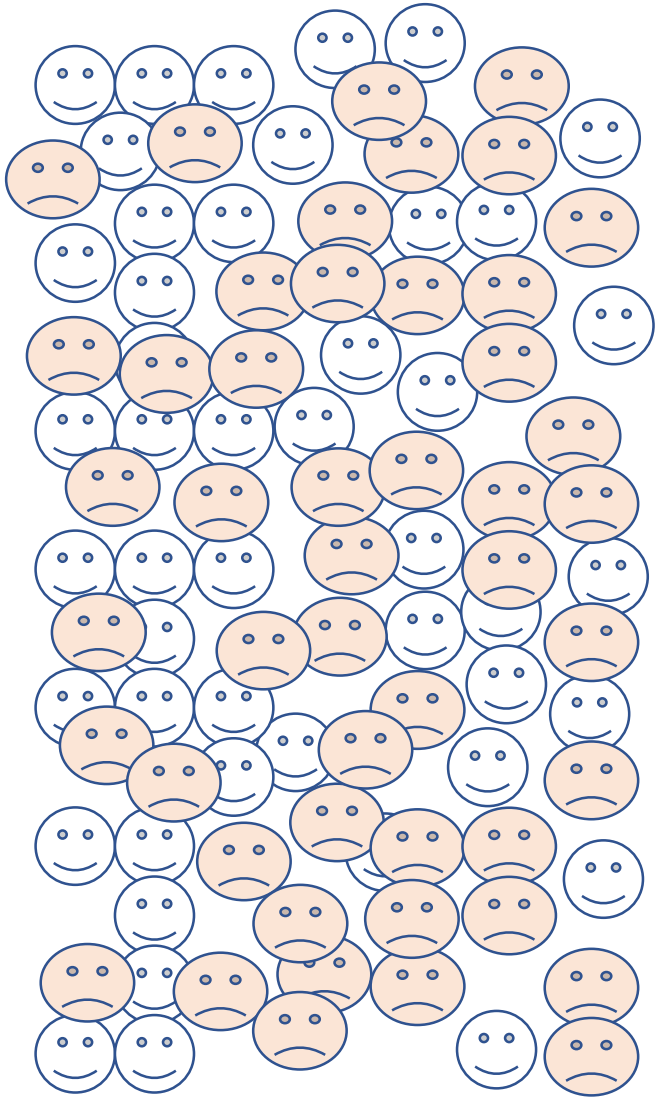
Vaccine seems to be safe and effective. But, pediatric generation is less infectious with very mild illness. We have limited data on immunization in young teenagers and have more cautious attitude.

N Engl J Med 2021; May 27, DOI: 10.1056/NEJMoa2107456

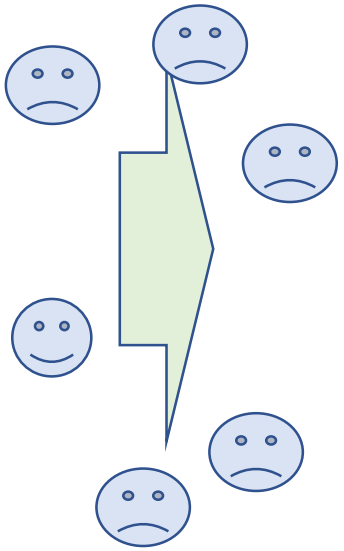
Phase III clinical trial design of Pfizer mRNA vaccine？

Vaccine group

Control group

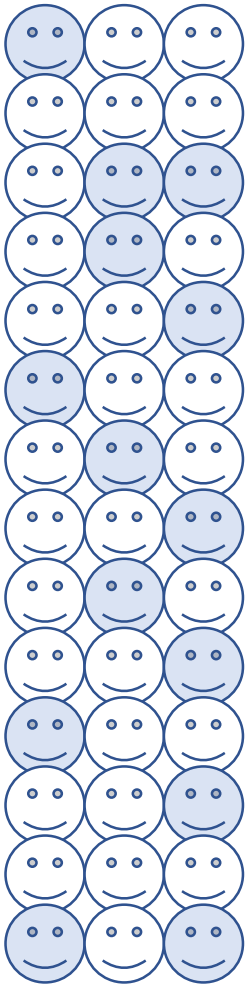


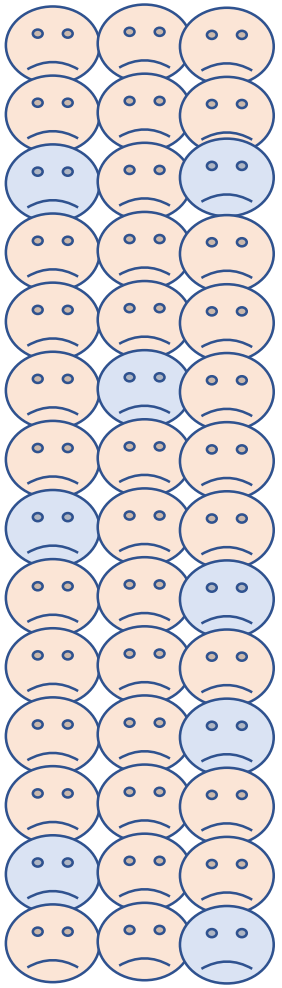
Patients



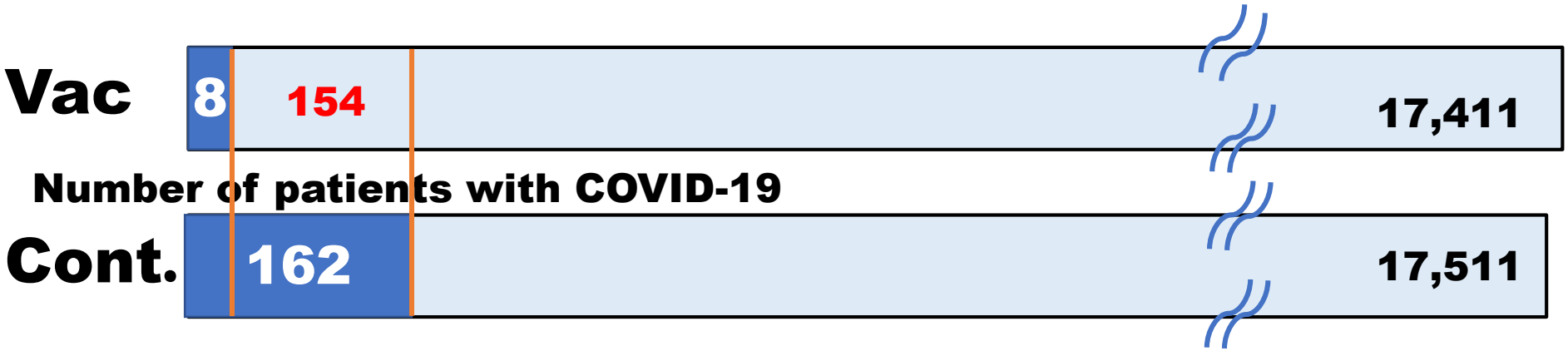
Event based Study: 170 cases With COVID-19

Control group	Vaccine group
162/17,511	8/17,411





Vaccine efficacy



Infection rate in vaccine group： 8/17,411=0.00046

Infection rate in control group ：162/17,511=0.00925

Risk ratio = 0.00925/0.00046=20.11

Efficacy：1-1/risk ratio=1-1/20.11=1-0.0497=0.95(95%)

Among 162 COVID-19 patients who did not have immunization, 95% of them (154 patients) would escape from infection if they were immunized.

How does vaccine induce specific immune response?

Immune response: host defense mechanisms against pathogens

Humoral immunity
Antibody

- Anti-toxin
- Antibodies against pathogen components
- Protection from infection

Cellular immunity
Cytotoxic T cells, helper T cells

- Damage infected cells
- Helper function to produce antibodies
- Prevention of further expansion of infection

Vaccine：prevent infection, onset of illness, and serious illness through host immune responses

Innate immunity recognizes molecular pattern

PAMPs:
pathogen-associated molecular pattern

DAMPs:
damage-associated molecular pattern

TLRs, RIG-I, C-lectin

Inflammasome

Induce cytokines and chemokines

IL-1β, IL-6, 18 TNF-α

IFN-α/β

IL-1β, IL-6, 18, TNF-α

Cytotoxic T cell prevent further expansion of infection

Helper T cells Command B cells to produce antibodies

Immunological memory

Helper T cells Command B cells to produce antibodies

Vaccination

Innate immunity

Acquired immunity
Immune memory

DC
MΦ
PAMPs

IFN-α/β

IL-1β,
IL-6,
TNF-α

Cellular immunity

Antibody

T, B Lymph

G-CSF
IL-8

Neutrophil

Inflammation

IL-1β, IL-6, TNF-α

NETs
autolysis

DNA
ROS

Innate immunity
DAMPs

IL-1β, IL-6, TNF-α

M1 macrophages

M2 macrophages

Inflamatory
nodule

G-CSF, IL-8
IL-1β, IL-6, TNF-α

Adverse events
Fever, local pain

Nakayama T, Vaccine 34: 5815–5818, 2016.

Vaccine effect (main reaction)

Vaccine induces acquired immune responses against pathogen to prevent infection, onset of illness, and serious illness.

Vaccine adverse reaction

Undesirable events following vaccination, being scientifically related to the vaccination.

Vaccine adverse events

Undesirable events following vaccination, their causal relation to the vaccination is scientifically uncertain. They are reported as questionable adverse reaction

Safety profile on common adverse reactions in clinical trial in Japan

Pfizer	1x %	2x %
Local pain	86.6% (2.4%)	79.3% (-)
Fatigue	40.3% (9.8%)	60.3% (2.4%)
Muscle pain	14.3% (2.4%)	16.4% (-)
Headache	32.8% (14.6%)	44.0% (12.2%)
Chill	25.2% (4.9%)	45.7% (2.4%)
Joint pain	14.3% (4.9%)	25.0% (-)
Fever >37.5°C	14.3% (-)	32.8% (-)

Vaccine group 120 subjects
Control group 40 subjects

Moderna	1x %	2x %
Local pain	82.7% (8.0%)	85.0% (2.0%)
Fatigue	18.7% (10.0%)	63.3% (8.0%)
Muscle pain	37.3% (4.0%)	49.7% (10.0%)
Headache	13.3% (-)	47.6% (10.0%)
Chill	5.3% (2.0%)	50.3% (-)
Joint pain	8.0% (-)	32.0% (-)
Fever >38°C	2% (2%)	40.1% (-)

Vaccine group 150 (147) subjects
Control group 50 subjects

Diagnosis of anaphylaxis should be based on Brighton Criterion

Anaphylaxis:

1) abrupt onset,
2) rapid progress of symptoms,
3) including two or more symptoms related to multi-organs
   hives, cardio-vascular illness, wheezing, difficulty in breath, etc.

Differential diagnosis

Immunization stress-related responses: ISRR

Acute reaction:

activation of sympathetic nerve

tachycardia, palpitation, shortness of breath, hyper ventilation
different sensation of heat and cold, sweating

activation of parasympathetic nerve Vagal reflex

bradycardia, hypotension, vertigo, dimmed vision,

Delayed reaction: fatigue, and different sensation

PEG is suspected as causative agent
Pdizer and Modera: PEG 2000 (MW:2000)
AstraZeneca: Polysorbate 80 (emulsifiers)

Incidence of anaphylaxis reported in VEARS

	Pfizer n=50	Moderna n=21
Median age (range)	38.5 (26-63)	39 (24- 63)
Number of female	47 (94%)	21 (100%)
Onset of symptom	10 (<1 - 120)	10 (<1 -45)
within 15 min	37 (74%)	18 (86%)
within 30 min	45 (90%)	19 (90%)
Past history of allergy	40 (80%)	18 (86%)
Past history of anaphylaxis	12 (24%)	5 (24%)
Shot (1x, 2x, unknown)	(42、3、5)	(19、1、1)
	9,943,247 Doses	7,581,429
	5.0/million	2.8/million

COVID-19 vaccine safety update
J Allergy Clin Immunol Pract 17; S2213-2198, 2020

Reported case 289/ 39,218,786 doses

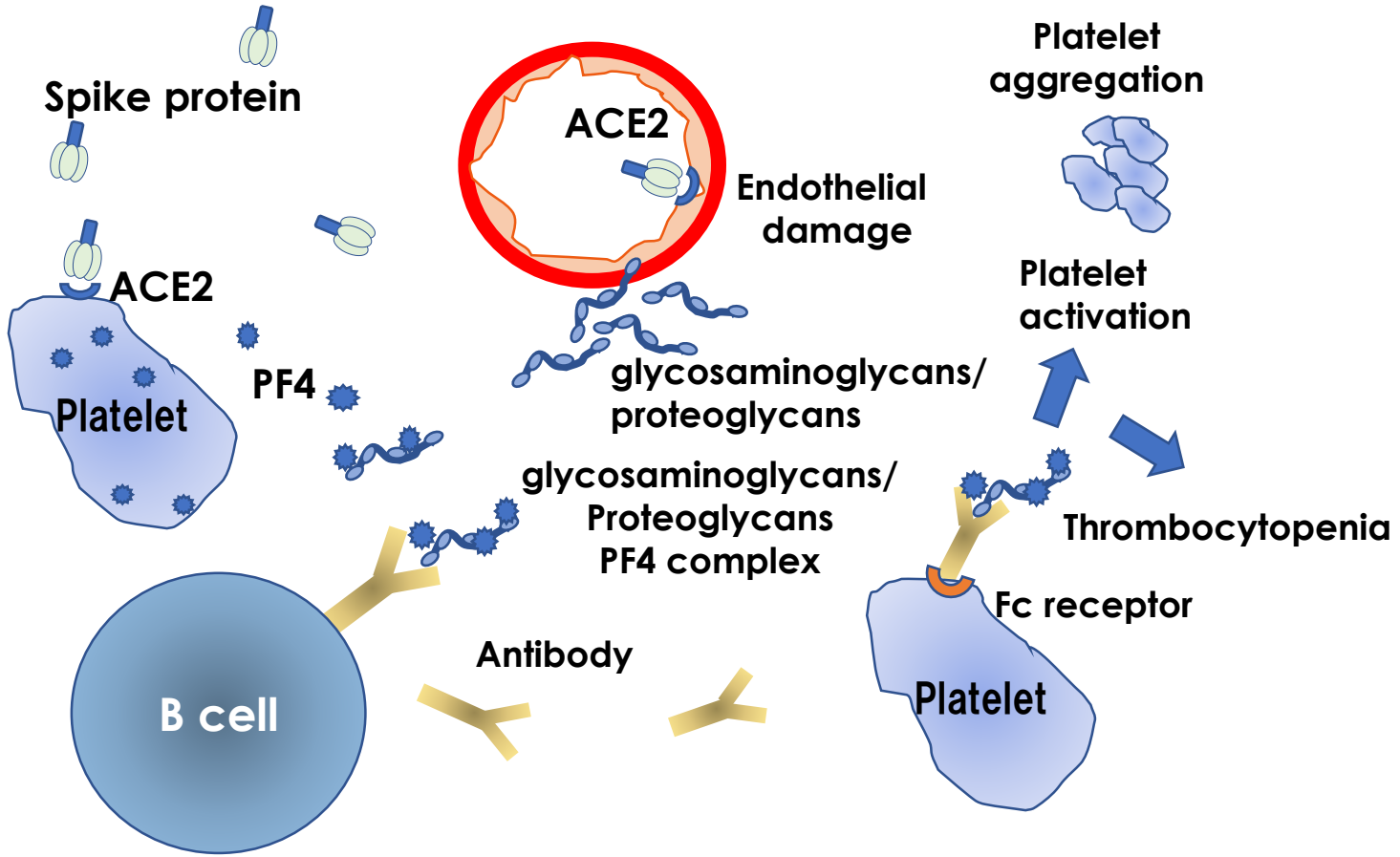
Incidence of anaphylaxis in Japan 7/million

Cardiomyopathy is discussed on mRNA vaccines

	Pfizer		Moderna	
	Reported case /dose	/million	Reported case /dose	/million
Japan	20/39,218,786	0.5/million	1/959,165	1.0/million
USA *	1,226/300 million	4.1/million	1,226/300 million	4.1/million
England	Carditis 60Pericarditis 42 /29 million	Carditis2.1/ Pericarditis 1.5/	Carditis 5、 pericarditis 2/ 0.88 million	Carditis 5.7/ Pericarditis 2.3/
EU	Carditis 122、 Pericarditis 126/ 160 million	Carditis 0.8/ Pericarditis 0.8/	Carditis 16、 Pericarditis 18/ 19 million	Carditis 0.8/ pericarditis 0.9/

＊In USA, cases were reported without discrimination.
Cardiomyopathy was reported higher in male at the second shot.

Thrombosis is discussed on AstraZeneca vaccine



Thrombosis was reported after immunization with AstraZeneca vaccine with 6.5 cases/million doses.
Otherwise, 35 cases with thrombosis was reported following in 54 million subjects immunized with Pfizer vaccine.

Vaccine adverse events (death after vaccination)

Pfizer vaccine (2021.2.27 – 6.27)

1st dose: 26,238,793, 2nd dose: 12,979,993

Suspected adverse reaction: 15,991 cases

serious reactions: 2,262 cases

Death: 453 (>65 years 420 cases, <65 years 31 cases, unknown 2 cases)

Pfizer	Death case/doses	Incidence/million	
Japan	453/39,218,786	17.3/million	Ministry
USA	510/28,374,410	18.0/million	CDC
England	439/29,000,000	15.1/million	MHRA
Moderna			
Japan	1		Ministry
USA	456/26,738,383	17.0/million	CDC
England	5/880,000	5.7/million	MHRA

Ministry of Health, Labour and Welfare, CDC, and MHRA

For reference

PPSV23 **3.2 cases/million**

Influenza vaccine **0.2 cases/million**

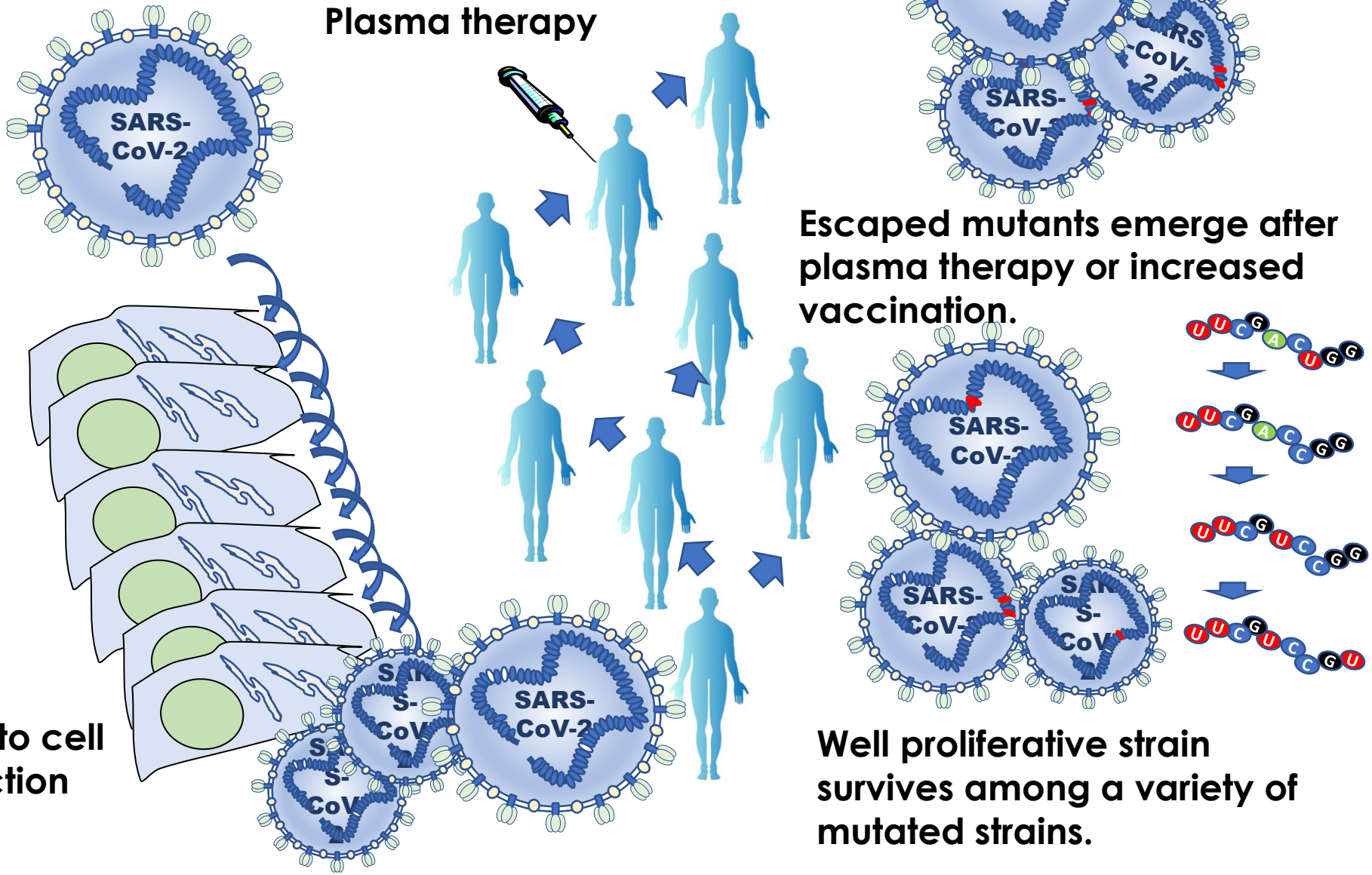
Number of COVID-19 infection and death in different age groups

Age	Population (thousands)	COVID Infection	Infection /million	COVID Death	Death /million	Death after vaccination/million
<10	9859	26904	2729	0	-	Overall incidence is 17.3/million
10-19	11171	59816	5355	0	-	
20-29	12628	176122	13947	8	0.63	
30-39	14303	118071	8255	27	1.68	
40-49	18520	115534	6238	106	5.72	
50-59	16278	103576	6363	301	18.80	
60-69	16227	66730	4112	939	57.87	
70-79	15927	58365	3665	2976	186.85	
>80	11249	57535	5115	8094	719.45	

Infection in Vaccine recipients
(breakthrough infection)
5,186/157 million 33/million

Vaccine efficacy; 96.2% (- 2 M)
decreased 6% per 2 months
90.1% (2 months after 2nd dose)
83.7 % (4-6 month)
Among 42,000 participants

Mutated strains emerged



Increasing number of mutated strains

So called	No. of countries	Strain Name	Mutations
England type	172	α	N501Y, A570D, D614G, P681H
South Africa type	120	β	K417N, E484K, N501Y, D614G
Brazil type	72	γ	K417N/T, E484K, N501Y, D614G
India type	29	δ	L452R, E484Q, D614G, P681R,

N501Y: Increased infectivity

E484K/Q :Escaped from neutralizing antibody

L452R: Escaped from CTL activity

CDC : SARS-CoV-2 Variants. Updated Jan.31. 21021

Risk related to the spread of new SARS-CoV-2 Variants of concern in the EU/EEA-first update. Jan. 21, 2021, ECDC

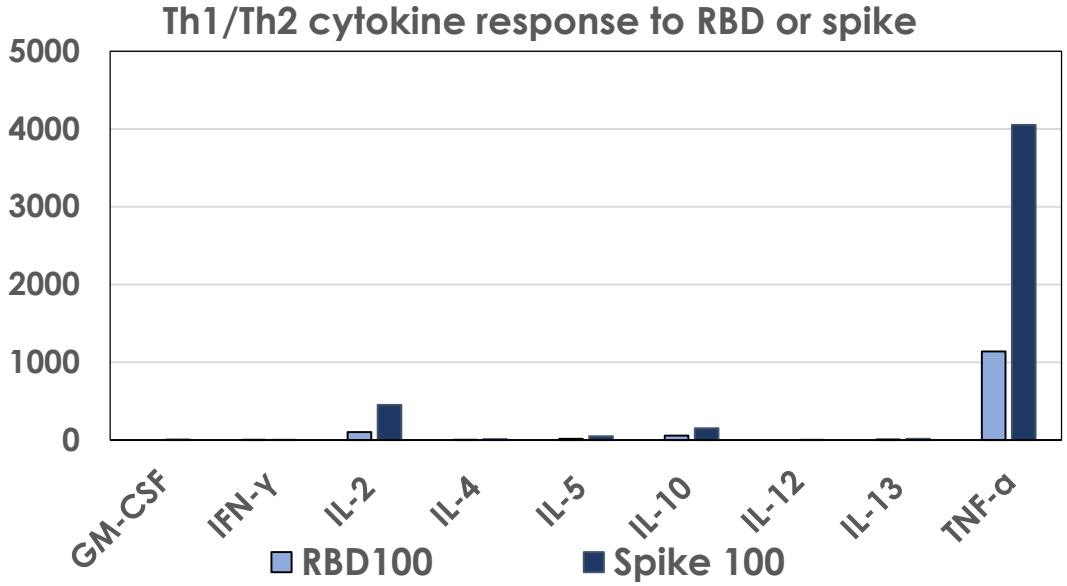
Different antigenicity of mutated strains

	Wuhan	EU	α	β	γ	California
TN 2X 1W	32	32	16	4	4	ND
TN 2X 1M	4	4	< 4	< 4	4	< 4
1 2X 1M	32	32	16	8	16	16
2 2X 1M	64	128	32	64	64	32
3 2X 1M	16	64	32	32	64	64
4 2X 1M	32	32	16	8	16	16
5 2X 1M	32	32	16	8	16	4
6 2X 1M	128	128	64	64	64	128
7 2X 1M	64	64	32	32	64	64
8 2X 1M	64	128	128	32	64	64
9 2X 1M	128	256	64	64	64	64
10 2X 1M	64	32	32	32	64	64
11 2X 1M	64	32	32	32	64	32
Natural ainfect 1X 1M	≥512	≥512	≥512	≥512	≥512	≥512

They had no adverse reactions after vaccination.

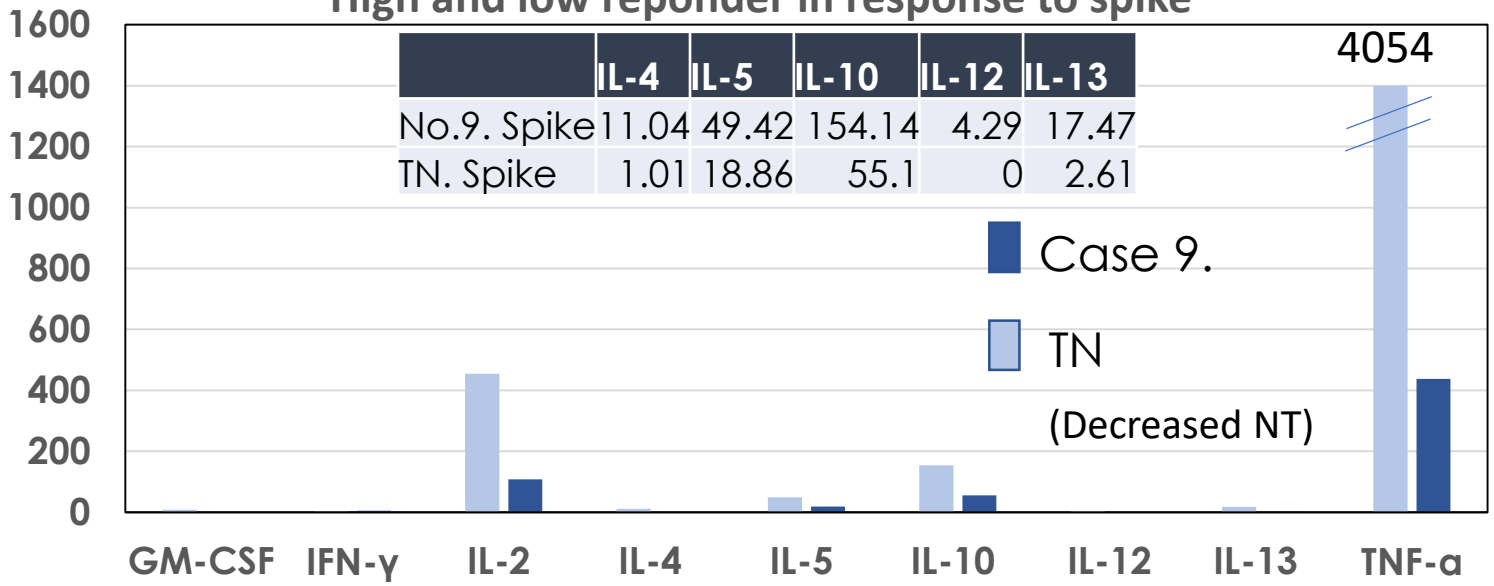
Low titer NT antibody against Wuhan is less cross reactivity against mutated strains.

Cell-mediated Immunity



Whole blood was cultured for 24 h stimulated with RBD and spike protein.
Higher cytokine production in response to spike antigen than RBD in Case No. 9 (NT antibody+).

As for TN, NT antibody decreased to 4 3 months after the 2nd dose, but positive for IFN-γ, IL-2.
Poor response of Th2 cytokines compared with Case 9.



	IL-4	IL-5	IL-10	IL-12	IL-13
No.9. Spike	11.04	49.42	154.14	4.29	17.47
TN. Spike	1.01	18.86	55.1	0	2.61

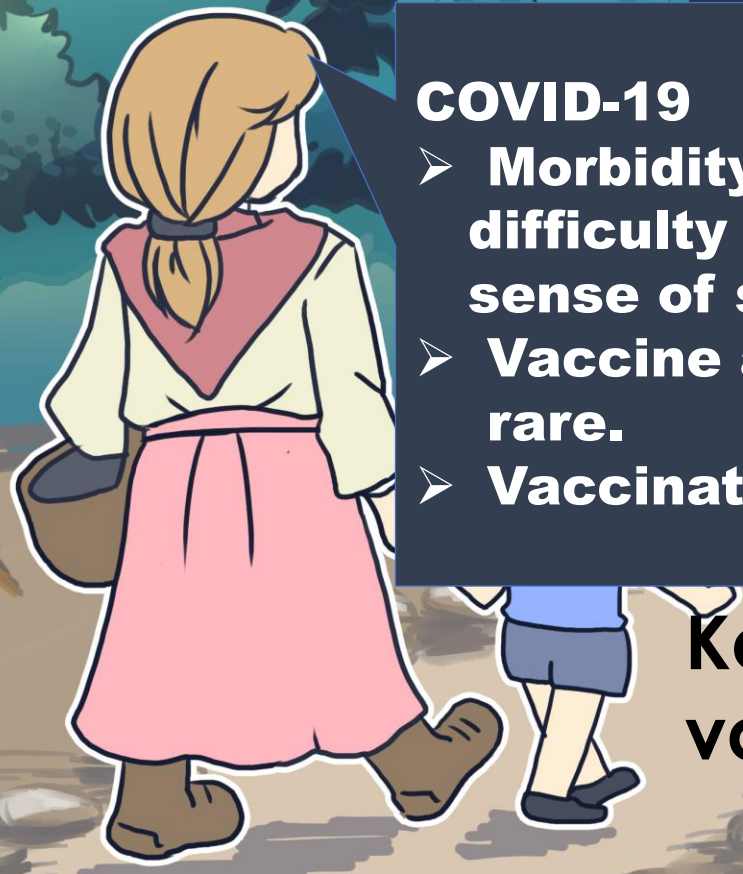
Cellular immune response stimulated with spike protein

	IFN-γ	IL-2	IL-4	IL-5	IL-10	TNF-α
No.6. 2X 3M	8.78	10.16	<	1.87	2.77	136.54
No.8. 2X 3M	4.88	4.38	<	1.38	<	86.88
No.7.2X 3M	7.44	8.55	<	2.36	1.49	83.79
No.1.2X 3M	2.33	3.79	<	<	<	50.54
No.9. 2X 1M	2.65	453.98	11.04	49.42	154.14	4054.32
No.9. 2X 3M	5.68	144.78	4.94	15.7	83.58	1123.24
TN 2X 1M	4.37	108.22	1.01	18.86	55.1	437.9
TN 2X 3M	<	<	<	0.34	4.51	25.77

In Jenner’time, why did mothers take their kids to smallpox vaccination?

Science is to properly understand.

ジェンナーの種痘所



COVID-19

- **Morbidity rate：0.8%、Mortality rate：1.8%**
  difficulty in breath, fatigue, disorder sense of smell and taste as complications
- **Vaccine adverse events are extremely rare.**
- **Vaccination effects >90%**

Kanji (Japanese letter) of vaccine is White God (**白神**) .

サータリさんより