MYOCARDITIS/PERICARDITIS AFTER mRNA COVID-19 VACCINE ADMINISTRATION: POTENTIAL MECHANISMS AND RECOMMENDED FUTURE ACTIONS

Date of most recent update: Feb 11, 2022

CONTRIBUTORS

Seda Arat, PhD
Senior Computational Toxicologist
Global Computational Safety Sciences
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Shambhunath Choudhary, DVM, PhD, DACVP, DABT
Associate Research Fellow
Global Pathology
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Petra Koza-Taylor, BSc (Hons)
Principal Computational Toxicologist
Global Computational Safety Sciences
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Michael Leach, DVM, PhD, DACVP (Lead on white paper)
Biologics Safety Lead
Portfolio and Regulatory Strategy
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Lila Ramaiah, DVM, PhD, DACVP
Senior Clinical Pathologist and Biomarker Strategist
Global Pathology
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Cynthia M Rohde, PhD, DABT
Associate Research Fellow
Portfolio and Regulatory Strategy
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Wen Shen, PhD
Senior Principal Scientist
Global Discovery, Investigative & Translational Sciences
Drug Safety Research & Development
Pfizer Worldwide Research & Development

Mitchell Thorn
Global Discovery, Investigative & Translational Sciences
Drug Safety Research & Development

Pfizer Worldwide Research & Development

Dina Tresnan, DVM, PhD, DACVM
Disease Area Cluster Lead
Safety Surveillance and Risk Management
Pfizer Worldwide Research, Development & Medicine

Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AIDS	Acquired immunodeficiency syndrome
ADME	Absorption, distribution, metabolism, excretion
AE	Adverse event
AESI	Adverse Events of Special Interest
ACE-2	Angiotensin-converting enzyme 2; host cell receptor for SARS-CoV-2
ALC-0159	Proprietary PEG-lipid included as an excipient in the LNP formulation used in the Pfizer-BioNTech COVID-19 Vaccine
ALC-0315	Proprietary amino-lipid included as an excipient in the LNP formulation used in the Pfizer-BioNTech COVID-19 Vaccine
BC	Brighton Collaboration
BLA	Biologics license application
BUN	Blood urea nitrogen
CBER	Center for Biologics Evaluation and Research
CDC	Centers for Disease Control and Prevention
^{3}H-CHE	[Cholesteryl-1,2-^{3}H(N)]-Cholesteryl Hexadecyl Ether, a non-exchangeable, non-metabolisable form of cholesterol that can be used a a radiolabeled tracer
CoV	Coronavirus
COVID-19	Coronavirus Disease 2019
CVB3	Coxsackievirus B3
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
DART	Developmental and reproductive toxicity
DCM	Dilated cardiomyopathy
DHPC	Direct Healthcare Provider Communication
DNA	Deoxyribonucleic acid
DOPC	Dioleoylphosphatidylcholine
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
EAM	Experimental autoimmune myocarditis
EEA	European Economic Area
EMB	Endomyocardial biopsy
EPAR	European public assessment report
EU	European Union
EUA	Emergency Use Authorization
GLP	Good Laboratory Practice
hATTR	Hereditary transthyretin-mediated amyloidosis
HCP	Healthcare providers
HIV	human immunodeficiency virus
HLA	Human leukocyte antigen
HPBL	Human peripheral blood lymphocyte
HSR	Hypersensitivity reaction
ICH	International Conference on Harmonization
IM	Intramuscular(ly)
IV	Intravenous(ly)
LNP	Lipid-nanoparticle

mRNA	Messenger RNA
NHP	Non-human primate
NLRP3	NOD Like Receptor family Protein 3
NOAEL	No observed adverse effect level
ORF	Open reading frame
PD-1	Programmed death 1
PEG	Polyethylene glycol
Ph EU	European Pharmacopoeia
PRAC	Pharmacovigilance Risk Assessment Committee
PRR	Pattern recognition receptors
PVP	Pharmacovigilance Plan
QW	Once weekly
RBC	Red blood cell
RNA	Ribonucleic acid
RIG-I/MDA5	Retinoic acid-inducible gene I and melanoma differentiation-associated protein 5
S	SARS-CoV-2 spike glycoprotein
S1	Subunit of the SARS-CoV-2 spike glycoprotein that contains a receptor-binding domain that recognizes and binds to the host receptor ACE-2
SARS	Severe Acute Respiratory Syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2; the coronavirus causing COVID-19
SMQ	Standardized MedDRA queries
siRNA	Small interfering RNA
SmPC	Summary of Product Characteristics
TLR	Toll-like receptor
USP-NF	United States Pharmacopeia and the National Formulary
USPI	United States product insert
UTR	Untranslated region
V8	Variant 8; P2 S
V9	Variant 9; P2 S
WBC	White blood cell
WHO	World Health Organization
YO	Years old

1. EXECUTIVE SUMMARY

BNT162b2 (Comirnaty®, PF-07302048, Pfizer-BioNTech COVID-19 vaccine) is a vaccine for active immunization against COVID-19. Myocarditis/myopericarditis has been associated in some, but not all, studies following administration of BNT162b2. A variety of potential mechanisms may be involved, and more than one may be acting in a given subject, or between subjects. No clear mechanism has been elucidated.

2. INTRODUCTION AND OBJECTIVES

BNT162b2 is a vaccine developed to prevent COVID-19 which is caused by the virus SARS-CoV-2. During clinical trials in which >20,000 individuals were vaccinated with 30 µg BNT162b2, side effects were limited primarily to vaccine associated reactogenicity However, upon more widespread administration after EUA, rare cases (estimated overall incidence of 2.13/100,000 in one study) of generally mild myocarditis, more frequent (10.69/100,000) in males 16-29 years of age, were reported; these resolved in most individuals with or without treatment (Mevorach 2021, Witberg 2021). Interestingly, other large studies have shown increased risk of myocarditis/myopericarditis after administration of the Moderna mRNA-1273 vaccine, but not with BNT162b2 (Husby 2021). In addition, Husby reported increased risk in females but not males. Heymans (2022) reviewed a variety of studies and concluded that COVID-19 infection caused 1,000-4,000 cases of myocarditis/100,000 people, while the vaccine cause 0.3-5 cases/100,000, indicating a much higher risk of myocarditis following natural infection compared with vaccination. Thus, the risk of myocarditis/myopericarditis varies between studies and our understanding is evolving at the present time, and likely will continue to evolve over the next several years. At the present time, myocarditis/myopericarditis has not been defined as an adverse drug reaction (ADR) by Pfizer, and thus a causal relationship to BNT162b2 vaccination has not been made by Pfizer. Despite rare cases of reported myocarditis/myopericarditis, the benefit-risk assessment for COVID-19 vaccination is considered to have a favorable balance for all age and sex groups; therefore, COVID-19 vaccination is recommended for everyone ≥12 years of age (Bozkurt 2021, Heymans 2022, Husby 2021). Interestingly, the "background" incidence of viral myocarditis in general is estimated at 1-10/100,00 people (Heymans 2022).

The objectives of this white paper are to 1) review the potential causes of myocarditis/myopericarditis, as well as determine the most likely mechanism(s) involved in the myocarditis/myopericarditis that has been associated in some reports with BNT162b2 administration, 2) outline potential nonclinical (in vitro and in vivo) models and investigations that could be conducted to better understand the myocarditis/myopericarditis observed, and 3) provide recommendations on next steps.

At the present time, this white paper should be considered a living document as information is rapidly evolving. Thus, this information and recommendations is this document should be considered in this light.

3. BACKGROUND

3.1. Composition of BNT162b2

BNT162b2 (frozen liquid formulation) components are listed in **Error! Reference source not found.**.

Table 1. Composition of BNT162b2 Drug Product, Multi-Dose Vial (225 µg/vial)

Name of Ingredient	Reference to Standard	Function	Concentration (mg/mL)	Amount per vial	Amount per 30 µg dose
BNT162b2 mRNA drug substance	In-house specification	Active ingredient	0.5	225 µg	30 µg
ALC-0315	In-house specification	Functional lipid	7.17	3.23 mg	0.43 mg
ALC-0159	In-house specification	Functional lipid	0.89	0.4 mg	0.05 mg
DSPC	In-house specification	Structural lipid	1.56	0.7 mg	0.09 mg
Cholesterol	USP-NF Ph. Eur.[a]	Structural lipid	3.1[b]	1.4 mg	0.2 mg
Sucrose	USP-NF, Ph. Eur.[c]	Cryoprotectant	103[b]	46 mg	6 mg
Sodium chloride	USP-NF, Ph. Eur.[c]	Buffer component	6	2.7 mg	0.36 mg
Potassium chloride	USP-NF, Ph. Eur.[c]	Buffer component	0.15	0.07 mg	0.01 mg
Dibasic sodium phosphate, dihydrate[d]	USP-NF, Ph. Eur.[c]	Buffer component	1.08	0.49 mg	0.07 mg
Monobasic potassium phosphate[e]	USP-NF, Ph. Eur.[c]	Buffer component	0.15	0.07 mg	0.01 mg
Water for Injection	USP-NF, Ph. Eur.[c]	Solvent/vehicle	q.s.	q.s.	q.s.

a. Incoming testing at each manufacturing site may initially be performed only in accordance with the receiving market's local compendia.
b. Values are rounded to maintain the same level of precision as the label claim, with trailing zeros not shown, where applicable. For example, 46 mg sucrose is rounded from 46.35 mg (103 mg/mL).
c. Grades of incoming materials are the same across sites as confirmed by the supplier Certificate of Analysis. However, incoming testing at each manufacturing site may initially be performed only in accordance with each site's local compendia.
d. Dibasic sodium phosphate, dihydrate is named as disodium phosphate dihydrate in the Ph. Eur.
e. Monobasic potassium phosphate is named as potassium dihydrogen phosphate in the Ph. Eur.
Abbreviations: ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate); ALC0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; q.s. = quantum satis (as much as may suffice).

Since EUA was granted, a second liquid frozen formulation has been developed for BNT162b2 (

Table 2. Composition of BNT162b2 Tris/Sucrose Drug Product, Multi-dose Vial (225 µg/vial)

). The mRNA and lipids comprising the LNP are the same; however, the excipients have been changed to provide greater stability at lower temperatures.

Table 2. Composition of BNT162b2 Tris/Sucrose Drug Product, Multi-dose Vial (225 µg/vial)

Name of Ingredients	Reference to Standard	Function	Concentration (mg/mL)	Amount per 0.3 mL dose
BNT162b2 mRNA drug substance	In-house specification	Active ingredient	0.1	30 µg
ALC-0315	In-house specification	Functional lipid	1.43	0.43 mg
ALC-0159	In-house specification	Functional lipid	0.18	0.05 mg
DSPC	In-house specification	Structural lipid	0.31	0.09 mg
Cholesterol	Ph. Eur.	Structural lipid	0.62	0.19 mg
Sucrose	USP-NF, Ph. Eur.	Cryoprotectant	103	31 mg[1]
Tromethamine (Tris base)	USP-NF, Ph. Eur.	Buffer component	0.20	0.06 mg
Tris (hydroxymethyl) aminomethane hydrochloride (Tris HCl)	In-house specification	Buffer component	1.32	0.4 mg
Water for Injection	USP-NF, Ph. Eur.	Solvent/vehicle	q.s.	q.s.

1. Values are rounded to maintain the same level of precision as the label claim, with trailing decimals not shown, where applicable. For example, 31 mg sucrose is rounded from 30.81.

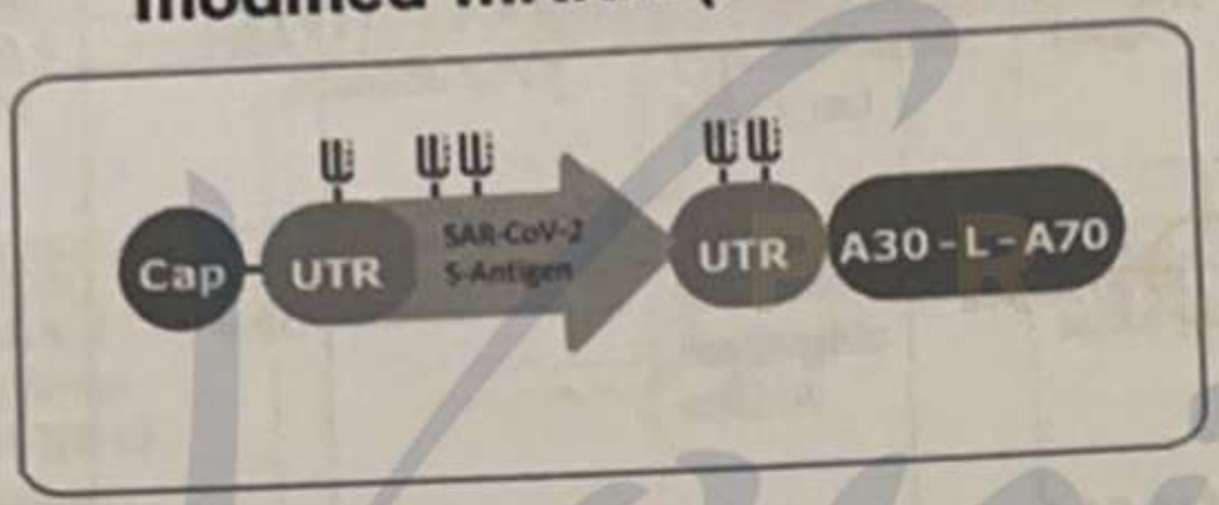
Abbreviations:
ALC-0315 = ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
ALC-0159 = 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine
q.s. = quantum satis (as much as may suffice)
HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
EDTA = edetate disodium dihydrate

3.1.1. BNT162b2 RNA

RNA-based vaccines are manufactured using a cell-free in vitro transcription process, which allows an easy and rapid production and the prospect of producing high numbers of vaccine doses within a shorter time period than possible with conventional vaccine approaches.

The active drug substance of Comirnaty is mRNA encoding the SARS-CoV-2 spike protein in the pre-fusion state. The mRNA contains a 5′ cap and UTR, the spike protein ORF, and a 3′ UTR and polyA tail (Figure 1).

Figure 1. Schematic of BNT162b2 Nucleoside-Modified mRNA



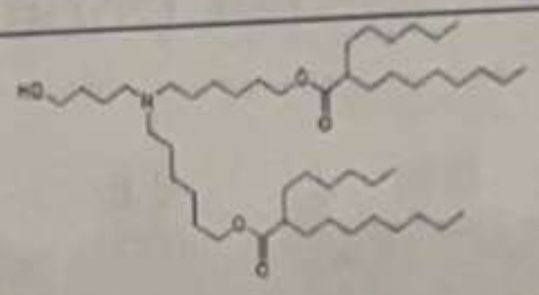
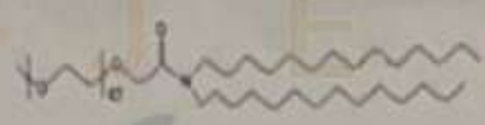
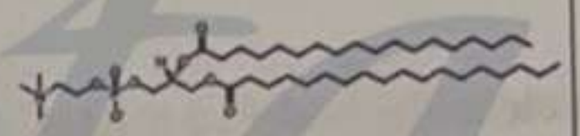
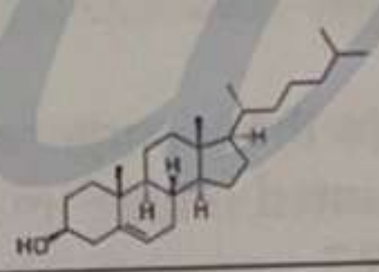
The BNT162b2 nucleoside-modified mRNA consists of a single-stranded, 5′-capped mRNA, with 5′ and 3′ untranslated regions and a poly-adenosine tail, that is translated upon entering the cell.

BNT162b2 also includes the incorporation of modified nucleosides (N1-methyl pseudouridine) with blunted innate immune sensor activating capacity (ss- and dsRNA sensors [TLR7 and TLR3, respectively] and RIG-I/MDA5 in cytosol) and thus augmented expression. These structural elements of the vector backbone of Comirnaty are optimized for prolonged and strong translation of the antigen-encoding RNA component. The LNP protects the RNA from degradation by RNAses and enables transfection of host cells after IM delivery.

3.1.2. BNT162b2 Lipid Nanoparticle and Lipid Components

The LNPs in BNT162b2 are comprised of four lipids (Table 2): (1) ALC-0315 (4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), a proprietary ionizable aminolipid, (2) ALC-0159 (2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide), a proprietary PEG-lipid, (3) DSPC, and (4) cholesterol. ALC-0315 is the major lipid component in BNT162b2 and is included in the LNP to confer distinct physicochemical properties that regulate particle formation, cellular uptake and endosomal release of the mRNA. ALC-0159 (PEG-lipid) stabilizes the particles, facilitates homogeneous particle sizes, and when administered, provides a transient steric barrier to minimize interactions with plasma proteins. DSPC and cholesterol are naturally occurring lipids, present in mammalian cell membranes, and are included in the LNP as structural lipids. Of the four lipids that form the LNP in BNT162b2, two are novel excipients (ALC-0315 [aminolipid] and ALC-0159 [PEG-lipid]). The BNT162b2 LNP diameter is <100 nm.

Table 3. Composition and Dose of BNT162b2 LNP

Lipid	Chemical Name	CAS No.	Structure	Dose (µg)	Dose (µg/kg)
ALC-0315	((4-hydroxybutyl)azanediyl) bis(hexane-6,1-diyl)bis(2-hexyldecanoate)	NA		430	8.6
ALC-0159	2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide	1849616-42-7		53.4	1.1
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine	816-94-4		93.6	1.9
Cholesterol	cholesterol	57-88-5		186	3.7

CAS = Chemical Abstracts Service; No. = number.

3.2. Further Information on Several Other COVID-19 Vaccines and a Relevant RNA Therapy

Table 3 provides relevant information on other COVID-19 vaccines made with nucleic acids as well as a relevant RNA therapy.

Table 4. Basic Characteristics and Dosing Information for Other Nucleic Acid COVID-19 Vaccines and an RNA Therapy

Name	Manufacturer	Type of Nucleic Acid Used	Target	LNP?	Dose, Route, Dosing Schedule, Number Doses
COVID-19 Vaccines					
mRNA-1273 (Moderna COVID-19 Vaccine; elasomeran, Spikevax)	Moderna	Modified-nucleoside mRNA	SARS-CoV-2 spike protein	Yes[a]	100 ug RNA/dose IM 0 and 28 days, Booster at > 6 mo 2 doses
Ad26.COV2-S [recombinant] (Janssen or Johnson& Johnson COVID-19 Vaccine)	Johnson and Johnson	Adenoviral vector (Chimpanzee Ad26)	SARS-CoV-2 spike protein	No	8.92 $\log_{10}$ infectious units IM Single dose Booster dose
AZD1222 (AZ COVID-19 Vaccine, Vaxzevria)	Oxford/Astra-Zeneca	Adenoviral vector (Chimpanzee ChAdOx1)	SARS-CoV-2 spike protein	No	2.5×10^8 infectious units IM Single dose
Relevant RNA Therapy					
Onpattro (patisiran)	Alnylam	siRNA	Transthyretin mRNA	Yes[b]	0.3 mg/kg (<100 kg), 30 mg (>100 kg) IV infusion Every 3 weeks

a.mRNA-1273 LNP is composed of cholesterol, DSPC, SM-102 (heptadecan-9-yl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate), 1,2-Dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000 (PEG2000 DMG).
b. The Onpattro LNP is composed of cholesterol, DSPC, DLin-MC3-DMA, and PEG2000-C-DMG.

3.3. Description of Myocarditis, Myopericarditis, and Pericarditis

Myocarditis is characterized microscopically as focal or widespread inflammatory cell infiltrates in the heart muscle, with or without cardiac myocyte injury (Lasrado et al, 2020; Lampejo et al, 2021). Pericarditis is characterized by inflammation of the outer membranous sac which surrounds the heart. The two conditions may occur together and be described as perimyocarditis or myopericarditis, depending on if myocarditis or pericarditis are predominant, respectively. In the context of mRNA-related heart effects, the terms myocarditis and myopericarditis are most often used.

Clinically, myocarditis can present in a variety of ways, ranging from mild symptoms of chest pain and palpitations associated with transient ECG changes to life-threatening cardiogenic shock and ventricular arrhythmia (Caforio et al, 2013). Myocarditis with mild symptoms and minimal ventricular dysfunction often resolve without specific treatment, and the majority of patients with biopsy confirmed myocarditis do not progress to dilated

cardiomyopathy, which has a poor prognosis. The underlying cause of the myocarditis will often determine prognosis (Carforio et al, 2007). Pericarditis may also present with chest pain and ECG changes, but also with pericardial effusion and pericardial friction rub (Lasrado et al, 2020). In most patients, pericarditis is self-limiting and will respond to anti-inflammatory medications. Clinical diagnosis of myocarditis and pericarditis has been defined by the Brighton Collaboration, which lays out criteria to determine the level of certainty of the myocarditis or pericarditis diagnosis
(https://brightoncollaboration.us/myocarditis-case-definition-update/). Myocarditis is definitively diagnosed by endomyocardial biopsy. It is important to note that incidental microscopic cardiac inflammatory infiltrates are not uncommon (18% of hearts), and inflammation with necrosis may also be seen (~5%) and may be considered contributory to the cause of death in some cases (Zhang 2013). Thus, there is a background of inflammatory infiltrates and even myocardial necrosis. However, less invasive methods may be used in combination to diagnose myocarditis and include clinical signs, elevations in cardiac biomarkers (eg, cTnI), ECG alterations, and heart abnormalities by imaging (eg, MRI, radiographs, echocardiogram). Pericarditis is definitely diagnosed by pericardial biopsy but, may also be diagnosed to varying degrees of certainty by clinical symptoms and physical exam findings, ECG changes, and cardiac imaging.

In most cases, the cause of myocarditis is not determined, but when identified, it is usually the result of a viral infection. Immune-mediated myocarditis may also occur in individuals with underlying autoimmune diseases such as lupus, or in those who may be genetically predisposed to cardiomyopathy. Other causes of myocarditis, such as hypersensitivity myocarditis, giant cell myocarditis, and sarcoidosis are uncommon. While myocarditis and pericarditis can occur at any age, young males <30 years of age are more commonly affected compared with men older than 50 or females of any age (Wang et al, 2021, Lasrado et al, 2020, Kyto et al, 2013). Testosterone has been implicated as a risk factor for coxsackievirus-related myocarditis susceptibility in rodents (Leyden et al, 1987).

Myocarditis may be classified in several ways, including by cause, microscopic findings, and clinicipathologic criteria (Blyszczuk 2019). Microscopically, the type of myocarditis can be defined by the cellular infiltrate identified from endomyocardial biopsies or autopsy heart tissue, when such information is available. Some microscopic classifications are shown below:

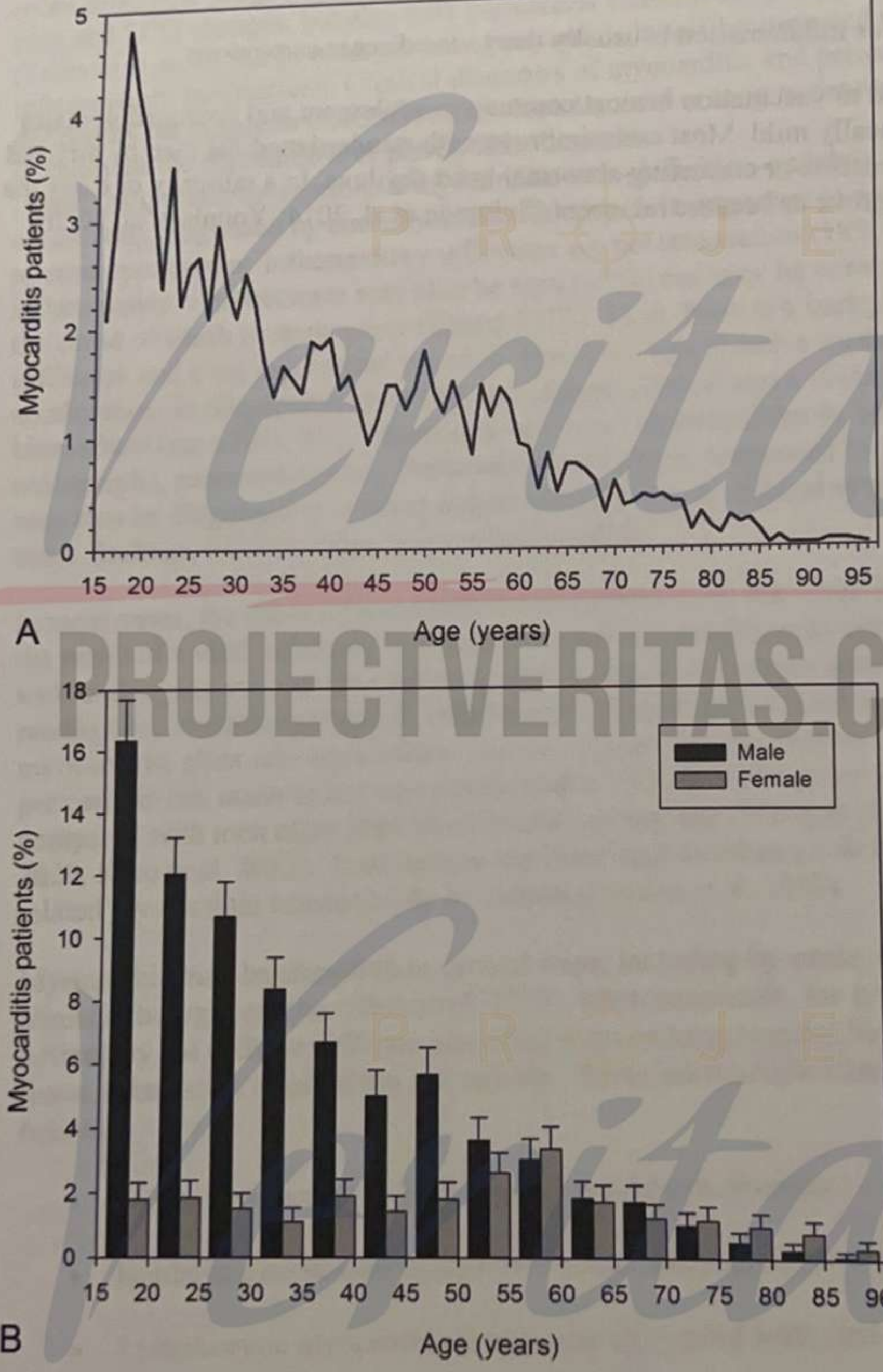
- Active myocarditis is associated with myocyte necrosis
- Borderline myocarditis includes inflammatory infiltrate without myocyte necrosis
- Lymphocytic myocarditis is typically associated with viral infections, toxicants, and autoimmunity.
- Eosinophilic infiltrates may be seen with parasitic infections, hypersensitivity reactions, and eosinophilic inflammatory diseases, and are often associated with myocyte necrosis.

- Giant cell infiltrates (giant cell myocarditis) may be associated with autoimmune diseases but, are generally idiopathic.

- Granulomatous inflammation is usually due to the disease sarcoidosis.

Myocarditis unrelated to vaccination is most common in adolescent and young adult males (Figure 2) and is typically mild. Most cases improve with standard medical therapy directed at improving heart function or correcting abnormal heart rhythms. In a minority of cases the symptoms do not improve or become recurrent (Solomon et al, 2014; Younis et al, 2020).

Figure 2. Age Distribution of All (n=3198) Myocarditis Patients



(A). Age group (16–20 years, 21–25 years, etc) distribution of patients by gender (B). Black bars mark men and grey bars women, error bars represent upper 95% CI (B) (Kyto et al, 2013).

The background incidence of clinical myocarditis in any age group, regardless of cause, is difficult to determine as endomyocardial biopsies, the gold standard for confirmation of myocarditis, are infrequently conducted (Caforio et al, 2013). In young individuals with

sudden death attributed to cardiac causes, microscopic diagnosis of myocarditis at autopsy has been reported with high variability, ranging from 2% to 42% in prevalence (Caforio et al, 2013). However, scant infiltrates are common in the heart unassociated with disease and have been reported in up to 48% of autopsy specimens from young individuals dying acutely from traumatic injuries (Kitulwatte et al, 2020; Claydon et al, 1989). In adults and children with dilated cardiomyopathy, biopsy-proven myocarditis has been reported at 9% to16% and 46%, respectively (Caforio et al, 2013). In cases where patients have mild signs/symptoms and minimal ventricular dysfunction, myocarditis often resolves without specific treatment. However, myocarditis in some individuals can progress to dilated cardiomyopathy, which generally has a poor prognosis.

Elfstrom et al (2007) investigated the risk of myocarditis, cardiomyopathy, and pericarditis in a general population cohort of approximately 14000 individuals with celiac disease and approximately 69000 age- and sex-matched reference individuals in Sweden. The analysis indicated that the median age of onset in the reference group was 22.5 years old for myocarditis and 57 years old for pericarditis. In individuals ≤15 yo, the incidence per 100,000 person-years was 3.45 for myocarditis and 3.16 for pericarditis. In individuals ≥16 yo, the incidence per 100,000 person-years was 4.4 for myocarditis and 18.0 for pericarditis.

Li X (2021) used observational data collected from 01 Jan 2017 through 21 Dec 2019 from 13 databases in eight countries (Australia, France, Germany, Japan, the Netherlands, Spain, the UK, and the US) to describe the background incidence of 15 pre-specified AESIs, during a time prior to the COVID-19 pandemic, that may be associated with COVID-19 vaccines (Li X 2021). While large variations were observed between databases in age and sex-specific estimates for each AESI, similar age and sex-related trends were observed in most databases and pooled rates. Table 5 outlines the background incidence rates per 100,000 person-years for myocarditis or pericarditis as described by Li X (2021). Overall, the incidence of myocarditis or pericarditis was higher in males compared with females.

Table 5. Background Incidence of Myocarditis or Pericarditis: Pooled Estimated Age and Sex Stratified Incidence Rates (Li X 2021)

	Incidence Rate per 100,000 Person Years (95% prediction interval)							
Age Cohort	1-5 y	6-17 y	18-34 y	35-54 y	55-64 y	65-74 y	75-84 y	≥85 y
Female	6 (1 to 25)	7 (2 to 21)	16 (8 to 32)	22 (9 to 53)	31 (13 to 72)	35 (12 to 97	39 (11 to 138)	34 (8 to 143)
Male	7 (1 to 32)	11 (5 to 24)	37 (16 to 88)	37 (16 to 87)	45 (20 to 102)	49 (17 to 139)	54 (15 to 193)	41 (9 to 193)

The background incidence of subclinical myocarditis has been estimated based on microscopic evaluation of the heart in autopsy specimens from individuals dying acutely of traumatic injuries. In a publication by Kitulwatte et al, scant myocardial inflammation was commonly present in the heart of individuals <40 years of age (Table 6; Kitulwatte et al, 2010). However, mild and moderate inflammation (which was associated with myocyte necrosis) was also present, with moderate inflammation only identified in individuals under

25 years of age (6% of that age group [3 out of 48 individuals]) (Table 6). The inflammatory cell infiltrates were primarily lymphocytic, with some mixed inflammation. These lesions were not associated with edema, fibrosis or myocyte hypertrophy. In another review of the incidence of myocarditis as an incidental finding at autopsy in men aged 15-25 years revealed 4 of 27 individuals had subclinical myocarditis (Claydon, 1989). The inflammation between individuals was variable and ranged from primarily lymphocytic to primarily neutrophilic and included one individual with lymphocytes, neutrophils, eosinophils, macrophages, and plasma cells. All individuals had some degree of myocytolysis (Claydon, 1989). These data indicate that subclinical myocarditis is a common finding in men under 30 years of age. Because most of the sudden death due to trauma cases were males, there was no discerning a sex difference in subclinical myocarditis from autopsy specimens.

Table 6. Incidence of Myocardial Inflammation at Autopsy in Different Age Groups from Individuals Dying of Traumatic Injuries. (81% Males, 19% Females with No History of Myocarditis)

Age Groups	<15 y	16-20 y	21-25 y	26-30 y	31-35 y	36-40 y	Total
None	3	12	7	7	5	2	36
Scant	1	5	13	14	9	6	48
Mild	1	1	2	2	4	3	13
Moderate	1	1	1	0	0	0	3
Total	6	19	23	23	18	11	100

3.4. mRNA Vaccine Clinical Trial and Post-Authorization Experience Relative to Myocarditis/Pericarditis

Within the participants 16 years of age and older from the Pfizer clinical trial dataset, two cases of pericarditis were reported through the data cut-off date of 18 June 2021. These cases originated from the Phase 3 clinical study C4591001 and both were deemed not related to study treatment by the Investigator. There were no cases of myocarditis reported as serious adverse events through the data cut-off date of 18 June 2021 (US Pharmacovigilance Plan for the BLA 28 July 2021).

Since April 2021, increased cases of myocarditis and pericarditis have been reported in the United States after mRNA COVID-19 vaccination (Pfizer-BioNTech and Moderna), particularly in adolescents and young adults (CDC 2021). There has not been a similar reporting pattern observed after administration of the Janssen COVID-19 Vaccine (Johnson & Johnson) (CDC 2021).

Cases of Myocarditis and Pericarditis are assessed internally as per Brighton Collaboration (BC) criteria Version_1.5.0_16.July.2021 and Version_1.0.0_15.July.2021), respectively. In summary, the criteria point to remarkable findings of diagnostic tools such cardiac biopsy, cardiac MRI, echocardiography, and electrocardiogram, as well as blood tests such as elevated Troponin. Until data lock period 30-September-2021, only less than 10% of the Myocarditis and Pericarditis cases reported through Pfizer's spontaneous reporting database were assessed as BC level 1 or confirmed Myocarditis and Pericarditis. The pattern of cases

conform, as per the label, to a pattern of Myocarditis cases occurring in majority of young males below 29 years of age within the first two weeks postvaccination, and recovering with standard treatment.

In most cases, patients who presented for medical care have responded well to medications and rest and had prompt improvement of symptoms. Reported cases have occurred predominantly in male adolescents and young adults >16 yo. Onset was typically within several days after mRNA COVID-19 vaccination (from Pfizer or Moderna), and cases have occurred more often after the second dose than the first dose. The CDC and its partners are investigating these reports of myocarditis and pericarditis following mRNA COVID-19 vaccination. The severity of myocarditis and pericarditis can vary. For the cases reported after mRNA COVID-19 vaccination, most who presented for medical care have responded well to conservative medications and rest.

Based on these data and those from other geographic regions including Israel and the EU, and at the request of Health Authorities, the product labels were updated to include the following:

The USPI and EUA Fact Sheets now include the following statement in Warning and Precaution: Post marketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose of the 2-dose primary series (USPI revised 12/2021). The observed risk is higher among males under 40 years of age than among females and older males. The observed risk is highest in males 12 through 17 years of age. Although some cases required intensive care support, available data from short-term follow-up suggest that most individuals have had resolution of symptoms with conservative management. Information is not yet available about potential long-term sequelae. The CDC has published considerations related to myocarditis and pericarditis after vaccination, including for vaccination of individuals with a history of myocarditis or pericarditis (CDC 2021).

The European Union (EU) Summary of Product Characteristics (SmPC) was updated to include myocarditis and pericarditis as adverse drug reactions in Section 4.8, as well as to Section 4.4 Special warnings and precautions. In addition, at the request of the Pharmacovigilance Assessment Committee (PRAC), a Direct Healthcare Provider Communication (DHPC) was distributed in all EEA countries to ensure that healthcare providers (HCPs) are aware of the potential for myocarditis and pericarditis associated with COVID-19 mRNA vaccine use. Myocarditis and pericarditis were also included as an Important identified risk in the EU RMP Version 2.3 (dated 04 August 2021, ongoing procedure) and in the US PVP Version 0.6 dated 28 July 2021.

Additional post-authorization/post-marketing studies to assess risks of myocarditis and pericarditis, including longer term follow up, are being undertaken.

3.5. Nonclinical Safety Data on mRNA COVID-19 Vaccines

BNT162b2 nonclinical data reveal no special hazard for humans based on conventional studies of repeat-dose toxicity and reproductive and developmental toxicity. Rats

administered BNT162b2 IM (receiving a total of three doses of 30 µg mRNA or 100 µg mRNA once weekly demonstrated some injection site edema and erythema, higher white blood cells (predominantly neutrophils and large unstained cells but, including monocytes and to a lesser extent basophils and eosinophils), and higher acute phase proteins consistent with an inflammatory response, as well as vacuolation of portal hepatocytes without evidence of liver injury. All effects were reversible. There were no vaccine-related microscopic findings in the heart. Similar results were seen with 2 other COVID-19 vaccines candidates evaluated in repeat-dose toxicity studies that used a similar study design: BNT162b1 at 30 or 100 µg mRNA/dose and BNT162b3 at 30 µg mRNA/dose. In a combined fertility and developmental study, female rats (44/group) were administered 4 IM doses (30 ug mRNA/dose) of BNT162b1, BNT162b2, or BNT162b3 (21 and 14 days prior to cohabitation with untreated males and on Gestation Days 9 and 20). There were no vaccine-related changes identified on mating performance, female fertility, pregnancy, or embry-fetal or postnatal survival, growth, or development.

In addition, no evidence of vaccine-related microscopic findings in the heart were identified in an immunogenicity and challenge study (VR-VTR-10671) in rhesus monkeys (Vogel et al, 2021). In that study, 6 monkeys were immunized with BNT162b2 IM (receiving two doses of 30 [human dose] or 100 µg mRNA once every 21 days) or BNT162b1 IM (receiving two doses of 100 µg mRNA once every 21 days) and subsequently challenged with 1.05 x 10^6 plaque-forming units of SARS-CoV-2 (strain USA-WA1/2020) between 41 and 55 days after the second dose of BNT162b2. Necropsies were conducted 7 or 8 days after the SARS-CoV-2 challenge and a microscopic evaluation of a limited tissue set was conducted, including the heart. There were no macroscopic observations in any of the tissues and the main microscopic finding was lung inflammation.

The Moderna COVID-19 vaccine (mRNA-1273), also an mRNA/LNP-based vaccine, was assessed in general toxicity studies in rats (Moderna COVID-19 Vaccine SmPC and EPAR Public Assessment Report). IM administration of up to 4 doses (doses ranging from 9 to 150 µg mRNA/dose; once every 2 weeks) caused transient and reversible injection site edema and erythema and transient and reversible changes in laboratory tests (including higher eosinophils, activated partial thromboplastin time, and fibrinogen). Similar to BNT162b2, there were no reports of vaccine-related microscopic findings in the heart with mRNA-1273.

None of the nonclinical studies with either the Moderna COVID-19 vaccine or BNT162b2 suggested a risk of myocarditis/pericarditis in humans.

3.5.1. ACE2 Expression Pattern

ACE2, the receptor for SARS-CoV-2 cell binding, is widely expressed in human tissues. Based on Human Protein Atlas data, ACE2 protein/RNA expression is greatest in the intestine (tissue enriched for RNA), kidney, gallbladder, pancreas, bronchus, nasopharynx, and reproductive tissues; and generally low in other tissues (Human Protein Atlas, 2021). However, several reports indicate ACE2 protein, RNA, and/or gene expression in the heart and that this expression may be upregulated in cases of heart damage such as cardiomyopathies (The Human Protein Atlas, 2021; Goulter et al, 2004; Chen et al, 2020; Hikmet et al, 2020; Li et al, 2020). Internal data (Zoomap) indicated a similar pattern of ACE2 expression in cynomolgus monkey, dog, and mouse; internal data for rat or rhesus are

not available (http://rstudio-hpc.pfizer.com/rsconnect/proteome_zoomap/?GENE=ACE2). External mRNA expression data for rhesus available at bgee.org indicates a similar pattern to human and other preclinical species. Table 7 shows transcript (mRNA) and protein sequence similarities between human and nonclinical species. ACE2 has at least 79% protein sequence homology between human and nonclinical species.

Table 7. ACE2 Homology Between Human and Animal Species

Species	Gene Name	Ensembl Gene ID	% Identity Ortholog to Human (Transcript | Protein)	% Identity Human to Ortholog (Transcript | Protein)	Ortholog Type
cyno	ACE2	ENSMFAG00000039194	80 | 95	35 | 95	one2one
dog	ACE2	ENSCAFG00000012112	87 | 84	34 | 84	one2one
rat	Ace2	ENSRNOG00000031665	85 | 83	26 | 79	one2one
mouse	Ace2	ENSMUSG00000015405	84 | 82	40 | 82	one2one
rabbit	ACE2	ENSOCUG00000017782	86 | 85	37 | 85	one2one

4. POTENTIAL MECHANISMS OF MYOCARDITIS AND PERICARDITIS

4.1. Known Causes of Myocarditis and Pericarditis

Myocarditis is inflammation of the heart muscle (myocardium) (Tschöpe et al, 2021), and pericarditis is swelling and inflammation of the thin, sac-like tissue surrounding the heart (pericardium) (Troughtom et al, 2004). In both cases, the body's immune system causes inflammation in response to a variety of infections and non-infectious triggers.

Myocarditis may be infectious, idiopathic, or autoimmune and may heal or progress to DCM. Among the infectious causes of myocarditis, viruses (eg, coxsackievirus, adenovirus, HIV, and hepatitis C, etc) are presumed to be the most common pathogen (Tschöpe et al, 2021). In a recent study from Israel, SARS-CoV-2 infection was also found to be associated with an increased risk of myocarditis, 11 events per 100,000 persons (Barda et al, 2021). Other

infectious agents that have been implicated are bacteria (eg. *Borrelia burgdorferi* – the cause Lyme disease), protozoa (eg, *Trypanosoma cruzi*), and fungi (eg, Candida) (Tschöpe et al, 2021; Cooper Jr, 2009). Non-infectious causes of myocarditis include a wide range of chemicals, toxic substances and drugs (such as immune checkpoint inhibitors, reported incidence of 0.04% to 1.14%), as well as systemic immune-mediated diseases (Palaskas et al, 2020; Tschöpe et al, 2021).

The damage to the myocardium is often progressive with three pathogenetically distinct phase. In acute phase I, initial insult (infectious or non-infectious) to the myocardium occurs. The further development of myocarditis in the subacute phase II is thought to be predominantly a result of autoimmune responses triggered by the initial injury. In the third phase (chronic), DCM develops in approximately one-third of the myocarditis cases and may progress despite cessation of the first two processes (Mason, 2003).The cause of pericarditis is often unknown. Acute pericarditis is often accompanied by some degree of myocarditis as the common etiologic agents are shared. Viral infections are the most common reason. Chronic and recurring pericarditis may be caused by autoimmune disorders (such as lupus, scleroderma, and rheumatoid arthritis). Other causes of pericarditis include heart attack and heart surgery, kidney failure, HIV/AIDS, cancer, tuberculosis and other health problems, injuries from accidents or radiation therapy and certain drugs, such as phenytoin (an anti-seizure medicine), warfarin and heparin (both blood-thinning medicines), and procainamide (a medicine to treat irregular heartbeats) (Imazio et al, 2015).

4.2. Vaccine-Related Myocarditis, Myopericarditis, Pericarditis, and Perimyocarditis

Several terms have been used for inflammation of the pericardium and myocardium as it relates to vaccinations, and the use of the terms does not always appear to be consistent. In clinical practice, both pericarditis and myocarditis often coexist (Imazio and Trinchero 2008). Myopericarditis indicates a primarily pericarditic syndrome, while perimyocarditis indicates primarily a myocarditic syndrome, but the terms may be used interchangeably (Imazio and Trinchero 2008). To confirm a diagnosis, microscopic evaluation is needed, but collection of heart tissue can be risky (Imazio and Trinchero 2008). Many cases presumably lack microscopic confirmation, and thus technically would be considered suspected or probable. **For the purposes of this section, the term myocarditis will be used.** In the current discussions in scientific literature around the mRNA vaccines, the terms myocarditis and myopericarditis appear most frequently.

Myocarditis has only rarely been reported in relationship to vaccination (Mei et al 2018). The true incidence of myocarditis after vaccination is likely underestimated because of the subclinical and nonspecific clinical manifestations in most cases (Kim et al 2019). Myocarditis following smallpox vaccination is the best studied collection of post-vaccination cases (see Table 8 for a summary of reviews on smallpox vaccine-related myocarditis). In European adults, there were 7 fatal and 59 non-fatal cases of post-vaccinial myocarditis in the 1950's and 1960's (reviewed by Cassimatis et al 2004). The estimated incidence ranged from 0.01% to 3%. In one of the studies noted by Cassimatus et al (2004), the total number of vaccinated subjects was 234 (Helle et al 1978, only abstract available). In other reports,

the abstracts (the only information available) did not specify the total number of subjects. Lymphocytic infiltration in the heart was reported in 2 fatal cases (Mathieu 1953 as described by Cassimatus et al 2004 [original article in French and not available]; Dalgraad 1957). It was not mentioned whether tissue from hearts from other fatal cases were available for microscopic examination. In children in the same timeframe, the European literature reported at least seven non-fatal cases of post-vaccinial myocarditis and two fatal cases, both with inflammatory infiltrates (but no eosinophils) in the myocardium (Larbre 1966, and Gatta and Pieroni 1976, as described by Cassimatus et al 2004 [original articles in French and Italian and not available]). The Australian literature reported 11 non-fatal and 1 fatal adult cases of post-vaccinial myocarditis (reviewed by Cassimatus et al 2004), the last with a mixed cardiac infiltrate (Finlay-Jones 1964). In contrast, cardiac complications were rarely described during that time period in the US, with only two non-fatal adult cases and two fatal adult cases. It was thought that this difference was due to the use of a different strain of vaccinia in the US (the New York City Board of Health strain [Dryvax, Wyeth Laboratories, Marietta, Pennsylvania]). However, between Dec 2002 and Dec 2003 the US military vaccinated over 540,000 personnel with this strain and higher than expected rate of myocarditis was observed (Cassimatus et al 2004, Eckart et al 2004). Detection of cases was based on surveys of initial small numbers of vaccinated individuals, or reporting of sick individuals (thus, asymptomatic cases would not have been detected). A total of 67 subjects developed myocarditis 10.4 ± 3.6 days after vaccination out of 540,824 vaccinated, or 12 cases/100,000 (0.012%). While the expected background rate of myocarditis was not provided in this study (Eckart et al 2004), a report slightly early in the vaccination campaign indicated a background rate of 2/100,000 (Arness et al 2004), and this number appears to be a good estimate of the background incidence in this population, and suggests an incidence ~6x that of the background. Given the higher incidence of myocarditis, it was thought that the lower rate reported previously in US might be due to variations in monitoring and reporting, and not actual differences in incidence (Cassimatus et al 2004).

Table 8. Selected Reviews on Smallpox Vaccine-Related Myocarditis/Myopericarditis (Case Reports are Not Included)

Approximate Time of Vaccination	1950's and 1960's	1960's	2002-2003	2004-2010
Location	Europe	Australia	United States (Military Vaccination Campaign)	United States (Generally Military and Their Beneficiaries)
Reference(s)	Summarized by Cassimatus et al 2004	Summarized by Cassimatus et al 2004, Finlay-Jones 1964	A number of publications described the myocarditis following vaccination as the issue evolved. Eckart et al 2004 provides the most complete data set from the end of the campaign. Some other publications also have useful commentary, in particular Halsell et al 2003 who published on information up to the early middle of the campaign, as well as Cassimatus et al 2004 and Arness et al 2004 who published on information up to the late middle of the campaign.	Engler et al 2015

In the case of the US military vaccinations, several articles were published in the midst of the vaccination campaign (Halsell et al 2003, Cassimatus et al 2004, Arness et al 2004, Eckart et al 2004). Halsell et al published earlier in the vaccination campaign on ~230,000 vaccinees, Cassimatus et al published in the late middle on ~450,000 vaccinees, Arness et al 2004 also published in the late middle on ~492,000 vaccinees, and Eckart et al published at the end of the campaign with 540,824 vaccinees. Thus, the same cases are presented in multiple publications and the incidence is slightly different across the publications. A number of additional articles were published, but do not add additional information (eg, Grabenstein and Winkenwerder 2003), and these are not presented in this white paper. Eckart et al contained the largest cohort and could be seen as the definitive report. Eckart et al reported 67 cases of suspected, probable, or confirmed myocarditis (67/540,824 = 12.4/100,000; background rate estimated at 2.16/100,000 in the related study by Arness et al 2004). Microscopic changes in the right ventricle following endomyocardial biopsy in one subject revealed infiltrates of eosinophils, lymphocytes, and macrophages associated with myocardial necrosis (Murphy et al 2003). Primary vaccinia infection was ruled out in this patient via PCR analysis. Treatment of this patient with steroids was effective, suggesting the cause was not infectious. While the

authors attributed the findings to smallpox vaccine, the patient also received pneumococcus, meningococcus, influenza, and anthrax vaccines in the same timeframe (Murphy 2003). If this was true of many of the other cases (which is unknown), it seems difficult to identify smallpox vaccine as the actual cause vs other vaccines. It should be noted that cardiac inflammation can also occur in natural smallpox infection (Cann et al 2013, Woodruff JF 1980). In a review, Cann et al (2013) notes that "By today's standards little is known about the systemic pathology of human smallpox.....descriptions of changes in major organ systems [other than cutaneous and mucosal lesions] are absent or incomplete and difficult to interpret". Cann et al (2013) also note that cardiac lesions as reported in the literature were "infrequent" or "rare" depending in different areas of the article (note, I did not review all the papers Cann referenced). Changes were described as multifocal myocardial and subendocardial hemorrhage, and lymphohistiocytic and eosinophilic myocarditis and epicarditis (Cann et al, 2013).

To address some of the questions, a prospective study evaluating the cardiac effects of smallpox vaccination or trivalent influenza vaccination was conducted (Engler et al 2015). Myocarditis (4 males) and pericarditis (1 female) was observed in smallpox vaccinees out of a total of 1081 subjects, and this incidence (0.46%) was over 200x the background population (background estimate of 0.0022% for clinical myocarditis); the 0.46% was higher than that reported by Eckart et al (2004). In addition, 31 smallpox vaccinees without cardiac symptoms had over 2x higher cardiac specific troponin (2.87%). This provides a total of 3.42% of subjects with heart effects in this study. No evidence of myopericarditis was seen in the influenza-vaccinated group. The peak inflammation following smallpox vaccination occurs around day 8-9 and included a predominantly Th1 cytokine pattern (IFN gamma, TNF, IL-6, etc) (Cohen et al, 2010; Simon et al, 2014), which matched the peak of myocardial injury (Engler et al, 2015), suggesting a link between the vaccine-induced inflammation and myocarditis or pericarditis. Due to the prospective nature of this study, it is probably the best data set regarding the true incidence of myocarditis following smallpox vaccination, and we are not aware of other prospective studies after smallpox vaccination. The higher incidence of myocarditis reported prospectively by Engler et al (2015) supports the suggestion that myocarditis following smallpox vaccination was underreported prior to the 2002-2003 vaccination campaign (Cassimatus et al 2004), and that assessments that rely in subjects to self report also likely underreport the actual incidence. On the other hand, it is also likely that underreported cases are mild and resolve without treatment.

Beyond smallpox , myocarditis associated with vaccination has been reported only very rarely. Vaccination against HPV; meningococcus; hepatitis A; typhoid; Japanese encephalitis virus; anthrax; combined diphtheria tetanus; polio (TdP) vaccines; and influenza have all been associated with myocarditis, but clear causality has not been shown (Mei et al 2018). Boccara et al 2001 reported on a case of acute myopericarditis after vaccination with TdP. Left ventricular endomyocardial biopsy did not show inflammatory infiltration or necrotizing myocarditis. The authors speculated that the findings represented a hypersensitivity reaction, and also noted that there was no definite evidence to support a causal link between the administration of vaccine and myopericarditis. Streifler et al first described recurrent pericarditis after influenza vaccination in 1981. Kim et al (2018) reported a case of acute fulminant myocarditis in a previously healthy young female following the administration of a quadrivalent inactivated influenza vaccination. Cheng et al (2016) reported a syndrome

known as autoimmune/inflammatory syndrome induced by adjuvants (ASIA), with signs including myocarditis following influenza vaccination with the MF59 adjuvant. The case reported by Kim et al (2018) received an inactivated quadrivalent influenza vaccine without adjuvant (GC Flu Quadrivalent, Green Cross), whereas the case reported by Cheng et al (2016) received an inactivated trivalent influenza vaccine with MF59 adjuvant (Fluad, Novartis). Thus, the presence of the adjuvant is not required to initiate myocarditis. A fatal case of myocarditis was reported in children between 6 to 23 months after administration of a trivalent inactivated influenza vaccine (Rosenberg, 2009). The authors concluded the causal relationship between influenza vaccination and development of myocarditis was not certain (Rosenberg, 2009). Overall, it is controversial whether myocarditis is causally related to influenza vaccination or merely a serendipitous occurrence. Engler et al (2015) did not report evidence of myocarditis following influenza vaccination of 189 subjects, although other new onset cardiac signs were seen in a small number of subjects. Helle et al (1978) report that up to 3% of Finnish military conscripts had evidence of myocarditis following vaccination against mumps, polio, tetanus, smallpox, diphtheria and type A meningococcal disease based on serial ECG changes suggestive of myocarditis.

The specific mechanism of post-vaccination myopericarditis is unclear. Cassimatus et al (2004) note that myocarditis remains a poorly understood entity and may be the final result of a process that can be comprised of multiple pathways (eg, ischemic, infectious, post-infectious, autoimmune, toxic). Vaccination with adjuvant influenza A results in systemic inflammation and impacts on heart rate variability suggesting the inflammation impacts the heart (Lanza et al 2011); the finding of systemic inflammation following vaccination is not surprising. Engler et al (2015) suggested a link between inflammation and myocardial effects. Cytokines such as IL-1 and TNF have been shown to play a role in experimental myocarditis (Lane et al 1993, Mann 2001, Lim et al 2002, Yamada et al 1994). Cytokines associated with sepsis, including TNF, IL-1, IL-2, IL-6, and IFN-gamma, are considered to be myocardial depressants following the acute response, which can be mixed stimulatory and depressant (reviewed by Prabhu 2004). As noted above, the timing of the peak inflammatory response to smallpox coincides with the peak incidence of myocarditis around day 8 to 9 (Cohen et al, 2010; Simon et al, 2014), suggesting there may be a relationship (Engler et al, 2015). although this timing is later than the 2 to 3 day range often reported with BNT162b2.

In conclusion, myocarditis or pericarditis has been associated with several vaccines. Most data come from a relatively small number of case reports. Substantially more information is available on myocarditis and pericarditis associated with the smallpox vaccine because it was administered to individuals in the military, allowing for better follow up. None of the existing information provides evidence of a clear, specific mechanism for inducing myocarditis. Hypotheses tend to center around induction of systemic inflammation by the vaccine, which leads to myocarditis or pericarditis by uncertain specific mechanisms. Many case reports do not consider other causes, and do not factor in the background incidence, which may impact conclusions.

4.3. Discussion of Potential Mechanisms Related to BNT162b2 Administration

4.3.1. Direct cardiotoxicity

It has been reported that modRNA can be delivered to damaged myocardium via LNPs (Evers et al 2022). However, based on the infrequent occurrence alone of the pericarditis and myocarditis after vaccination as well as the most frequent timing when the observations occur (after the second dose), it is highly unlikely that administration of BNT162b2 is having a direct cardiotoxic effect. In addition, repeat-dose toxicity and biodistribution studies in animals do not support this conclusion.

The components of BNT162b2 are not known cardiotoxins. The vaccine is composed of mRNA, comprised of naturally occurring nucleosides, as well as lipids. Cholesterol and DSPC are naturally occurring lipids. ALC-0159 and ALC-0315 are similar to lipids (DLin-MC3-DMA and PEG2000-C-DMG) used in Onpattro (patisiran), an siRNA/LNP therapy for treatment of transthyretin-mediated amyloidosis, which did not show evidence of direct cardiotoxicity (Onpattro Public Assessment Report). The other excipients of the vaccine include phosphate or tris buffers, NaCl, and sucrose.

4.3.2. Viral/bacterial, underlying condition, drug/toxin-related

4.3.2.1. Acute/active viral infection

In the published reports of myocarditis after mRNA COVID-19 vaccine administration, SARS-CoV-2 polymerase chain reaction and viral serology for other main cardiotropic viruses were negative, and therefore, although we cannot rule out the viral myocarditis completely, there is no strong evidence linking myocarditis after mRNA COVID-19 vaccine administration to SARS-CoV-2 or other viral infections (Abu Mouch et al, 2021, Mclean et al, 2021; D'Angelo et al, 2021; Albert et al, 2021; Muthukumar et al, 2021; Montgomery et al, 2021).

4.3.2.2. Genetic predisposition and pre-existing conditions

Possible predisposing factors for the development of myocarditis with COVID-19 mRNA vaccines, including genetic factors, baseline comorbidities, immunity or autoimmunity profile, have not been fully explored. In the reported case studies where evaluation of such predisposing factors were described, relevant information is briefly summarized below.

Prior history of myocarditis - In a case report, a 29-year-old man had an episode of recurrent acute myocarditis six days after the received the first dose of Moderna COVID-19 vaccine (mRNA-1273) (Tano et al, 2021). Although the causal nature of this recurrent myocarditis remains elusive, this case appears to be very peculiar as it occurred after the first dose of COVID-19 vaccine in a subject with a history of previously healed myocarditis (confirmed by cardiac magnetic resonance imaging) which had been treated with anti-inflammatory drugs with complete clinical resolution in 2013.

Prior history of SARS-CoV-2 infection – According to Bozkurt et al., approximately 11% of patients who developed myocarditis after COVID-19 vaccination previously had COVID-19 (Bozkurt et al, 2021). SARS-CoV-2 infection has been linked to the onset of autoimmune diseases (Talotta et al, 2021; Vojani et al, 2020), which may predispose individuals to

myocarditis after COVID-19 vaccination (see below "Pre-existing systemic immune-mediated diseases").

Pre-existing systemic immune-mediated diseases - Myocarditis has been described in association with many systemic immune-mediated diseases such as lupus, sarcoidosis, and others. In one case report, a patient who developed myocarditis after COVID-19 mRNA vaccination has a past medical history of asthma, autoimmune hypothyroidism, and chronic atrophic gastritis. A vaccine-triggered autoimmune reaction manifesting as acute myocarditis was speculated in a cased individual (Bautista et al, 2021); however, no autoantibody tests were performed in this cased individual. In another report, none of the individuals with pre-existing autoantibodies or anti-cytokine antibodies experienced adverse events, nor did levels of pre-existing autoantibodies or anti-cytokine antibodies change in response to vaccination (Arunachalam et al, 2021). In addition, in the USA, of the 8.5 million people with autoimmune diseases, 80% are women. This is in contrast the male predominance in myocarditis and pericarditis incidence post COVID-19 vaccination, arguing against pre-existing systemic immune-mediated diseases as a main cause of myocarditis and pericarditis after COVID-19 vaccination.

Genetic factors - Although there have been no reports of genetic mutations as a cause of myocarditis, many studies suggested that genes regulating inflammation may be important in determining susceptibility to disease. Genetic polymorphisms (eg. HLA allele polymorphism, Martinetti et al, 2011), epigenetic signatures (Movassagh et al, 2011a; Movassagh et al, 2011b), and sex chromosome complement (Robinson et al, 2011) may influence the type and severity of inflammation during myocarditis. Panel testing of genes potentially linked to cardiomyopathy was included in a case report, but were negative (Muthukumar et al, 2021). However, potential predisposing genetic factors for the development of myocarditis with COVID-19 vaccines have not yet been fully explored and understood.

Sex-related effects

Myocarditis has been considered as a male predominance disease historically (Fairweather D et al. 2013). Reported female to male ratio of myocarditis is between 1:1.5 and 1:1.7 (Mason et al, 1995; Caforio et al, 2007; Magnani et al, 2006). Reported female to male ratio of DCM is between 1:1.3 and 1:1.5 (Bagger et al, 1984; Gillum et al, 1986; Coughlin et al, 1993).

The reasons for male predominance in myocarditis and pericarditis incidence post COVID-19 vaccination remain unknown. In acute inflammatory reactions (such as sepsis or burns), women have a better clinical course and survival, while in chronic inflammatory diseases (such as severe asthma and chronic obstructive pulmonary disease) women fare worse (discussed in Lefèvre et al, 2019 introduction). Both hormonal and genetic reasons have be postulated. Testosterone was reported to play a role through multiple mechanisms including inhibition of anti-inflammatory cells (Fairweather et al, 2013; Lyden, 1987; Girón-González et al, 2000; Frisancho-Kiss et al, 2009) and commitment to a Th1-type immune response (Huber et al, 1994). However, differences in immune markers in prepubertal boys and girls suggest that sex hormones alone do not explain the differences (Lefèvre et al, 2019). Genetic mechanisms are postulated because women have two XX chromosomes vs one in men, and the X chromosome encodes for a variety or proteins involved in immune reactions, including

toll-like receptors (Lefèvre et al, 2019; Souyris et al, 2019). In female cells, normally one X chromosome is inactivated to equalize gene dosage with males, but it is estimated that 15 – 23% of X-linked genes escape inactivation so that both alleles are expressed (Souyris et al 2019), which could lead to differential immune responses in males vs females. In vitro studies have shown that stimulation of whole blood from males leads to greater cytokine release compared with females, and that the differences cannot be completely explained by concentrations of estradiol or testosterone (Lefèvre et al, 2019). On the other hand, immune cells from females have been shown to have higher TLR7-driven responses vs immune cells from males (Souyris et al, 2019); because TLR7 detects single-stranded RNA, and BNT162b2 is single-stranded RNA, this would be expected to result in greater inflammatory responses to vaccination in females. Thus, the role of sex in inflammatory responses is complex and not well understood. Another contributing factor of male predominance in myocarditis and pericarditis incidence post COVID-19 vaccination could be underdiagnosis in women.

4.3.3. Immune-mediated mechanisms

The involvement of host immune responses in myocarditis and inflammatory cardiomyopathy is well established. An example is viral myocarditis: in addition to the direct damages generated during viral infection, host immune responses may produce indirect lesions of the cardiac muscle by killing cardiomyocytes that are infected (antiviral immunity) or uninfected (autoimmunity) (Zhao et al, 2018). Although the vaccine-induced immune response is linked mainly to protective immunity, an undesired or exaggerated immune responses may potentially augment the risk of inflammation and immunopathology.

A system-level analysis of innate and adaptive immunity to an mRNA vaccine revealed that BNT162b2 vaccination stimulated antiviral immunity with little type I IFN response after the first dose, and a notably enhanced innate response after the secondary immunization (Arunachalam et al, 2021).

4.3.3.1. mRNA-LNP platform associated mechanisms of host immune activation

COVID-19 mRNA vaccines contain LNP-encapsulated nucleoside-modified mRNA encoding the viral spike glycoprotein of SARS-CoV-2. The increased cases of myocarditis and pericarditis have been reported mainly after mRNA COVID-19 vaccination, while there has not been a similar reporting pattern observed after administration of COVID-19 vaccines using adenovirus vector vaccine platform. It is therefore worth discussing potential immune-mediated mechanisms related with the mRNA vaccine platform.

One of the major hurdles during the development of the mRNA therapeutic platform is the degree of reactogenicity. The presence of unmodified uridine chemistry and dsRNA impurities contribute to the mRNA-LNP-associated immune activation in vitro and in vivo (Nelson et al, 2020). Non-self nucleic acids are usually identified by two families of PRRs: the endosomal membrane-bound TLR and cytoplasmic sensors of viral nucleic acids. Although the BNT162b2 mRNA vaccine is optimized to reduce its detection by the innate immune system through the addition of nucleoside modifications and minimizing double-strand RNA impurity, it is possible, especially in certain individuals with genetic predisposition and underlying conditions that the immune responses to mRNA may not be

sufficiently turned down and drive the activation of an innate and adaptive immune response (Pelka et al, 2016). This may lead to the excessive activation of proinflammatory cascades which contribute to the development of myocarditis.

Lipid components in the LNP may also activate host immune responses following systemic or local administration (Hou et al, 2021). For example, PEG-lipids may stimulate the complement system. Cationic and ionizable lipids have also been reported to stimulate the secretion of pro-inflammatory cytokines and reactive oxygen species. Although the immune responses to these lipids has not yet been fully understood, complement system and Toll-like receptors may participate in innate immune activation (Hou et al, 2021). Also, minor differences in biophysical characteristics of LNP (eg. particle size, homogeneity, shape and liposome lamellarity), may also have an effect.

4.3.3.2. Vaccine-associated autoimmunity

Vaccine-associated autoimmunity is a well-known phenomenon attributed most often to either the cross-reactivity between antigens or the effect of adjuvant. For example, HPV vaccine is linked to Guillain-Barré syndrome and other neuropathies (Watad et al, 2017). For COVID-19 mRNA vaccines, this matter becomes more complicated due to the nucleic acid formulation and presence of LNP as discussed above. SARS-CoV-2 infection has been linked to the development of autoantibodies and the onset of autoimmune diseases (Talotta et al, 2021; Vojani et al, 2020). This observation raises the speculation that a similar scenario might occur following COVID-19 vaccination (Talotta et al, 2021). However, it is worth noting that immune responses to COVID-19 infection and the BNT162b2 vaccination are different (Arunachalam et al, 2020; Arunachalam et al, 2021). In one study, none of the healthy volunteers are reported to develop autoantibodies after BNT162b2 vaccination (Arunachalam et al, 2021).

In one published report of myocarditis after COVID-19 vaccination (Muthukumar et al, 2021), a 52-year-old man developed symptoms consistent with myocarditis 3 days after receiving the second dose of Moderna COVID vaccine (mRNA-1273). This patient was negative for active SARS-CoV-2 infection and has no previous history of SARS-CoV-2 infection. The SARS-CoV-2 spike IgM and IgG neutralizing antibody levels were comparable in this patient versus a vaccinated individual without myocarditis, suggesting that myocarditis in this cased individual is not vaccine antibody mediated. Also, the patient was negative in a genetic panel testing of 121 genes potentially linked to cardiomyopathy, arguing against an existing genetic predisposition to cardiomyopathy. Screening of cytokine response in the case patient revealed elevated levels of 4 cytokines (IL-1ra, IL-5, IL-16, and MIG), diminished levels of 1 cytokine LIF (leukemia inhibitory factor), and 3 other cytokines (IL-10, MIF, and VEGF) with bidirectional pattern (increase or decrease) relative to an unvaccinated individual or a vaccinated individual without myocarditis. Also, an elevated frequency of a distinct subset of NK cells (CD3negCD16posCD56pos) was observed. Moreover, higher levels of antibodies against some self-antigens such as aquaporin 4, endothelial cell antigen, and proteolipid protein 1 were reported in this patient. Therefore, in this particular case, autoantibody generation may be one of the mechanisms for this individual to develop myocarditis after vaccination.

4.3.3.3. Delayed hypersensitivity

Published reports to date do not suggest a delayed hypersensitivity reaction (D'Angelo et al, 2021; Bozkurt B et al, 2021). None of the case reports published to date had evidence of eosinophilia in peripheral blood or immune complex deposition or eosinophilic infiltrates in endomyocardial biopsy samples arguing against hypersensitivity, allergic or eosinophilic myocarditis (Bozkurt et al, 2021).

4.3.4. Molecular mimicry to spike protein

Another potential mechanism for myocarditis and pericarditis is molecular mimicry between the spike protein of SARS-CoV-2 and self-antigens. A recent preprint (Marrama et al, 2021) examined whether previously identified myocarditis antigens (35 antigens were explored) contained any sequence homology to SARS-CoV-2 spike protein-derived peptides. The authors found no significant enrichment and concluded that increased occurrence of myocarditis after SARS-CoV-2-spike vaccination is not supported to be mediated by a cross-reactive adaptive immune response.

In order to further explore the phenomenon of molecular mimicry, we undertook a study (21IT091) to determine if there are human endogenous proteins that contain peptides with strong homology to the spike protein. Such homology could potentially lead to immune responses raised against the vaccine to cross react with human proteins. The SARS-CoV-2 spike protein sequence (1,275 amino acids) that was used to construct BNT162b2 was used to search the public protein database UniProt for human proteins containing homologous peptides. Since the focus of the study was to understand if there was an association to peri or myocarditis, heart expression data (Human Protein Atlas and internal Zoomap) was also considered for any potential endogenous proteins containing homologous peptides.

No peptides ≥9 amino acids with 100% similarity in human endogenous proteins were identified. Only two proteins containing 8-mers with 100% similarity were identified; unconventional myosin-XVI (MYO16), a cytoplasmic protein and amiloride-sensitive sodium channel subunit alpha (SCNN1A) that is a plasma membrane protein. Neither of these proteins are predominantly expressed in the heart, MYO16 is most highly expressed in the cerebral cortex, testes and frontal lobe at the mRNA level (Zoomap). SCNN1A has broad tissue expression and is very low in heart (Zoomap). Therefore, these two proteins are not considered to be potential antigen mimics that could lead to peri or myocarditis.

The typical length of MHC-I peptide epitopes is 9-mers and MHC-II peptide epitopes is 15-mers respectively, however the percent similarity required for cross-reactivity is uncertain. Therefore, proteins containing shorter peptides with 75-100% similarity were evaluated further. There were 22 heptapeptides and 354 hexapeptides identified with 100% homology to human proteins. These results are very similar to those reported by Kundac and Shoenfeld, 2020. Since it is not certain that 100% homology is necessary for cross-reactivity, human proteins containing peptides (6-10-mers) with 75-100% homology to the spike protein were also considered. Exploring tissue distribution of these proteins (1689 total) showed that only 45 were predominantly (although not exclusively) expressed in the heart. In order to understand if any of the homologous peptide sequences were likely to be antigenic, the immunogenicity potential was explored with predictive algorithms in the Immune Epitope

Database: T-cell epitope prediction (http://tools.iedb.org/main/tcell/). This resulted in six proteins of which only one, nebulette (NEBL) has a heart-specific expression profile and may be involved in cardiac myofibril assembly. To determine if NEBL is a true antigenic mimic and leads to cross reactivity needs to be tested.

In conclusion, antigen mimicry is an unlikely mechanism of vaccine induced peri or myocarditis. It is also possible that multiple mechanisms could be involved between individuals, or within the same individual. Additional experiments need to be performed to determine the mechanism(s) involved.

5. ANIMAL MODELS OF MYOCARDITIS AND PERICARDITIS

Myocarditis models

Myocarditis has an assortment of causes, including infectious, autoimmune, and toxic. Many animal models have been developed to help elucidate the pathophysiology of disease induction by these various mechanisms. Infectious disease models of myocarditis include viral (e.g., coxsackievirus B3 [CVB3]-induced), bacterial (e.g., Borrelia-induced), or protozoal (e.g., Trypomastigotes-induced). Non-infectious disease models include autoimmune myocarditis (eg, experimental autoimmune myocarditis [EAM], which is induced by administration of cardiac myosin or Complete Freund's Adjuvant), and toxic models to understand drug-induced toxicity (eg, PD-1 deficient or CTLA-4 knockout mice to study immune check point inhibitor-related myocarditis) (Blyszczuk 2019; Tschöpe et al, 2021; Upadhrasta et al, 2019).

Most often, mice and rats are the species used for myocarditis models. The relatively low cost, ease of handling, short disease induction times, and rapidity of development of phenotypes are the primary reasons for their use in myocarditis studies. Although larger animals such as dogs, NHPs, guinea pigs, sheep, and pigs may have more direct relevance to humans, they have considerable disadvantages compared to mouse or rat in terms of expense, space, reagents, and ethics. Because of this, large animal models have been rarely used to study myocardial disease (Ross et al, 2013).

Disease course in both infectious and non-infectious animal models depend upon the genetic background (i.e., species and strain) and sex. Mice are often used. In the CVB3-induced myocarditis model, BALB/c, A.BY/SnJ, and A/J mice, but not C57BL/6, progress to a phenotype of inflammatory dilated cardiomyopathy (DCM), characterized by chronic myocarditis, myocardial fibrosis, and cardiomyopathy (Fairweather et al, 2007). Similarly, mice on BALB/c, A/J or ASW background which have more mast cells expressing TLR4 are susceptible to EAM, while mice on C57BL/6 background are resistant (Blyszczuk 2019). Chronic inflammation develops in male animals more often than females. Male mice with CVB3-induced myocarditis develop more severe myocardial inflammation than females; the detrimental immune response in male individuals is driven by a predominant M1 macrophage response, while female animals show a stronger M2 macrophage response (Di Florio et al, 2020). Cardiac inflammation during CVB3-induced myocarditis in mice was increased by testosterone and reduced by 17β-estradiol (Coronado et al 2019). In the EAM model, male

Lewis rats showed an impaired inflammatory response and an exaggerated collagen deposition affecting the cardiac function while females demonstrated a protective response (Barcena, 2021). Animal age is another determinant for the development of myocarditis. Murine adenovirus-1 was shown to induce myocarditis in C57Bl/6 mice age in an age dependent manner causing neonatal mice to develop lethal infection; on the other hand, infection was not lethal in adult mice (McCarthy et al, 2015). Finally, housing conditions or environmental factors may also influence the myocarditis outcome in these animals. For example, in CVB3-induced models mice exposed to bisphenol A leached from plastic cages and water bottles had increased myocarditis and pericarditis compared with mice housed in glass cages that drank out of glass water bottles (Bruno et al, 2019).

Both infectious and non-infectious models have their advantages and disadvantages. Infectious models combine the immune response involved in pathogen clearance with autoimmune responses and hence more closely reflect the physiological processes in the human disease than do models of EAM. For example, high titers of heart-specific autoantibodies (eg, troponin autoantibodies) have been detected in mouse models following CVB3 infection; heart-specific autoantibodies are also commonly detected in human myocarditis patients (Blyszczuk 2019). Like humans, autoimmune responses (eg, to cardiac myosin) following infection in animal models contribute to the cardiac pathology (Huber, 1997; Blyszczuk 2019). However, handling of zoonotic infectious agents is hazardous and requires special containment, and it is often difficult to uncouple autoimmune responses from immune responses involved in pathogen clearance in infectious models. Non-infectious models facilitate the investigation of the progression of myocarditis to inflammatory cardiomyopathy and DCM and allow interrogation of the involvement of specific components of the immune system in the disease process without complications from the infectious agent or autoimmune responses that are often generated in infectious models.

The course of myocarditis among humans varies widely, depending upon the individual genetic makeup, causative agents, autoimmune response, course of inflammation, progression to DCM/inflammatory DCM, age, and sex (Di Florio et al, 2020; Elamm et al, 2012; Tschöpe et al, 2021). These factors should be carefully considered along with animal specific factors described above (such as strain, housing conditions) when developing an animal model of myocarditis. Although many animal models have been developed, they rarely if ever reproduce the full spectrum of human myocarditis. Animal models have been rarely used to study vaccine-associated myocarditis and/or pericarditis. A recent study showed myopericarditis (WBC infiltration with myocardial degeneration) in a BALB/c mouse model upon intravenous administration of the BNT162b2 mRNA vaccine (Li C et al, 2021). However, the IV dose used in this study was extremely high (almost 500 times the dose given to humans) and the animals did not develop heart lesions with the clinically recommended IM route of administration, making it unclear if this model is translatable to humans.

Animal models for COVID-19-related myocarditis were reviewed early in the pandemic (Cleary et al, 2020a; Cleary et al, 2020b; Muñoz-Fontela et al, 2020). SARS-Cov-2 spike protein does not effectively bind mouse or rat ACE2 receptors, and therefore normal mice are not suitable models for evaluating SARS-Cov-2 infection by wild type virus (Zhao et al 2020, Piplani et al 2021). This also means that normal mice or rats are likely not suitable for assessing direct effects of the spike protein binding to ACE2 receptors, or downstream effects. However, recently emerging SARS-Cov-2 variants containing N501Y can infect mice and rats and lead to disease (Shuai et al 2021). Transgenic mouse models where human ACE2 receptors are expressed have been developed (Muñoz-Fontela et al 2020). In contrast to the mouse, the spike protein of "original" SARS-Cov-2 does bind to ACE2 receptors in the Syrian hamster, ferret, rabbit, rhesus monkey, and cynomolgus monkey (as well as other species) (Zhao et al 2020, Piplani et al 2021), making them potential models for assessing effects related to spike protein binding to ACE2 receptors (in addition to being models to study infection [Cleary et al, 2020a; Cleary et al, 2020b; Muñoz-Fontela et al 2020]). It should also be noted that spike protein has been shown to circulate in exosomes (Bansal et al 2021), and these could fuse with cardiomyocytes (Eguchi et al 2019) and allow spike protein to enter the cell.

Pericarditis models

Unlike myocarditis, the number of animal models developed and tested for pericarditis is rather limited. There are few reports in the literature on animal models of pericarditis, including right ventricular perimyocarditis induced by CVB3 infection in BALB/c mice (Matsumori et al 1980), pericarditis in sheep induced by injection of heat-killed staphylococci and Freund's adjuvant directly into the peritoneal cavity (Leak et al, 1987), development of pericarditis in TGF-β1–KO mice (Kulkarni et al, 1995), and pericardiectomy-induced pericarditis in dogs and pigs (Page et al, 1986; Loging et al, 199). None of these earlier models demonstrated the presence of pericardial constriction which is a rare but significant complication of acute pericarditis. To study constrictive pericarditis associated with autoimmune heart disease IFN-γ–knockout mice model was developed. This model develops grossly detectable adhesive pericarditis and is characterized by increased pericardial inflammation and fibrosis (Afanasyeva et al, 2004). Recently, a rat model of constrictive pericarditis has been developed by injecting a solution of 1-mg/mL lipopolysaccharides [0.5 mL] and a 10% talc suspension [0.5 mL] (Wang et al, 2020). The role of NLRP3 inflammasome in pericarditis was studied recently by developing a mouse model of acute pericarditis through the intrapericardial injection of zymosan A (Mauro et al, 2021).

Conclusion: Currently there are no good, established animal models to study vaccine-associated myocarditis and/or pericarditis. Good animal models should exhibit adequate homology (genetic similarity) or analogy (functional similarity), and be translatable to humans (Swearengen, 2018). Development of a good animal model first requires identifying the relationship of the vaccine to a known mechanism(s), which can then be reproduced in the animal. Although various vaccines have been associated with myocarditis (details in

section 4.2), the incidence is generally very low and underlying cause/pathogenesis is unknown. The mechanism and pathogenesis of reported COVID-19 vaccine-related myocarditis is also unknown at the present time. Although it should be possible to determine the effect of vaccination on a model with underlying autoimmunity or DCM, the translatability to humans is unclear. Thus, there is currently no good model of vaccine-associated myocarditis and/or pericarditis in animals, although model development could be explored.

6. OTHER POTENTIAL INVESTIGATIONS INTO CAUSE OF MYOCARDITIS/MYOPERICARDITIS

The diagnosis of myocarditis/myopericarditis is primarily based on clinical presentation, histopathology and immunohistochemistry (including anti-CD3, T lymphocytes; anti-CD68, macrophages; anti-CD20, B lymphocytes; and anti HLA-DR, antigen presenting cells including macrophages, dendritic cells and B-cells) of endomyocardial biopsy samples (gold standard), advanced non-invasive imaging methods (ECG, nuclear imaging, cardiovascular magnetic resonance [CMR] imaging), presence of inflammatory biomarkers (C-reactive protein, erythrocyte sedimentation rate), cardiac biomarker elevations (cardiac troponins and natriuretic peptides), inflammatory and immune cell markers and cell ratios, microRNAs and antibodies (Tschöpe et al, 2021). Circulating exosome analysis could provide an innovative future approach to myocarditis/myopericarditis diagnosis. The presence of serum exosomes containing a specific miRNA profile (panel including has-miR-30a, hsa-miR192, hsa-miR-146a, hsa-miR-155, and hsa-miR-320a may serve as more sensitive indicators of myocarditis than cTnI, but would not distinguish vaccine-related myocarditis from other causes (Yingying et al, 2021).

There are no biomarkers that provide a definitive diagnosis of vaccine-related myocarditis (vaccine as cause of myocarditis). The diagnosis of vaccine-related myocarditis is based on clinical presentation and exclusion of other causes of myocarditis, including microbial infection, systemic disease (eg, coronary artery disease, kidney failure, cancer, tuberculosis), exposure to cardiotoxic substances (eg, phenytoin, warfarin, heparin, procainamide), and systemic immune-mediated diseases. Infectious myocarditis is confirmed by viral genome analysis of EMB samples via quantitative PCR. Autoimmune myocarditis has negative PCR for infectious agents with or without serum cardiac autoantibodies (anti-heart autoantibodies (AHAs) and anti-intercalated disk autoantibodies (AIDAs) (Caforio et al, 2013). In addition, the idiosyncratic nature of myocarditis/myopericarditis suggests that genetic, host, and environmental factors contribute to susceptibility, progression, and prognosis. Provocation tests could ascertain the causal relation between vaccination and myocarditis/myopericarditis, but these are generally not performed for ethical reasons.

Although there are no definitive tests to prove a causal link between the administration of vaccine and myo/myopericarditis, human tissues may provide insight into the mechanisms involved. Potential investigations to characterize immune-related mechanisms include circulating antibody characterization from affected and unaffected vaccinated and unvaccinated individuals, inflammatory cytokine analysis, complement activation, evaluation of circulating leukocyte frequencies, absolute numbers and phenotypes, HLA typing on DNA

extracted from peripheral blood and heart, and HLA class I and II expression on cardiac tissue. Immunophenotyping of circulating immune cells may be informative because changes in peripheral T cell subsets including regulatory T cells, Th1, Th17 cells and autoreactive alpha-myosin heavy chain autoreactive T cells have been associated with myocarditis (Vdovenko et al, 2018, Zarak-Crnkovic et al, 2019, Myers et al, 2016) . In addition, circulating dendritic cells subsets have been shown to be reduced in humans with myocarditis (Pistulli et al, 2020). If available and obtainable, stored pre- and post-vaccination serum specimens from affected and unaffected military personnel could be assessed for pre-existing serum anti-heart antibodies to rule out autoimmune mechanisms. In addition to tissue analysis, a variety of in vitro assays may be utilized to elucidate myocarditis/myopericarditis mechanisms, including auto-reactive T cell and B cell proliferation assays, cytokine induction by cardiac antigens, cytotoxic response assays, and surface plasmon resonance assays to characterize potential autoantibodies involved in myocarditis (Landsberger et al, 2008).

Tissue cross-reactivity assays comparing binding of healthy convalescent plasma or affected plasma to healthy human heart tissue or affected heart tissue could demonstrate an antibody-mediated immune mechanism. A similar analysis could assess binding to extracted myocardial proteins.

If in silico investigations suggest that molecular mimicry could play a role in vaccine-associated myocarditis/myopericarditis, clinical specimens could be interrogated for infiltrating lymphocytes in myocarditis/myopericarditis and injection site by T cell receptor next generation sequencing (CDR3 region, the antigen-binding portion of the T cell receptor beta chain) could address the distribution, clonality and diversity of T cell receptor, and determine if clones are shared between cardiac lymphocytes and injection site lymphocytes. In addition to the role of T cells, mechanisms that involve other cellular components of the immune system including B cells, NK cells, dendritic cells, and macrophages can be explored both histologically and using in vitro assays.

7. SUMMARY OF POTENTIAL MYOCARDITIS/PERICARDITIS MECHANISM(S) AND RECOMMENDED ACTIVITIES

Myocarditis and/or pericarditis have previously been linked with vaccine administration, especially smallpox vaccination; however, a definitive mechanism has not been identified. In reviewing the potential known causes of myocarditis or pericarditis in general, an immune-mediated mechanism seems most likely. It is possible that the innate immune response generated by the mRNA COVID-19 vaccines could lead to myocarditis in individuals with an underlying condition/predisposition to myocarditis.

There are no established models for vaccine-related myocarditis and translatability of any results generated in vitro or in vivo in animal models to patients is unclear. Until a better understanding of what mechanism may be leading to myocarditis in patients, it is recommended that additional in vitro or nonclinical in vivo studies be conducted with great caution, they may not generate useful data, and there is a chance that spurious or irrelevant findings may cause issues. It is recommended that additional investigations be considered with samples (eg, blood, serum, heart tissue, etc) or information (eg, genetics) from humans that experienced myocarditis after vaccination is obtained.

At the present time, there are a number of discussions on what additional work could be done, and what should be done. Rather than include that rapidly evolving information in this white paper, colleagues should reach out to contributors to this document for the latest information.

8. REFERENCES

Abu Mouch S, Roguin A, Hellou E, Ishai A, Shoshan U, Mahamid L, Zoabi M, Aisman M, Goldschmid N, Berar Yanay N. Myocarditis following COVID-19 mRNA vaccination. Vaccine. 2021;39:3790–3793. doi: 10.1016/j.vaccine.2021.05.087

Afanasyeva M, Georgakopoulos D, Fairweather D, Caturegli P, Kass DA, Rose NR. Novel model of constrictive pericarditis associated with autoimmune heart disease in interferon-gamma-knockout mice. Circulation. 2004 Nov 2;110(18):2910-7.

Albert E, Aurigemma G, Saucedo J, Gerson DS. Myocarditis following COVID-19 vaccination. Radiol Case Rep. 2021;16:2142–2145. doi: 10.1016/j.radcr.2021.05.033

Arness MK, Eckart RE, Love SS, Atwood JE, Wells TS, Engler RJ, Collins LC, Ludwig SL, Riddle JR, Grabenstein JD, Tornberg DN. Myopericarditis following smallpox vaccination. Am J Epidemiol. 2004 Oct 1;160(7):642-51. doi: 10.1093/aje/kwh269. PMID: 15383408.

Bansal, S., Perincheri, S., Fleming, T., Poulson, C., Tiffany, B., Bremner, R. M., & Mohanakumar, T. (2021). Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines. Journal of Immunology, 207(10), 2405-2410. https://doi.org/10.4049/jimmunol.2100637

Barcena ML, Jeuthe S, Niehues MH, et al. Sex-Specific Differences of the Inflammatory State in Experimental Autoimmune Myocarditis. Front Immunol. 2021;12:686384.

Barda N, Dagan N, Ben-Shlomo Y, et al. Safety of the BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Setting. N Engl J Med. 2021;385(12):1078-1090.

Bautista GJ, Pena OP, Bonilla Fernandez JA, Cardenes LA, Ramirez BL, Caballero DE. Acute myocarditis after administration of the BNT162b2 vaccine against COVID-19. Rev Esp Cardiol (Engl Ed). Published online April 27, 2021;S1885-5857(21)00133-X. doi: 10.1016/j.rec.2021.04.005.

Blyszczuk, P. Myocarditis in humans and in experimental animal models. Front. Cardiovasc. Med. 6, 64 (2019).

Bozkurt B, Kamat I, and Hotez PJ. Myocarditis With COVID-19 mRNA Vaccines. Circulation. 2021;144:471–484. DOI: 10.1161/CIRCULATIONAHA.121.056135

Boccara F, Benhaiem-Sigaux N, Cohen A. Acute myopericarditis after diphtheria, tetanus, and polio vaccination. Chest. 2001 Aug;120(2):671-2. doi: 10.1378/chest.120.2.671. PMID: 11502677.

Bruno KA, et al. BPA alters estrogen receptor expression in the heart after viral infection activating cardiac mast cells and T cells leading to perimyocarditis and fibrosis. Front. Endocrinol. 2019;10:598. doi: 10.3389/fendo.2019.00598.

Caforio AL, Calabrese F, Angelini A, Tona F, Vinci A, Bottaro S, Ramondo A, Carturan E, Iliceto S, Thiene G, Daliento L. A prospective study of biopsy-proven myocarditis: prognostic relevance of clinical and aetiopathogenetic features at diagnosis. Eur Heart J. 2007 Jun;28(11):1326-33. doi: 10.1093/eurheartj/ehm076. Epub 2007 May 9. PMID: 17493945.

Caforio ALP, Pankuweit S, Arbustini E, et al. Current state of knowledge on aetiology, diagnosis, management, and therapy of myocarditis: a position statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases. Eur Heart J. 2013;34(33):2636-48, 2648a-2648d.

Cassimatis DC, Atwood JE, Engler RM, Linz PE, Grabenstein JD, Vernalis MN. Smallpox vaccination and myopericarditis: a clinical review. J Am Coll Cardiol. 2004 May 5;43(9):1503-10. doi: 10.1016/j.jacc.2003.11.053. PMID: 15120802.

CDC. Clinical considerations: Myocarditis and pericarditis after receipt of mRNA COVID-19 vaccines among adolescents and young adults. 2021. https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html

Chamling B, Vehof V, Drakos S, Weil M, Stalling P, Vahlhaus C, Mueller P, Bietenbeck M, Reinecke H, Meier C, and Yilmaz A. Occurrence of acute infarct-like myocarditis following COVID-19 vaccination: just an accidental co-incidence or rather vaccination-associated autoimmune myocarditis? Clin Res Cardiol (2021). https://doi.org/10.1007/s00392-021-01916-w

Chen HX, Chen ZH, Shen HH. [Structure of SARS-CoV-2 and treatment of COVID-19]. Sheng Li Xue Bao. 2020 Oct 25;72(5):617-630. Chinese. PMID: 33106832.

Cheng MP, Kozoriz MG, Ahmadi AA, Kelsall J, Paquette K, Onrot JM. Post-vaccination myositis and myocarditis in a previously healthy male. Allergy Asthma Clin Immunol. 2016 Feb 11;12:6. doi: 10.1186/s13223-016-0114-4. PMID: 26877725; PMCID: PMC4751718.

Claydon SM. Myocarditis as an incidental finding in young men dying from unnatural causes. Med Sci Law. 1989;29(1):55-8.

Cleary, S. J., Magnen, M., Looney, M. R., & Page, C. P. (2020a). Update on animal models for COVID-19 research. British Journal of Pharmacology, 177(24), 5679-5681. https://doi.org/10.1111/bph.15266.

Cleary, S. J., Pitchford, S. C., Amison, R. T., Carrington, R., Robaina Cabrera, C. L., Magnen, M., Looney, M. R., Gray, E., & Page, C. P. (2020b). Animal models of mechanisms of SARS-CoV-2 infection and COVID-19 pathology. British Journal of Pharmacology, 177(21), 4851-4865. https://doi.org/10.1111/bph.15143.

Cohen JI, Hohman P, Fulton R, Turk SP, Qin J, Thatcher K, Hornung RL. Kinetics of serum cytokines after primary or repeat vaccination with the smallpox vaccine. J Infect Dis. 2010 Apr 15;201(8):1183-91. doi: 10.1086/651453. PMID: 20214479; PMCID: PMC2839010.

Cooper, LT Jr. Myocarditis. N Engl J Med. 2009 Apr 9; 360(15): 1526–1538.

Coronado MJ, Bruno KA, Blauwet LA, et al. Elevated Sera sST2 Is Associated With Heart Failure in Men ≤50 Years Old With Myocarditis. J Am Heart Assoc. 2019;8(2):e008968. doi:10.1161/JAHA.118.008968

Coughlin SS, Comstock GW, Baughman KL. Descriptive epidemiology of idiopathic dilated cardiomyopathy in Washington County, Maryland, 1975-1991. J Clin Epidemiol. 1993;46:1003–8.

D'Angelo T, Cattafi A, Carerj ML, Booz C, Ascenti G, Cicero G, Blandino A, Mazziotti S. Myocarditis after SARS-CoV-2 vaccination: a vaccine induced reaction? Can J Cardiol. Published online June 9, 2021;S0828- 282X(21)00286-5. doi: 10.1016/j.cjca.2021.05.010

Di Florio DN, Sin J, Coronado MJ, Atwal PS, Fairweather D. Sex Differences in Inflammation, Redox Biology, Mitochondria and Autoimmunity. Redox Biol (2020) 31:101482.

Eckart RE, Love SS, Atwood JE, Arness MK, Cassimatis DC, Campbell CL, Boyd SY, Murphy JG, Swerdlow DL, Collins LC, Riddle JR, Tornberg DN, Grabenstein JD, Engler RJ; Department of Defense Smallpox Vaccination Clinical Evaluation Team. Incidence and follow-up of inflammatory cardiac complications after smallpox vaccination. J Am Coll Cardiol. 2004 Jul 7;44(1):201-5. doi: 10.1016/j.jacc.2004.05.004. PMID: 15234435.

Eguchi, S., Takefuji, M., Sakaguchi, T., Ishihama, S., Mori, Y., Tsuda, T., Takikawa, T., Yoshida, T., Ohashi, K., Shimizu, Y., Hayashida, R., Kondo, K., Bando, Y. K., Ouchi, N., & Murohara, T. (2019). Cardiomyocytes capture stem cell-derived, anti-apoptotic microRNA-214 via clathrin-mediated endocytosis in acute myocardial infarction. Journal of Biological Chemistry, 294(31), 11665-11674. https://doi.org/10.1074/jbc.RA119.007537.

Elamm C, Fairweather D, Cooper LT. Pathogenesis and diagnosis of myocarditis. Heart. 2012 Jun;98(11):835-40.

Elfström P, Hamsten A, Montgomery SM, et al. Cardiomyopathy, pericarditis and myocarditis in a population-based cohort of inpatients with coeliac disease. J Intern Med. 2007;262(5):545-54.

Engler RJ, Nelson MR, Collins LC Jr, Spooner C, Hemann BA, Gibbs BT, Atwood JE, Howard RS, Chang AS, Cruser DL, Gates DG, Vernalis MN, Lengkeek MS, McClenathan BM, Jaffe AS, Cooper LT, Black S, Carlson C, Wilson C, Davis RL. A prospective study of the incidence of myocarditis/pericarditis and new onset cardiac symptoms following smallpox and influenza vaccination. PLoS One. 2015 Mar 20;10(3):e0118283. doi: 10.1371/journal.pone.0118283. PMID: 25793705; PMCID: PMC4368609.

Evers, M. J. W., Du, W., Yang, Q., Kooijmans, S. A. A., Vink, A., van Steenbergen, M., Vader, P., de Jager, S. C. A., Fuchs, S. A., Mastrobattista, E., Sluijter, J. P. G., Lei, Z., & Schiffelers, R. (2022). Delivery of modified mRNA to damaged myocardium by systemic administration of lipid nanoparticles. Journal of Controlled Release, 343, 207-216. https://doi.org/10.1016/j.jconrel.2022.01.027.

Fairweather D, Cooper LT, Jr, and Blauwet LA. Sex and Gender Differences in Myocarditis and Dilated Cardiomyopathy. Curr Probl Cardiol. 2013 Jan; 38(1): 7–46.

Fairweather D, Rose NR. Coxsackievirus-induced myocarditis in mice: a model of autoimmune disease for studying immunotoxicity. Methods. (2007) 41:118–22.

Finlay-Jones LR. Fatal myocarditis after vaccination against smallpox. Report of a case. N Engl J Med. 1964 Jan 2;270:41-2. doi: 10.1056/NEJM196401022700108. PMID: 14062126.

Frisancho-Kiss S, Coronado MJ, Frisancho JA, Lau VM, Rose NR, Klein SL, Fairweather D. Gonadectomy of male BALB/c mice increases Tim-3(+) alternatively activated M2 macrophages, Tim-3(+) T cells, Th2 cells and Treg in the heart during acute coxsackievirus-induced myocarditis.Brain Behav Immun. 2009; 23:649–657. doi: 10.1016/j.bbi.2008.12.002

Gatta AM, Pieroni P. Miocardite acuta mortale dopo vaccinazione antivaiolosa [Acute fatal myocarditis following smallpox vaccination]. Minerva Pediatr. 1976 Sep 8;28(27):1691-6. Italian. PMID: 1012203.

Gillum RF. Idiopathic cardiomyopathy in the United States, 1970-1982. Am Heart J. 1986;111:752–5.

Girón-González JA, Moral FJ, Elvira J, García-Gil D, Guerrero F, Gavilán I, Escobar L. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared with women.Eur J Endocrinol. 2000; 143:31–36. doi: 10.1530/eje.0.1430031

Goulter AB, Goddard MJ, Allen JC, Clark KL. ACE2 gene expression is up-regulated in the human failing heart. BMC Med. 2004 May 19;2:19. doi: 10.1186/1741-7015-2-19. PMID: 15151696; PMCID: PMC425604.

Grabenstein JD, Winkenwerder W Jr. US military smallpox vaccination program experience. JAMA. 2003 Jun 25;289(24):3278-82. doi: 10.1001/jama.289.24.3278. PMID: 12824209.

Halsell JS, Riddle JR, Atwood JE, Gardner P, Shope R, Poland GA, Gray GC, Ostroff S, Eckart RE, Hospenthal DR, Gibson RL, Grabenstein JD, Arness MK, Tornberg DN; Department of Defense Smallpox Vaccination Clinical Evaluation Team. Myopericarditis following smallpox vaccination among vaccinia-naive US military personnel. JAMA. 2003 Jun 25;289(24):3283-9. doi: 10.1001/jama.289.24.3283. PMID: 12824210.

Heymans, S., & Cooper, L. T. (2022). Myocarditis after COVID-19 mRNA vaccination: clinical observations and potential mechanisms. Nature Reviews: Cardiology, 19(2), 75-77. https://doi.org/10.1038/s41569-021-00662-w.

Helle EP, Koskenvuo K, Heikkilä J, Pikkarainen J, Weckström P. Myocardial complications of immunisations. Ann Clin Res. 1978 Oct;10(5):280-7. PMID: 736507.

Hikmet F, Méar L, Edvinsson Å, Micke P, Uhlén M, Lindskog C. The protein expression profile of ACE2 in human tissues. Mol Syst Biol. 2020 Jul;16(7):e9610. doi: 10.15252/msb.20209610. PMID: 32715618; PMCID: PMC7383091.

Hou X, Zaks T, Langer R, and Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021 Aug 10;1-17. doi: 10.1038/s41578-021-00358-0.

Huber SA. Autoimmunity in myocarditis: relevance of animal models. Clin Immunol Immunopathol. 1997 May;83(2):93-102.

Huber SA, Pfaeffle B. Differential Th1 and Th2 cell responses in male and female BALB/c mice infected with coxsackievirus group B type 3.J Virol. 1994; 68:5126–5132. doi: 10.1128/JVI.68.8.5126-5132.1994

Husby, A., Hansen, J. V., Fosbøl, E., Thiesson, E. M., Madsen, M., Thomsen, R. W., Sørensen, H. T., Andersen, M., Wohlfahrt, J., Gislason, G., Torp-Pedersen, C., Køber, L., & Hviid, A. (2021). SARS-CoV-2 vaccination and myocarditis or myopericarditis: population - based cohort study. BMJ, 375, e068665. https://doi.org/10.1136/bmj-2021-068665

Human Protein Atlas. Available at: https://www.proteinatlas.org/ENSG00000130234-ACE2/tissue. Accessed on: 20 Sep 2021.

Imazio M, Trinchero R. Myopericarditis: Etiology, management, and prognosis. Int J Cardiol. 2008 Jun 23;127(1):17-26. doi: 10.1016/j.ijcard.2007.10.053. Epub 2008 Jan 24. PMID: 18221804.

Kanduc D, Shoenfeld Y. Molecular mimicry between SARS-CoV-2 spike glycoprotein and mammalian proteomes: implications for the vaccine Immunol Res. 2020 Oct;68(5):310-313. doi: 10.1007/s12026-020-09152-6. Epub 2020 Sep 18.PMID: 32946016.

Kim YJ, Bae JI, Ryoo SM, Kim WY. Acute Fulminant Myocarditis Following Influenza Vaccination Requiring Extracorporeal Membrane Oxygenation. Acute Crit Care. 2019 May;34(2):165-169. doi: 10.4266/acc.2017.00045. Epub 2018 Nov 7. PMID: 31723923; PMCID: PMC6786665.

Kitulwatte ID, Kim PJH, Pollanen MS. Sudden death related myocarditis: a study of 56 cases. Forensic Sci Med Pathol. 2010;6(1):13-9.

Kulkarni AB, Ward JM, Yaswen L, et al. Transforming growth factor-beta 1 null mice: an animal model for inflammatory disorders. Am J Pathol. 1995; 146: 264–275.

Kytö V, Sipilä J, Rautava P. The effects of gender and age on occurrence of clinically suspected myocarditis in adulthood. Heart. 2013;99(22):1681-4.

Lampejo T, Durkin SM, Bhatt N, Guttmann O. Acute myocarditis: aetiology, diagnosis and management. Clin Med (Lond). 2021 Sep;21(5):e505-e510. doi: 10.7861/clinmed.2021-0121. PMID: 34507935; PMCID: PMC8439515.

Landsberger, M. et al. Potential role of antibodies against cardiac Kv channel-interacting protein 2 in dilated cadiomyopathy, American Heart Journal, July 2008, Volume 156, Number 1.

Lane JR, Neumann DA, Lafond-Walker A, Herskowitz A, Rose NR. Role of IL-1 and tumor necrosis factor in coxsackie virus-induced autoimmune myocarditis. J Immunol. 1993 Aug 1;151(3):1682-90. PMID: 8335952.

Lanza GA, Barone L, Scalone G, Pitocco D, Sgueglia GA, Mollo R, Nerla R, Zaccardi F, Ghirlanda G, Crea F. Inflammation-related effects of adjuvant influenza A vaccination on platelet activation and cardiac autonomic function. J Intern Med. 2011 Jan;269(1):118-25. doi: 10.1111/j.1365-2796.2010.02285.x. Epub 2010 Oct 22. PMID: 20964738.

Larbre F, Thivolet J, Hermier M, Cotton JB, Gaudin G, Richoux. Myocardite aiguë mortelle après vaccination antivariolique [Fatal acute myocarditis after smallpox vaccination]. Pediatrie. 1966 Apr-May;21(3):345-50. French. PMID: 5930422.

Lasrado N, Yalaka B, Reddy J. Triggers of Inflammatory Heart Disease. Front Cell Dev Biol. 2020; 8:192.

Leak LV, Ferrans VJ, Cohen SR, et al. Animal model of acute pericarditis and its progression to pericardial fibrosis and adhesions: ultrastructural studies. Am J Anat. 1987; 180: 373–390.

Lefèvre, N., Corazza, F., Valsamis, J., Delbaere, A., De Maertelaer, V., Duchateau, J., & Casimir, G. (2019). The Number of X Chromosomes Influences Inflammatory Cytokine Production Following Toll-Like Receptor Stimulation. Frontiers in Immunology, 10, 1052. https://doi.org/10.3389/fimmu.2019.01052

Li, C., Chen, Y., Zhao, Y., Lung, D. C., Ye, Z., Song, W., Liu, F. F., Cai, J. P., Wong, W. M., Yip, C. C., Chan, J. F., To, K. K., Sridhar, S., Hung, I. F., Chu, H., Kok, K. H., Jin, D. Y., Zhang, A. J., & Yuen, K. Y. (2021). Intravenous injection of COVID-19 mRNA vaccine can induce acute myopericarditis in mouse model. Clinical Infectious Diseases. https://doi.org/10.1093/cid/ciab707

Li M-Y, Li L, Zhang Y, et al. Expression of the SARS-CoV-2 cell receptor gene ACE2 in a wide variety of human tissues. Infect Dis Poverty. 2020;9(1):45

Li X, Ostropolets A, Makadia R, et al. Characterizing the incidence of adverse events of special interest for COVID-19 vaccines across eight countries: a multinational network cohort study. medRxiv. 2021;2021.03.25.21254315. doi: 10.1101/2021.03.25.21254315. Preprint.

Lim BK, Choe SC, Shin JO, Ho SH, Kim JM, Yu SS, Kim S, Jeon ES. Local expression of interleukin-1 receptor antagonist by plasmid DNA improves mortality and decreases myocardial inflammation in experimental coxsackieviral myocarditis. Circulation. 2002 Mar 19;105(11):1278-81. PMID: 11901035.

Loging JA, New RB, Baicu SC, et al. Effects of angiotensin type-I receptor blockade on pericardial fibrosis. J Surg Res. 1999; 87: 101–107

Lyden DC, Olszewski J, Feran M, Job LP, Huber SA. Coxsackievirus B-3-induced myocarditis. Effect of sex steroids on viremia and infectivity of cardiocytes. *Am J Pathol.* 1987;126(3):432-438.

Magnani JW, Danik HJ, Dec GW, Jr, DiSalvo TG. Survival in biopsy-proven myocarditis: a long-term retrospective analysis of the histopathologic, clinical, and hemodynamic predictors. *Am Heart J.* 2006;151:463–70.

Mann DL. Tumor necrosis factor and viral myocarditis: the fine line between innate and inappropriate immune responses in the heart. Circulation. 2001 Feb 6;103(5):626-9. doi: 10.1161/01.cir.103.5.626. PMID: 11156870.

Marrama D, Sette A, and Peters, B. Lack of evidence of significant homology of SARS-CoV-2 spike sequences to myocarditis-associated antigens (preprint; available at http://www.iedb.org/downloader.php?file_name=doc/10-03-21%20Homology%20of%20SARS-CoV-2%20spike%20to%20myocarditis-associated%20antigens%20%28V2%29.pdf

Martinetti M, Dugoujon JM, Caforio AL, Schwarz G, Gavazzi A, Graziano G, et al. HLA and immunoglobulin polymorphisms in idiopathic dilated cardiomyopathy. Hum Immunol. 1992;35(3):193–9.

Mason, JW. Myocarditis and dilated cardiomyopathy: An inflammatory link. Cardiovascular Research 2003. 60 (1): 5–10

Mason JW, O'Connell JB, Herskowitz A, Rose NR, McManus BM, Billinham ME, et al. A clinical trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. *N Engl J Med.* 1995;333:269–75.

Matsumori A, Kawai C. Coxsackie virus B3 perimyocarditis in BALB/c mice: experimental model of chronic perimyocarditis in the right ventricle. J Pathol. 1980 Jun;131(2):97-106.

Mauro AG, Bonaventura A, Vecchié A, Mezzaroma E, Carbone S, Narayan P, Potere N, Cannatà A, Paolini JF, Bussani R, Montecucco F, Sinagra G, Van Tassel BW, Abbate A, Toldo S. The Role of NLRP3 Inflammasome in Pericarditis: Potential for Therapeutic Approaches. JACC Basic Transl Sci. 2021 Feb 22;6(2):137-150.

McCarthy MK, Procario MC, Twisselmann N, et al. Proinflammatory effects of interferon gamma in mouse adenovirus 1 myocarditis. J Virol. 2015; 89(1): 468- 479.

McLean K, Johnson T. Myopericarditis in a previously healthy adolescent male following COVID-19 vaccination: a case report. Acad Emerg Med. Published online June 16, 2021. doi: 10.1111/acem.14322

Mei, R., Raschi, E., Forcesi, E., Diemberger, I., De Ponti, F., & Poluzzi, E. (2018). Myocarditis and pericarditis after immunization: Gaining insights through the Vaccine Adverse Event Reporting System. International Journal of Cardiology, 273, 183-186. https://doi.org/10.1016/j.ijcard.2018.09.054

Moderna COVID-19 vaccine Public Assessment Report. Available at: https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf. Accessed on 17Jan2022.

Moderna COVID-19 vaccine SmPc. Available at: https://www.ema.europa.eu/en/documents/product-information/spikevax-previously-covid-19-vaccine-moderna-epar-product-information_en.pdf. Accessed on: 17Jan2022.

Montgomery J, Ryan M, Engler R, Hoffman D, McClenathan B, Collins L, Loran D, Hrncir D, Herring K, Platzer M, et al. Myocarditis following immunization with mRNA COVID-19 vaccines in members of the US military. JAMA Cardiol. Published online June 29, 2021. doi: 10.1001/jamacardio.2021.2833

Movassagh M, Vujic A, Foo R (2011a) Genome-wide DNA methylation in human heart failure. *Epigenomics* 3:103-109.

Movassagh M, Choy MK, Knowles DA, Cordeddu L, Haider S, Down T, Siggens L, Vujic A, Simeoni I, Penkett C, Goddard M, Lio P, Bennett MR, Foo RS (2011b) Distinct epigenomic features in end-stage failing human hearts. Circulation 124:2411-2422.

Mevorach, D., Anis, E., Cedar, N., Bromberg, M., Haas, E. J., Nadir, E., Olsha-Castell, S., Arad, D., Hasin, T., Levi, N., Asleh, R., Amir, O., Meir, K., Cohen, D., Dichtiar, R., Novick, D., Hershkovitz, Y., Dagan, R., Leitersdorf, I., Ben-Ami, R., Miskin, I., Saliba, W., Muhsen, K., Levi, Y., Green, M. S., Keinan-Boker, L., & Alroy-Preis, S. (2021). Myocarditis after BNT162b2 mRNA Vaccine against Covid-19 in Israel. *New England Journal of Medicine*, *385*(23), 2140-2149. https://doi.org/10.1056/NEJMoa2109730.

Muñoz-Fontela, C., Dowling, W. E., Funnell, S. G. P., Gsell, P. S., Riveros-Balta, A. X., Albrecht, R. A., Andersen, H., Baric, R. S., Carroll, M. W., Cavaleri, M., Qin, C., Crozier, I., Dallmeier, K., de Waal, L., de Wit, E., Delang, L., Dohm, E., Duprex, W. P., Falzarano, D., Finch, C. L., Frieman, M. B., Graham, B. S., Gralinski, L. E., Guilfoyle, K., Haagmans, B. L., Hamilton, G. A., Hartman, A. L., Herfst, S., Kaptein, S. J. F., Klimstra, W. B., Knezevic, I., Krause, P. R., Kuhn, J. H., Le Grand, R., Lewis, M. G., Liu, W. C., Maisonnasse, P., McElroy, A. K., Munster, V., Oreshkova, N., Rasmussen, A. L., Rocha-Pereira, J., Rockx, B., Rodríguez, E., Rogers, T. F., Salguero, F. J., Schotsaert, M., Stittelaar, K. J., Thibaut, H. J., Tseng, C. T., Vergara-Alert, J., Beer, M., Brasel, T., Chan, J. F. W., García-Sastre, A., Neyts, J., Perlman, S., Reed, D. S., Richt, J. A., Roy, C. J., Segalés, J., Vasan, S. S., Henao-Restrepo, A. M., & Barouch, D. H. (2020). Animal models for COVID-19. Nature, 586(7830), 509-515. https://doi.org/10.1038/s41586-020-2787-6.

Murphy JG, Wright RS, Bruce GK, Baddour LM, Farrell MA, Edwards WD, Kita H, Cooper LT. Eosinophilic-lymphocytic myocarditis after smallpox vaccination. Lancet. 2003 Oct 25;362(9393):1378-80. doi: 10.1016/S0140-6736(03)14635-1. PMID: 14585641.

Muthukumar A, Narasimhan M, Li QZ, Mahimainathan L, Hitto I, Fuda F, Batra K, Jiang X, Zhu C, Schoggins J, et al. In-depth evaluation of a case of presumed myocarditis after the second dose of COVID-19 mRNA vaccine. Circulation. 2021;144:487–498. doi: 10.1161/CIRCULATIONAHA.121.056038

Myers JM, Cooper LT, Kem DC, Stavrakis S, Kosanke SD, Shevach EM, Fairweather D, Stoner JA, Cox CJ, Cunningham MW. Cardiac myosin-Th17 responses promote heart failure in human myocarditis. JCI Insight. 2016 Jun 16;1(9):e85851. doi: 10.1172/jci.insight.85851. PMID: 27366791; PMCID: PMC4924810.

Nelson J, Sorensen EW, Mintri S, Rabideau AE, Zheng W, Besin G, Khatwani N, Su SV, Miracco EJ, Issa WJ, Hoge S, Stanton MG, and Joyal LJ. Impact of mRNA chemistry and manufacturing process on innate immune activation. Sci. Adv. 2020; 6: eaaz6893

Ogata AF, Cheng C-A, Desjardins M, et al. Circulating SARS-CoV-2 Vaccine Antigen Detected in the Plasma of mRNA-1273 Vaccine Recipients. Clin Infect Dis. 2021;ciab465.

Onpattro EPAR Public Assessment Report. Available at: https://www.ema.europa.eu/en/documents/assessment-report/onpattro-epar-public-assessment-report_.pdf. Accessed on 17Jan2022.

Page PL, Plumb VJ, Okumura K, et al. A new animal model of atrial flutter. J Am Coll Cardiol. 1986; 8: 872–879.

Palaskas N., Lopez-Mattei J., Durand JB., Iliescu, C., and Deswal, A. Immune Checkpoint Inhibitor Myocarditis: Pathophysiological Characteristics, Diagnosis, and Treatment. J Am Heart Assoc. 2020; 21;9(2):e013757

Pelka K., Shibata T., Miyake K., Latz E. Nucleic acid-sensing TLRs and autoimmunity: novel insights from structural and cell biology. Immunol. Rev. 2016;269:60–75. doi: 10.1111/imr.12375.

Piplani, S., Singh, P. K., Winkler, D. A., & Petrovsky, N. (2021). In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin. Scientific Reports, 11(1), 13063. https://doi.org/10.1038/s41598-021-92388-5.

Pistulli R, Andreas E, König S, Drobnik S, Kretzschmar D, Rohm I, Lichtenauer M, Heidecker B, Franz M, Mall G, Yilmaz A, Schulze PC. Characterization of dendritic cells in human and experimental myocarditis. ESC Heart Fail. 2020 Oct;7(5):2305-2317. doi: 10.1002/ehf2.12767. Epub 2020 Jul 3. PMID: 32619089; PMCID: PMC7524053.

Prabhu SD. Cytokine-induced modulation of cardiac function. Circ Res. 2004 Dec 10;95(12):1140-53. doi: 10.1161/01.RES.0000150734.79804.92. PMID: 15591236.

Rosenberg M, Sparks R, McMahon A, Iskander J, Campbell JD, Edwards KM. Serious adverse events rarely reported after trivalent inactivated influenza vaccine (TIV) in children 6-23 months of age. Vaccine. 2009 Jul 9;27(32):4278-83. doi: 10.1016/j.vaccine.2009.05.023. Epub 2009 May 28. PMID: 19450636.

Ross A. Breckenridge, Animal Models of Myocardial Disease, Editor(s): P. Michael Conn, Animal Models for the Study of Human Disease, Academic Press, 2013, Pages 145-171.

Shuai, H., Chan, J. F., Yuen, T. T., Yoon, C., Hu, J. C., Wen, L., Hu, B., Yang, D., Wang, Y., Hou, Y., Huang, X., Chai, Y., Chan, C. C., Poon, V. K., Lu, L., Zhang, R. Q., Chan, W. M., Ip, J. D., Chu, A. W., Hu, Y. F., Cai, J. P., Chan, K. H., Zhou, J., Sridhar, S., Zhang, B. Z., Yuan, S., Zhang, A. J., Huang, J. D., To, K. K., Yuen, K. Y., & Chu, H. (2021). Emerging SARS-CoV-2 variants expand species tropism to murines. EBioMedicine, 73, 103643. https://doi.org/10.1016/j.ebiom.2021.103643.

Simon WL, Salk HM, Ovsyannikova IG, Kennedy RB, Poland GA. Cytokine production associated with smallpox vaccine responses. Immunotherapy. 2014;6(10):1097-112. doi: 10.2217/imt.14.72. PMID: 25428648; PMCID: PMC4263415.

Solomon, C. G. & LeWinter, M. M. Acute Pericarditis. *New Engl J Medicine* **371**, 2410–2416 (2014).

Souyris, M., Mejía, J. E., Chaumeil, J., & Guéry, J. C. (2019). Female predisposition to TLR7-driven autoimmunity: gene dosage and the escape from X chromosome inactivation. Seminars in Immunopathology, 41(2), 153-164. https://doi.org/10.1007/s00281-018-0712-y.

Streifler JJ, Dux S, Garty M, Rosenfeld JB. Recurrent pericarditis: a rare complication of influenza vaccination. Br Med J (Clin Res Ed). 1981 Aug 22;283(6290):526-7. doi: 10.1136/bmj.283.6290.526-a. PMID: 6790054; PMCID: PMC1507927.

Swearengen JR. Choosing the right animal model for infectious disease research. Animal Model Exp Med. 2018 Jul 28;1(2):100-108.

Talotta R, Robertson E. Autoimmunity as the comet tail of COVID-19 pandemic. World J Clin Cases 2020; 8: 3621–3644.

Tano GD, Moschini L, Calvaruso EV, et al. Recurrent Myocarditis After the First Dose of SARS-CoV-2 mRNA-1273 Vaccine. Epub ahead of print 2021. DOI: 10.21203/rs.3.rs-699585/v1

Troughton RW, Asher, CR, and Klein, AL. Pericarditis. The Lancet 2004; 363 (9410): 717-727.

Tschöpe C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, Hare JM, Heidecker B, Heymans S, Hübner N, Kelle S, Klingel K, Maatz H, Parwani AS, Spillmann F, Starling RC, Tsutsui H, Seferovic P, Van Linthout S. Myocarditis and inflammatory cardiomyopathy: current evidence and future directions. Nat Rev Cardiol. 2021 Mar;18(3):169-193.

Upadhrasta S, Elias H, Patel K, Zheng L. Managing cardiotoxicity associated with immune checkpoint inhibitors. Chronic Dis Transl Med. 2019;5(1):6-14.

Vdovenko D, Eriksson U. Regulatory Role of CD4+ T Cells in Myocarditis. J Immunol Res. 2018 Jun 21;2018:4396351. doi: 10.1155/2018/4396351. PMID: 30035131; PMCID: PMC6032977.

Vogel AB, Kanevsky I, Che Y, et al. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. Nature. 2021 Apr;592(7853):283-289.

Vojani A and Kharrazian D. Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases. Clin Immunol. 2020 Aug; 217: 108480.

Wang X, Qiao W, Xiao Y, Sun L, Ren W. Experimental Research on the Evaluation of Left Ventricular Function by Layered Speckle Tracking in a Constrictive Pericarditis Rat Model. J Ultrasound Med. 2020 Nov;39(11):2219-2229.

Watad, A., David, P., Brown, S., and Shoenfeld, Y. Autoimmune/Inflammatory Syndrome Induced by Adjuvants and Thyroid Autoimmunity. Front Endocrinol (Lausanne). 2016; 7: 150.

Witberg, G., Barda, N., Hoss, S., Richter, I., Wiessman, M., Aviv, Y., Grinberg, T., Auster, O., Dagan, N., Balicer, R. D., & Kornowski, R. (2021). Myocarditis after Covid-19 Vaccination in a Large Health Care Organization. *New England Journal of Medicine*, *385*(23), 2132-2139. https://doi.org/10.1056/NEJMoa2110737

Woodruff JF. Viral myocarditis. A review. Am J Pathol. 1980 Nov;101(2):425-84. PMID: 6254364; PMCID: PMC1903609.

Yamada T, Matsumori A, Sasayama S. Therapeutic effect of anti-tumor necrosis factor-alpha antibody on the murine model of viral myocarditis induced by encephalomyocarditis virus. Circulation. 1994 Feb;89(2):846-51. doi: 10.1161/01.cir.89.2.846. PMID: 8313574.

Yingying Zhang, Xueqin Li, Deguo Wang, Xiaogan Jiang, Mengying Zhang, Kun L. Serum exosome microRNA panel as a noninvasive biomarker for molecular diagnosis of fulminant myocarditis,Molecular Therapy - Methods & Clinical Development,Volume 20, 2021.

Younis, A. *et al.* Epidemiology Characteristics and Outcome of Patients With Clinically Diagnosed Acute Myocarditis. *Am J Medicine* **133**, 492–499 (2020).

Zarak-Crnkovic M, Kania G, Jaźwa-Kusior A, Czepiel M, Wijnen WJ, Czyż J, Müller-Edenborn B, Vdovenko D, Lindner D, Gil-Cruz C, Bachmann M, Westermann D, Ludewig B, Distler O, Lüscher TF, Klingel K, Eriksson U, Błyszczuk P. Heart non-specific effector CD4+ T cells protect from postinflammatory fibrosis and cardiac dysfunction in experimental autoimmune myocarditis. Basic Res Cardiol. 2019 Dec 20;115(1):6. doi: 10.1007/s00395-019-0766-6. PMID: 31863205; PMCID: PMC6925074.

Zhang, M., Tavora, F., Zhang, Y., Ripple, M., Fowler, D., Li, L., Zhao, Z., & Burke, A. (2013). The role of focal myocardial inflammation in sudden unexpected cardiac and noncardiac deaths--a clinicopathological study. International Journal of Legal Medicine, 127(1), 131-138. https://doi.org/10.1007/s00414-011-0634-x.

Zhao L et al. Roles of Host Immunity in Viral Myocarditis and Dilated Cardiomyopathy. J Immunol Res. 2018: 5301548.

Zhao, X., Chen, D., Szabla, R., Zheng, M., Li, G., Du, P., Zheng, S., Li, X., Song, C., Li, R., Guo, J. T., Junop, M., Zeng, H., & Lin, H. (2020). Broad and Differential Animal Angiotensin-Converting Enzyme 2 Receptor Usage by SARS-CoV-2. Journal of Virology, 94(18). https://doi.org/10.1128/jvi.00940-20.

Refs not used

Bagger JP, Baandrup U, Rasmussen K, Moller M, Vesterlund T. Cardiomyopathy in western Denmark. *Br Heart J.* 1984;52:327–31

Keinath K, Church T, Kurth B, Hulten E. Myocarditis secondary to smallpox vaccination. BMJ Case Rep. 2018 Mar 22;2018:bcr2017223523. doi: 10.1136/bcr-2017-223523. PMID: 29572367; PMCID: PMC5878341.

Mora LF, Khan AH, Sperling LS. Cardiac complications after smallpox vaccination. South Med J. 2009 Jun;102(6):615-9. doi: 10.1097/SMJ.0b013e31819fe55b. PMID: 19434043.