PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, 23(1), 31-46
https://prospects.wum.edu.pl/

Review Article

BIONTECH/PFIZER (BNT162B2) COVID-19 mRNA VACCINE: MANUFACTURING, IMMUNOGENICITY, EFFICACY AND SAFETY

Esraa Alkhalil[1]*, Bashar Jarad[1], Tala Saleh[1], Wafaa Aldarwish[1], and Dima Joujeh[1]

[1] *Department of Biotechnology Engineering, Faculty of Technical Engineering, University of Aleppo, Aleppo, Syria.*

* Correspondence, e-mail: esraa.alkhalil@alepuniv.edu.sy

Received: 17.10.2024 / Accepted: 11.12.2024 / Published: 06.02.2025

ABSTRACT

The investigation into the Pfizer vaccine for the Coronavirus (COVID-19) is a vital area of study, especially given the ongoing global pandemic. The safety, efficacy, and overall credibility of the Pfizer vaccine remain contentious issues. This research seeks to deliver a thorough and unbiased examination of the vaccine's production process, its functional mechanism, and its immunogenic properties. It also presents the latest scientific findings concerning the vaccine's safety and efficacy based on recent clinical trials. The analysis is conducted with impartiality, ensuring that data and scientific outcomes are reported without prejudice.

BNT162b2 is a nucleoside-modified mRNA vaccine formulated with lipid nanoparticles, designed to encode the spike protein of SARS-CoV-2, which stimulates an immune response in those vaccinated. The Pfizer-BioNTech mRNA COVID-19 vaccine incorporates an advanced cap 1 analog, a specific 5′ UTR region derived from the human α-globin gene, and an optimized downstream Kozak consensus sequence. The mRNA sequence has been enhanced by introducing N1-methyl-Ψ, substituting all uridines, including those in stop codons. The 3′-UTR of the BNT162b2 mRNA includes sequences from the amino-terminal enhancer of split mRNA and mitochondrial 12S rRNA, alongside a 30-mer poly(A) tail and a 10-nucleotide linker, which together improve and extend protein expression.

As of May 30, 2024, the U.S. National Library of Medicine (Clinicaltrials.gov) lists 1,314 clinical trials globally related to COVID-19 mRNA vaccines, with 135 specifically focusing on BNT162b2, most of which are in Phase II and III. Numerous trials have confirmed the vaccine's effectiveness and safety, with no major adverse effects reported in the majority of studies. Access to reliable information on the Pfizer-BioNTech vaccine is vital for public awareness and informed vaccination decisions, emphasizing the importance of ongoing trials to update medical literature, especially as the vaccine is evaluated across various age groups and against new SARS-CoV-2 variants.

KEYWORDS: SARS-CoV-2, Nucleoside-modified mRNA, Immuno response mechanism, Clinical trials.

Article is published under the CC BY license.

1. Introduction

On March 11, 2020, the World Health Organization (WHO) classified COVID-19 as a global pandemic [1]. By November 10, 2024, the total number of reported COVID-19 cases worldwide, caused by the SARS-CoV-2 virus, had risen to 776,841,264, with 7,075,468 deaths worldwide [2].

The common symptoms of COVID-19 infection include fever, dry cough, myalgia, and shortness of breath [3]. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) primarily infects multiciliated cells in the trachea or nasopharynx, or sustentacular cells in the nasal olfactory mucosa during natural human infection [4].

Coronaviruses (CoVs) are enveloped positive-sense RNA viruses, distinguished by their club-shaped surface spikes, a large RNA genome, and a distinctive replication mechanism [5].

Coronaviruses can readily adapt to new environments through mutation and recombination, enabling them to efficiently modify their host range and tissue tropism. As a result, these viruses represent persistent and long-term health threats [6]. Vaccination remains the most effective strategy for protecting against COVID-19. It prepares the immune system to respond rapidly to the virus, helping to prevent illness. Vaccines achieve this by developing immune memory in T and B lymphocytes, which recognize the virus and provide prolonged protection following the administration of both doses [7].

Before the development and approval of COVID-19 vaccines, extensive knowledge about the structure and function of coronaviruses significantly contributed to advancing vaccine production capabilities [8]. The process of vaccine development is time-intensive, as vaccines must demonstrate both effectiveness and a high degree of safety. Unlike medications used to treat sick individuals, vaccines are administered to healthy people, necessitating stringent safety standards [9].

mRNA technology provides a highly flexible platform for vaccine and therapeutic development. This approach enables the encoding and expression of virtually any protein, supporting the creation of prophylactic and therapeutic vaccines for a wide array of diseases, including infections and cancer, as well as protein replacement therapies [10].

The first two COVID-19 vaccines to receive conditional marketing authorization (CMA) from the European Medicines Agency or emergency use authorization (EUA) from the U.S. Food and Drug Administration (FDA) were the nucleoside-modified mRNA vaccines developed by BioNTech/Pfizer (BNT162b2) and Moderna (mRNA-1273) [11].

This review provides detailed information about the manufacturing of the BNT162b2 mRNA vaccine, including the iterative optimization of mRNA structural components to improve the stability. It also introduces the current knowledge about its mechanism of action, immunogenicity, effectiveness and safety according to latest clinical trials.

2. Materials and Methods

Data was collected from online databases such as Google Scholar and PubMed, using the keywords 'BNT162b2', 'BioNTech/Pfizer', 'mRNA vaccine', 'manufacturing', 'immunogenicity', 'efficacy', 'safety', 'mechanism of action'. We were limited to articles published in English. The online search was customized between January 2024 and June 2024.

3. The Structure of Coronavirus COV-SARS-2

Coronaviruses are members of the *Coronaviridae* family within the *Nidovirales* order, and are classified into four genera: α-, ß-, γ-, and δ-coronaviruses. While α- and ß-coronaviruses primarily infect mammals, γ-coronaviruses target avian species, and δ-coronaviruses can infect both mammals and aves [12]. SARS-CoV-2 belongs to the beta genus of coronaviruses [13].

Coronaviruses are approximately spherical and moderately pleomorphic, with virions averaging 80–120 nm in diameter. Their genomes consist of non-segmented, single-stranded RNA of positive sense, equivalent to mRNA [14]. These genomes are approximately 30,000 nucleotides in length, with a GC content of 38%, 13–15 open reading frames (ORFs) (12 functional), and 11 protein-coding genes encoding 12 proteins [12]. The final third of the genome encodes four structural proteins common to all coronaviruses: S (spike glycoprotein), M (membrane glycoprotein), E (envelope protein), and N (nucleocapsid phosphoprotein) [15] (Fig. 1).

SARS-CoV-2 virions are surrounded by a lipid bilayer with spike protein trimers protruding from their surface [16-17].

The S protein contains of two subunits: S1, which facilitates viral attachment to angiotensin converting enzyme 2 (ACE2) receptor on host cells, and S2 which mediates the fusion of viral and host cell membranes. This process enables viral entry via endocytosis and the release of the virions into the host cell cytoplasm [7]. ACE2 is a type I membrane protein expressed in various tissues, including the lungs, heart, kidneys, and intestines [18].

4. Adaptive Immunity to SARS-COV-2 Infection

The outcome of SARS-CoV-2 infection is heavily influenced by the immune system's orchestrated response [19]. Understanding the immune reactions to this virus is crucial for the controlling, preventing, and treating of COVID-19 [20].

Upon exposure to the virus, the initial interaction takes place in the upper respiratory tract through the nasal epithelium [21]. SARS-CoV-2 gains entry into host cells via the S protein through receptor-mediated endocytosis. After replication and packaging, the virus is released from the infected cell to infect new cells.

Subsequently, the virus encounters the innate immune system. The main cellular components of innate immunity, including epithelial cells, dendritic cells, and macrophages, respond to the virus [7].

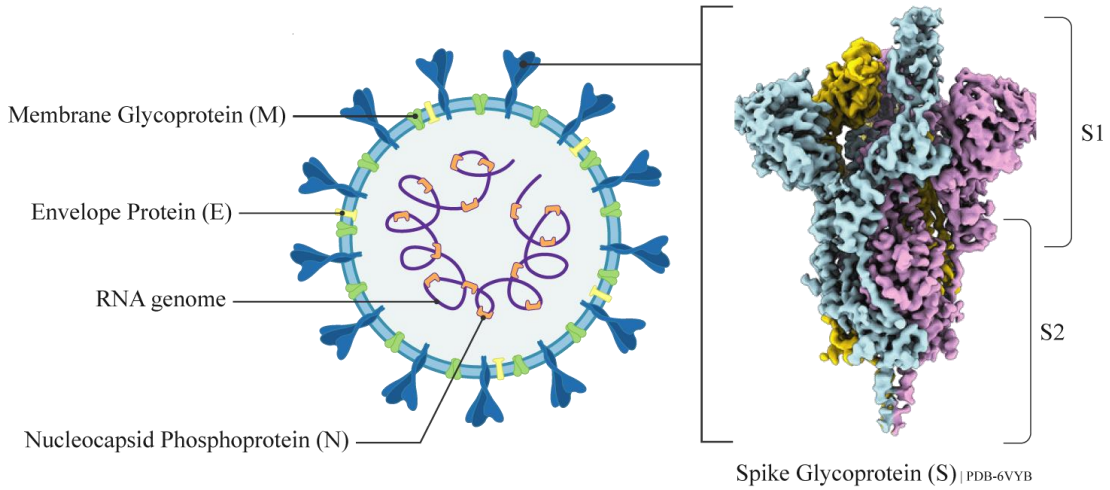


Fig. 1. Coronavirus structure.

Macrophages from the innate response can ingest and destroy some viruses, releasing viral components, that activate the adaptive immune response through T- and B-lymphocytes, generating killer T-cells and specific antibodies to combat COVID-19 infection and eliminate infected cells [21-22].

Each protein of SARS-CoV-2 can trigger an immune response, resulting in the production of antibodies [23]. The antibody responses specific to SARS-CoV-2 primarily target two proteins: the spike protein (S) and the nucleocapsid protein (N). It has been suggested that IgG antibodies targeting the spike protein are more specific, while antibodies targeting the nucleocapsid protein may offer greater sensitivity, particularly in the early stages of infection [24].

5. mRNA Vaccines Against SARS-COV-2

Currently, over 180 vaccine candidates based on various platforms are under development for SARS-CoV-2 [25]. However, the most significant breakthrough was the development of mRNA vaccines, which were rapidly developed and approved in response to the COVID-19 pandemic. The mRNA technology allows for the intracellular production of the desired vaccine antigen [26]. The leading vaccine candidates use lipid nanoparticles to encapsulate mRNA that encodes the full spike protein (S) of the virus or its subunits, such as S1 and S2, as well as the receptor-binding domain (RBD). This mRNA is typically translated in its native trimeric form [27].

6. Manufacturing of BNT162b2 mRNA vaccine

The structure of mRNA vaccine closely resembles that of eukaryotic mRNA, consisting of a single-stranded molecule with a 5′ cap, a 3′ poly (A) tail, 5′ and 3′ untranslated regions (5′-UTRs and 3′-UTRs), and an open reading frame (ORF) [28] (Fig. 2).

Due to the ease of controlling, rapidly synthesizing, and simplicity of in vitro mRNA synthesis, the use of various mRNA types has expanded [29].

There are two primary types of RNA vaccines: conventional non-replicating mRNA and self-amplifying RNA. In conventional mRNA vaccines, the mRNA encodes the target gene along with 5′ and 3′ UTRs to enhance gene expression. In self-amplifying RNA vaccines, the mRNA not only encodes the target gene but also specific RNA virus replication genes, promoting the production of abundant intracellular RNA [30].

To ensure the production of high-quality mRNA, several manufacturing steps are required (Fig. 3). These steps are divided into upstream processing, which involves the enzymatic synthesis of mRNA, and downstream processing, which includes the unit operations required for purifying the final mRNA product [28].

6.1. DNA Template Design

The in vitro transcription (IVT) process to generate mRNA relies on a DNA template that encodes the desired genetic sequence and contains an RNA polymerase promoter site. The DNA template must include the T7 RNA polymerase promoter sequence, a 5′-UTR, an open reading frame (ORF), and a 3′-UTR [31]. For functional translation of the mRNA, a poly (A) tail is essential. This tail can be incorporated into the plasmid, added through PCR, or enzymatically polyadenylated post-transcription [32].

The DNA templates can be either linearized plasmids or PCR products. Plasmid DNA templates (PDT) are typically generated through microbial fermentation. After sequencing a pathogen's genome, the sequence for the target antigen is designed and inserted into a plasmid DNA construct [33]. Pfizer, for example, produces PDT through *E. coli* fermentation [34]. However, preparing PDT is a time-consuming process, taking several days to weeks, with no guarantee of identifying the correct clone. This method is also expensive [35].

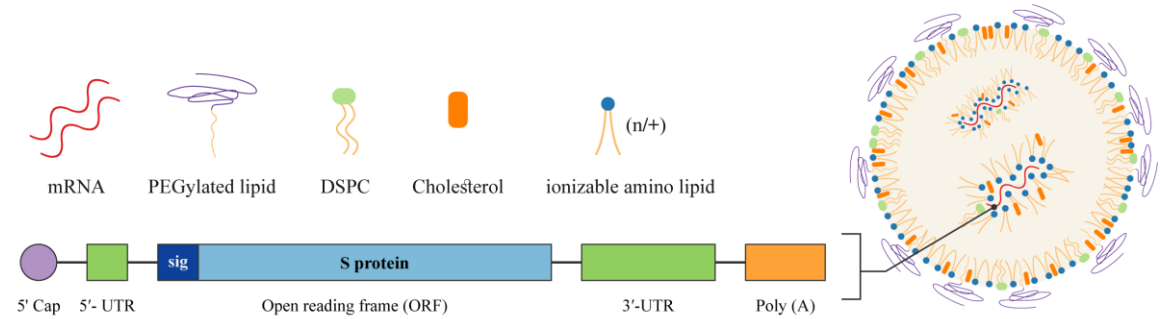
In contrast, synthetic DNA templates (SDT) are produced using assembly polymerase chain reaction (aPCR) with synthetic oligonucleotides as starting materials. Oligo 1 contains the T7 promoter and 5′-UTR, oligo 2 encodes the cDNA for the mRNA of interest, and oligo 3 includes the 3′-UTR and a short poly (A) sequence. The resulting SDT is then amplified by PCR and used as a template for the IVT of mRNA. This approach offers a cost-effective, and time-efficient workflow for producing in vitro transcribed mRNA [36].

6.2. In Vitro Transcription (IVT) of mRNA

IVT is a well-established process that enables the synthesis of RNA molecules directed by DNA templates, utilizing RNA polymerases and nucleoside triphosphates (NTPs). The activity of the polymerase is typically evaluated by quantifying the mRNA product during the reaction [37]. IVT can produce long RNA transcripts, often exceeding several kilobases in length, with high yields [38].

6.3. mRNA Capping

Capping is a critical step for stabilizing mRNA and improving its translation efficiency by protecting it from exonuclease degradation [39]. To achieve this, synthetic mRNAs are designed with modified cap analogs that closely resemble fully processed mRNAs, thus preventing activation of the innate immune system [40]. The core 5′ cap structure comprises an N7-methylated guanosine linked to the terminal 5′ nucleotide of the mRNA via a 5′-to-5′ triphosphate linkage (m7GpppN), referred to as Cap-0 [41]. Capped transcripts can be generated using a cap analog during the IVT process [31].



GAGAAΨ*AAAC	ΨAGΨAΨΨCΨΨ	CΨGGΨCCCCA	CAGACΨCAGA	GAGAACCCGC	CACCAΨGΨΨC	GΨGΨΨCCΨGG	ΨGCΨGCΨGCC	ΨCΨGGΨGΨCC	AGCCAGΨGΨG	100
ΨGAACCΨGAC	CACCAGAACA	CAGCΨGCCΨC	CAGCCΨACAC	CAACAGCΨΨΨ	ACCAGAGGCG	ΨGΨACΨACCC	CGACAAGGΨG	ΨΨCAGAΨCCA	GCGΨGCΨGCA	200
CΨCΨACCCAG	GACCΨGΨΨCC	ΨGCCΨΨΨCΨΨ	CAGCAACGΨG	ACCΨGGΨΨCC	ACGCCAΨCCA	CGΨGΨCCGGC	ACCAAΨGGCA	CCAAGAGAΨΨ	CGACAACCCC	300
GΨGCΨGCCCΨ	ΨCAACGACGG	GGΨGΨACΨΨΨ	GCCAGCACCG	AGAAGΨCCAA	CAΨCAΨCAGA	GGCΨGGAΨCΨ	ΨCGGCACCAC	ACΨGGACAGC	AAGACCCAGA	400
GCCΨGCΨGAΨ	CGΨGAACAAC	GCCACCAACG	ΨGGΨCAΨCAA	AGΨGΨGCGAG	ΨΨCCAGΨΨCΨ	GCAACGACCC	CΨΨCCΨGGGC	GΨCΨACΨACC	ACAAGAACAA	500
CAAGAGCΨGG	AΨGGAAAGCG	AGΨΨCCGGGΨ	GΨACAGCAGC	GCCAACAACΨ	GCACCΨΨCGA	GΨACGΨGΨCC	CAGCCΨΨΨCC	ΨGAΨGGACCΨ	GGAAGGCAAG	600
CAGGGCAACΨ	ΨCAAGAACCΨ	GCGCGAGΨΨC	GΨGΨΨΨAAGA	ACAΨCGACGG	CΨACΨΨCAAG	AΨCΨACAGCA	AGCACACCCC	ΨAΨCAACCΨC	GΨGCGGGAΨC	700
ΨGCCΨCAGGG	CΨΨCΨCΨGCΨ	CΨGGAACCCC	ΨGGΨGGAΨCΨ	GCCCAΨCGGC	AΨCAACAΨCA	CCCGGΨΨΨCA	GACACΨGCΨG	GCCCΨGCACA	GAAGCΨACCΨ	800
GACACCΨGGC	GAΨAGCAGCA	GCGGAΨGGAC	AGCΨGGΨGCC	GCCGCΨΨACΨ	AΨGΨGGGCΨA	CCΨGCAGCCΨ	AGAACCΨΨCC	ΨGCΨGAAGΨA	CAACGAGAAC	900
GGCACCAΨCA	CCGACGCCGΨ	GGAΨΨGΨGCΨ	CΨGGAΨCCΨC	ΨGAGCGAGAC	AAAGΨGCACC	CΨGAAGΨCCΨ	ΨCACCGΨGGA	AAAGGGCAΨC	ΨACCAGACCA	1000
GCAACΨΨCCG	GGΨGCAGCCC	ACCGAAΨCCA	ΨCGΨGCGGΨΨ	CCCCAAΨAΨC	ACCAAΨCΨGΨ	GCCCCΨΨCGG	CGAGGΨGΨΨC	AAΨGCCACCA	GAΨΨCGCCΨC	1100
ΨGΨGΨACGCC	ΨGGAACCGGA	AGCGGAΨCAG	CAAΨΨGCGΨG	GCCGACΨACΨ	CCGΨGCΨGΨA	CAACΨCCGCC	AGCΨΨCAGCA	CCΨΨCAAGΨG	CΨACGGCGΨG	1200
ΨCCCCΨACCA	AGCΨGAACGA	CCΨGΨGCΨΨC	ACAAACGΨGΨ	ACGCCGACAG	CΨΨCGΨGAΨC	CGGGGAGAΨG	AAGΨGCGGCA	GAΨΨGCCCCΨ	GGACAGACAG	1300
GCAAGAΨCGC	CGACΨACAAC	ΨACAAGCΨGC	CCGACGACΨΨ	CACCGGCΨGΨ	GΨGAΨΨGCCΨ	GGAACAGCAA	CAACCΨGGAC	ΨCCAAAGΨCG	GCGGCAACΨA	1400
CAAΨΨACCΨG	ΨACCGGCΨGΨ	ΨCCGGAAGΨC	CAAΨCΨGAAG	CCCΨΨCGAGC	GGGACAΨCΨC	CACCGAGAΨC	ΨAΨCAGGCCG	GCAGCACCCC	ΨΨGΨAACGGC	1500
GΨGGAAGGCΨ	ΨCAACΨGCΨA	CΨΨCCCACΨG	CAGΨCCΨACG	GCΨΨΨCAGCC	CACAAAΨGGC	GΨGGGCΨAΨC	AGCCCΨACAG	AGΨGGΨGGΨG	CΨGAGCΨΨCG	1600
AACΨGCΨGCA	ΨGCCCCΨGCC	ACAGΨGΨGCG	GCCCΨAAGAA	AAGCACCAAΨ	CΨCGΨGAAGA	ACAAAΨGCGΨ	GAACΨΨCAAC	ΨΨCAACGGCC	ΨGACCGGCAC	1700
CGGCGΨGCΨG	ACAGAGAGCA	ACAAGAAGΨΨ	CCΨGCCAΨΨC	CAGCAGΨΨΨG	GCCGGGAΨAΨ	CGCCGAΨACC	ACAGACGCCG	ΨΨAGAGAΨCC	CCAGACACΨG	1800
GAAAΨCCΨGG	ACAΨCACCCC	ΨΨGCAGCΨΨC	GGCGGAGΨGΨ	CΨGΨGAΨCAC	CCCΨGGCACC	AACACCAGCA	AΨCAGGΨGGC	AGΨGCΨGΨAC	CAGGACGΨGA	1900
ACΨGΨACCGA	AGΨGCCCGΨG	GCCAΨΨCACG	CCGAΨCAGCΨ	GACACCΨACA	ΨGGCGGGΨGΨ	ACΨCCACCGG	CAGCAAΨGΨG	ΨΨΨCAGACCA	GAGCCGGCΨG	2000
ΨCΨGAΨCGGA	GCCGAGCACG	ΨGAACAAΨAG	CΨACGAGΨGC	GACAΨCCCCA	ΨCGGCGCΨGG	AAΨCΨGCGCC	AGCΨACCAGA	CACAGACAAA	CAGCCCΨCGG	2100
AGAGCCAGAA	GCGΨGGCCAG	CCAGAGCAΨC	AΨΨGCCΨACA	CAAΨGΨCΨCΨ	GGGCGCCGAG	AACAGCGΨGG	CCΨACΨCCAA	CAACΨCΨAΨC	GCΨAΨCCCCA	2200
CCAACΨΨCAC	CAΨCAGCGΨG	ACCACAGAGA	ΨCCΨGCCΨGΨ	GΨCCAΨGACC	AAGACCAGCG	ΨGGACΨGCAC	CAΨGΨACAΨC	ΨGCGGCGAΨΨ	CCACCGAGΨG	2300
CΨCCAACCΨG	CΨGCΨGCAGΨ	ACGGCAGCΨΨ	CΨGCACCCAG	CΨGAAΨAGAG	CCCΨGACAGG	GAΨCGCCGΨG	GAACAGGACA	AGAACACCCA	AGAGGΨGΨΨC	2400
GCCCAAGΨGA	AGCAGAΨCΨA	CAAGACCCCΨ	CCΨAΨCAAGG	ACΨΨCGGCGG	CΨΨCAAΨΨΨC	AGCCAGAΨΨC	ΨGCCCGAΨCC	ΨAGCAAGCCC	AGCAAGCGGA	2500
GCΨΨCAΨCGA	GGACCΨGCΨG	ΨΨCAACAAAG	ΨGACACΨGGC	CGACGCCGGC	ΨΨCAΨCAAGC	AGΨAΨGGCGA	ΨΨGΨCΨGGGC	GACAΨΨGCCG	CCAGGGAΨCΨ	2600
GAΨΨΨGCGCC	CAGAAGΨΨΨA	ACGGACΨGAC	AGΨGCΨGCCΨ	CCΨCΨGCΨGA	CCGAΨGAGAΨ	GAΨCGCCCAG	ΨACACAΨCΨG	CCCΨGCΨGGC	CGGCACAAΨC	2700
ACAAGCGGCΨ	GGACAΨΨΨGG	AGCAGGCGCC	GCΨCΨGCAGA	ΨCCCCΨΨΨGC	ΨAΨGCAGAΨG	GCCΨACCGGΨ	ΨCAACGGCAΨ	CGGAGΨGACC	CAGAAΨGΨGC	2800
ΨGΨACGAGAA	CCAGAAGCΨG	AΨCGCCAACC	AGΨΨCAACAG	CGCCAΨCGGC	AAGAΨCCAGG	ACAGCCΨGAG	CAGCACAGCA	AGCGCCCΨGG	GAAAGCΨGCA	2900
GGACGΨGGΨC	AACCAGAAΨG	CCCAGGCACΨ	GAACACCCΨG	GΨCAAGCAGC	ΨGΨCCΨCCAA	CΨΨCGGCGCC	AΨCAGCΨCΨG	ΨGCΨGAACGA	ΨAΨCCΨGAGC	3000
AGACΨGGACC	CΨCCΨGAGGC	CGAGGΨGCAG	AΨCGACAGAC	ΨGAΨCACAGG	CAGACΨGCAG	AGCCΨCCAGA	CAΨACGΨGAC	CCAGCAGCΨG	AΨCAGAGCCG	3100
CCGAGAΨΨAG	AGCCΨCΨGCC	AAΨCΨGGCCG	CCACCAAGAΨ	GΨCΨGAGΨGΨ	GΨGCΨGGGCC	AGAGCAAGAG	AGΨGGACΨΨΨ	ΨGCGGCAAGG	GCΨACCACCΨ	3200
GAΨGAGCΨΨC	CCΨCAGΨCΨG	CCCCΨCACGG	CGΨGGΨGΨΨΨ	CΨGCACGΨGA	CAΨAΨGΨGCC	CGCΨCAAGAG	AAGAAΨΨΨCA	CCACCGCΨCC	AGCCAΨCΨGC	3300
CACGACGGCA	AAGCCCACΨΨ	ΨCCΨAGAGAA	GGCGΨGΨΨCG	ΨGΨCCAACGG	CACCCAΨΨGG	ΨΨCGΨGACAC	AGCGGAACΨΨ	CΨACGAGCCC	CAGAΨCAΨCA	3400
CCACCGACAA	CACCΨΨCGΨG	ΨCΨGGCAACΨ	GCGACGΨCGΨ	GAΨCGGCAΨΨ	GΨGAACAAΨA	CCGΨGΨACGA	CCCΨCΨGCAG	CCCGAGCΨGG	ACAGCΨΨCAA	3500
AGAGGAACΨG	GACAAGΨACΨ	ΨΨAAGAACCA	CACAAGCCCC	GACGΨGGACC	ΨGGGCGAΨAΨ	CAGCGGAAΨC	AAΨGCCAGCG	ΨCGΨGAACAΨ	CCAGAAAGAG	3600
AΨCGACCGGC	ΨGAACGAGGΨ	GGCCAAGAAΨ	CΨGAACGAGA	GCCΨGAΨCGA	CCΨGCAAGAA	CΨGGGGAAGΨ	ACGAGCAGΨA	CAΨCAAGΨGG	CCCΨGGΨACA	3700
ΨCΨGGCΨGGG	CΨΨΨAΨCGCC	GGACΨGAΨΨG	CCAΨCGΨGAΨ	GGΨCACAAΨC	AΨGCΨGΨGΨΨ	GCAΨGACCAG	CΨGCΨGΨAGC	ΨGCCΨGAAGG	GCΨGΨΨGΨAG	3800
CΨGΨGGCAGC	ΨGCΨGCAAGΨ	ΨCGACGAGGA	CGAΨΨCΨGAG	CCCGΨGCΨGA	AGGGCGΨGAA	ACΨGCACΨAC	ACAΨGAΨGAC	ΨCGAGCΨGGΨ	ACΨGCAΨGCA	3900
CGCAAΨGCΨA	GCΨGCCCCΨΨ	ΨCCGΨCCΨG	GGΨACCCCGA	GΨCΨCCCCCG	ACCΨCGGGΨC	CCAGGΨAΨGC	ΨCCCACCΨCC	ACCΨGCCCCA	CΨCACCACCΨ	4000
CΨGCΨAGΨΨC	CAGACACCΨC	CCAAGCACGC	AGCAAΨGCAG	CΨCAAAACGC	ΨΨAGCCΨAGC	CACACCCCCA	CGGGAAACAG	CAGΨGAΨΨAA	CCΨΨΨAGCAA	4100
ΨAAACGAAAG	ΨΨΨAACΨAAG	CΨAΨACΨAAC	CCCAGGGΨΨG	GΨCAAΨΨΨCG	ΨGCCAGCCAC	ACCCΨGGAGC	ΨAGCAAAAAA	AAAAAAAAAA	AAAAAAAAAA	4200
AAAAGCAΨAΨ	GACΨAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAAAAAAAA	AAAA		4284

*Ψ = 1-methyl-3'-pseudouridylyl

Fig. 2. Schematic structure of in vitro transcribed BNT162b2 mRNA (Purple: 5′-cap. Green: 5′-and 3′-UTR sequences. Blue: coding sequence of SARS-CoV-2 spike glycoprotein. Orange: poly(A) tail), and mRNA-LNP structure. Bottom: Sequence of the BNT162b2 mRNA vaccine from Pfizer/BioNTech, where Ψ indicates N1-methyl-3′-pseudouridine.

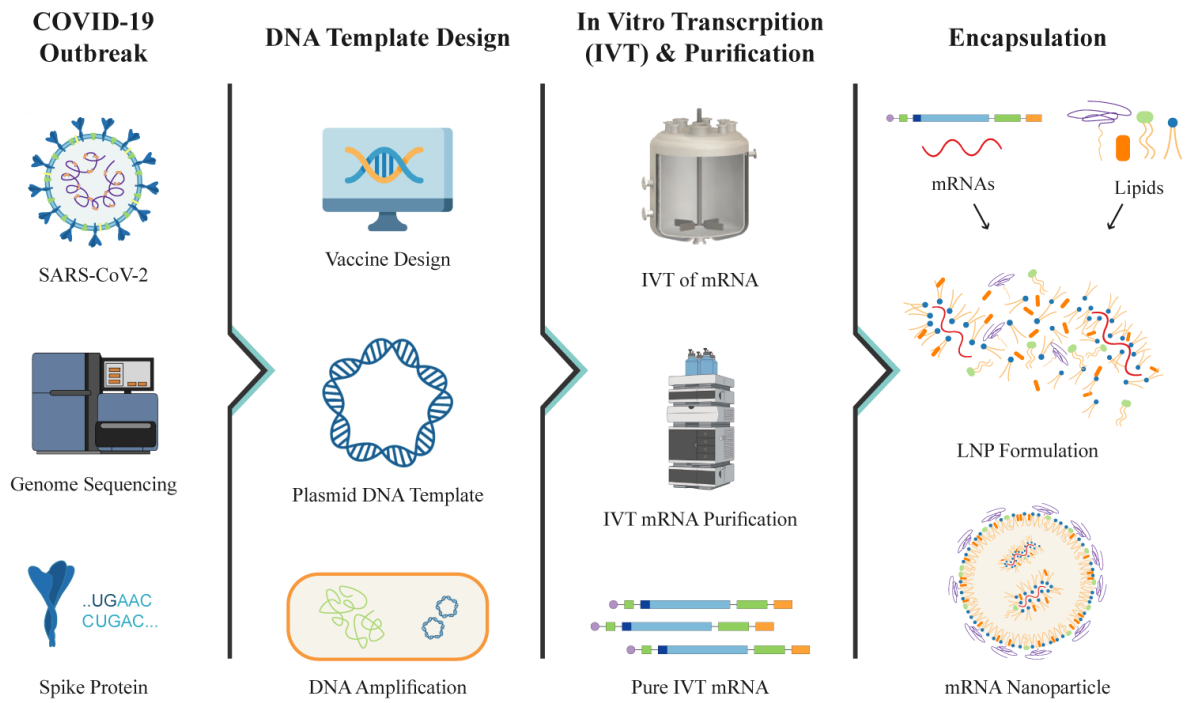


Fig. 3. Schematic illustration of BNT162b2 mRNA vaccine manufacturing process: once an outbreak declared, the genome of the pathogen was determined. Vaccine antigen sequence (spike glycoprotein) was determined, followed by construction of plasmid DNA template, in vitro transcription, capping, purification, and encapsulation of the mRNA.

6.4. Optimization of BNT162b2 mRNA Vaccine

Each structural component of the mRNA vaccine can be optimized to enhance mRNA stability, translation efficiency, and immune-stimulatory properties [42].

The RNA sequence of the BNT162b2 vaccine consists of 4284 nucleotides [43], and has a molecular weight of approximately 1388 kDa [42], and encompasses five main elements. Below, we describe the modifications made to the BNT162b2 mRNA compositions to enhance stability.

5' Cap: The Pfizer-BioNTech mRNA COVID-19 vaccine now utilizes an advanced cap 1 analog [39]. This improved 5′-cap structure (m7 (3′OMeG) (5′) ppp (5′) (2′OMeA) pG, (cap 1) [43] is created through methylation of the 2'-hydroxyl group on cap 0, providing almost 100% capping efficiency [39], and aiding in ribosome recruitment and RNA protection from degradation [43].

5′- UTR: The BNT162b2 mRNA contains a 5′ UTR region (ΨCΨΨCΨGGΨCCCCACAGACΨCAGAGAGAACCCGCC) derived from the highly expressed human gene α-globin, along with an optimized downstream Kozak consensus GCCACCAUG in place of the standard ACCAUG (where AUG is the start codon) [44-45].

Open reading frame (ORF): The BNT162b2 mRNA contains an optimized ORF that encodes the spike protein of SARS-CoV-2 from nucleotides 55 to 3879, including the signal peptide from nucleotides 55 to 102 [42]. In 2020, Pfizer-BioNTech incorporated N1-methyl-Ψ into BNT162b2 mRNA sequence, replacing all uridines, including those in the stop codons. This modification can alter RNA structure by improving base-pairing, base stacking, and increasing backbone rigidity (through hydrogen bonding interactions) [46].

Additionally, two amino acid mutations, K986P and V987P (where lysine at position 986 and valine at position 987 were substituted with proline), were introduced [47], to stabilize the spike protein in its prefusion conformation, optimizing both its expression and immunogenicity [48].

3′-UTR: The 3′-UTR of the BNT162b2 mRNA spans from nucleotides 3880 to 4174 [42], and consists of sequences from the amino-terminal enhancer of split mRNA and mitochondrial encoded 12S rRNA, which enhance protein expression by stabilizing the RNA [43].

Poly A tail: For IVT of RNA from a DNA template, a predetermined poly(A) tail length is preferred, particularly for clinical applications [49]. Poly(A) tails longer than 100 base pairs are considered optimal for therapeutic mRNAs. However, DNA sequences encoding long poly(A) stretches can destabilize DNA plasmids used for transcription. To address this, a short UGC linker is included in the poly(A) tail [33-39].

The BNT162b2 vaccine incorporates a 30-mer poly(A) tail, a 10-nucleotide linker sequence (GCAUAUGACU), and an additional 70 adenosine residues from nucleotides 4175 to 4284 to enhance and prolong protein expression [42].

6.5. IVT mRNA Purification

During in vitro synthesis of mRNA, various elements, including a DNA plasmid, RNA polymerase, metal ion coenzyme factors, nucleotide starting materials [39], and aberrant mRNA molecules formed during the IVT [28], may unintentionally intermix in the final product. Efficiently removing these impurities is crucial for enhancing mRNA translation levels, and preventing undesirable immune response, ultimately resulting in the production of non-immunogenic IVT mRNA with improved translation

efficiency [39].

Consequently, following the synthesis of mRNA through IVT, it is essential to purify it from the reaction mixture. Conventional purification methods at the laboratory scale typically involve DNA removal via DNAse digestion followed by lithium chloride (LiCl) precipitation. However, these methods do not effectively eliminate unwanted mRNA species, such as double-stranded RNA and truncated RNA fragments [28].

Chromatographic separation is a key technique for the selective, adaptable, and scalable purification of biological substances.

Recent advancements in chromatographic purification have greatly improved the preparation and purification of mRNA vaccines [50]. HPLC is widely recognized as the benchmark for mRNA purification in laboratory settings [39]. HPLC purification eliminates double-stranded RNA and other impurities from in vitro-transcribed RNAs containing Ψ or m5C/Ψ, resulting in RNA with significantly higher translation efficiency — up to 1000 times more than non-HPLC purified RNA [51].

To address the challenges of purifying IVT mRNA, various chromatography purification techniques have been employed.

Size-exclusion HPLC (SEC) separation is based on differences in the size or hydrodynamic radius of the molecules. Reversed-phase ion-pairing HPLC (RP-IP-HPLC) uses lipophilic cations, such as quaternary ammonium compounds, which ion-pair with the negatively charged sugar–phosphate backbone of the oligonucleotide [52].

The ion-exchange HPLC (IE-HPLC) method involves a contacting step, where the sample is brought into contact with an ion-exchange sorbent containing a positively charged functional group linked to solid phase media. The sample, which is in an aqueous-based solution, allowing the mRNA to bind to the sorbent's positively charged functional group [53].

Recently, affinity chromatography methods have gained popularity for RNA preparation [52]. These methods exploits the hybridization affinity between the poly-A tail of the mRNA and a poly-dT chain attached to a chromatographic stationary phase [54].

6.6. Encapsulation

mRNA, which degrades naturally within approximately two days in the body [55], requires protection to maintain its stability during transport into target cells. Lipid nanoparticles (LNPs) play a critical role in safeguarding mRNA from degradation [56]. These nanoparticles encapsulate the mRNA and organize it into stable lipid bilayers for effective cellular uptake [57] (Fig. 2). The process involves LNPs adsorption to the cell membrane, uptake via endocytosis, and subsequent release of the mRNA inside the cell [58].

As of June 2021, all SARS-CoV-2 mRNA vaccines developed or approved for clinical use rely on LNPs [33]. Specifically, the BNT162b2 mRNA is encapsulated using patented LNPs designed to enhance delivery efficiency, as evidenced by clinical trial results (NCT04368728).

The LNP formulation comprises four key components in a ratio of 50:10:38.5:1.5 mol/mol [57]:

1. An ionizable or cationic lipid [ALC-0315 ((4-hydroxybutyl) azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate))] (BNT162b2) [59], which encapsulates the polyanionic mRNA [42], and provides positive charges to facilitate membrane crossing [60].

2. Phospholipid, such as 1,2-distearoyl-snglycero-3-phosphocholine (DSPC), which mimics cell membrane lipids.

3. Cholesterol: to stabilize the LNPs lipid bilayer structure [42].

4. Polyethylene glycol (PEG)-lipid [ALC-0159 (2-[(polyethylene glycol)−2000]-N,N-ditetradecylacetamide (PEG2000-DMA) in BNT162b2] [59], which provides a hydration layer, improves colloidal stability, and reduces protein absorption [42].

7. Mechanism of Action of BNT162b2 Vaccine

The BNT162b2 vaccine is administered intramuscularly, ideally into the deltoid muscle [61]. Once administered, the vaccine is taken up by muscle cells or antigen-presenting cells (APCs) such as dendritic cells or macrophages through a process called endocytosis [57] (Fig. 4.1-2). The mRNA contained in the vaccine is then released into the cytoplasm of the host cells, not the nucleus, where it can access ribosomes to produce viral spike proteins [55]. The antigenic protein, when translated, can activate the immune system through various mechanisms [33]. Muscle tissues are richly supplied with blood vessels, which help recruit and circulate various types of immune cells, including infiltrating APCs, at the injection site. Additionally, resident APCs in the skin, muscles, and lymph nodes are capable of processing the expressed antigens and capturing mRNA nanoparticles [62].

7.1. Mechanism of Endosomal Escape of Delivered mRNA

Due to their size, charge, and hydrophilicity, RNA molecules cannot easily diffuse through cellular membranes and instead are taken up into cells via endocytosis. However, the endosome itself poses a challenge as it is composed of a lipid bilayer barrier, which traps and retains approximately 99% of RNA therapeutics, with only a small fraction entering the cytoplasm [63].

To overcome this, ionizable lipids are combined with mRNA to form nanoparticles in an acidic environment [33]. The acidic conditions within endosomes lead to the protonation of ionizable lipids, converting them into cationic lipids [64], which interact with the anionic head of phospholipids in the endosomal membrane [65]. This interaction promotes membrane fusion and disruption [64], as the hydrophobic tail of the cationic lipid and phospholipid extends, breaking apart the bilayer phospholipid structure and facilitating the release of mRNA into the cytoplasm [65].

Various mechanisms have been proposed to facilitate the escape of mRNA from endosomes, including pore formation in the endosomal membrane, pH-buffering effects of protonable groups, and fusion with the lipid bilayer [66]. The fusion process between lipid membranes,

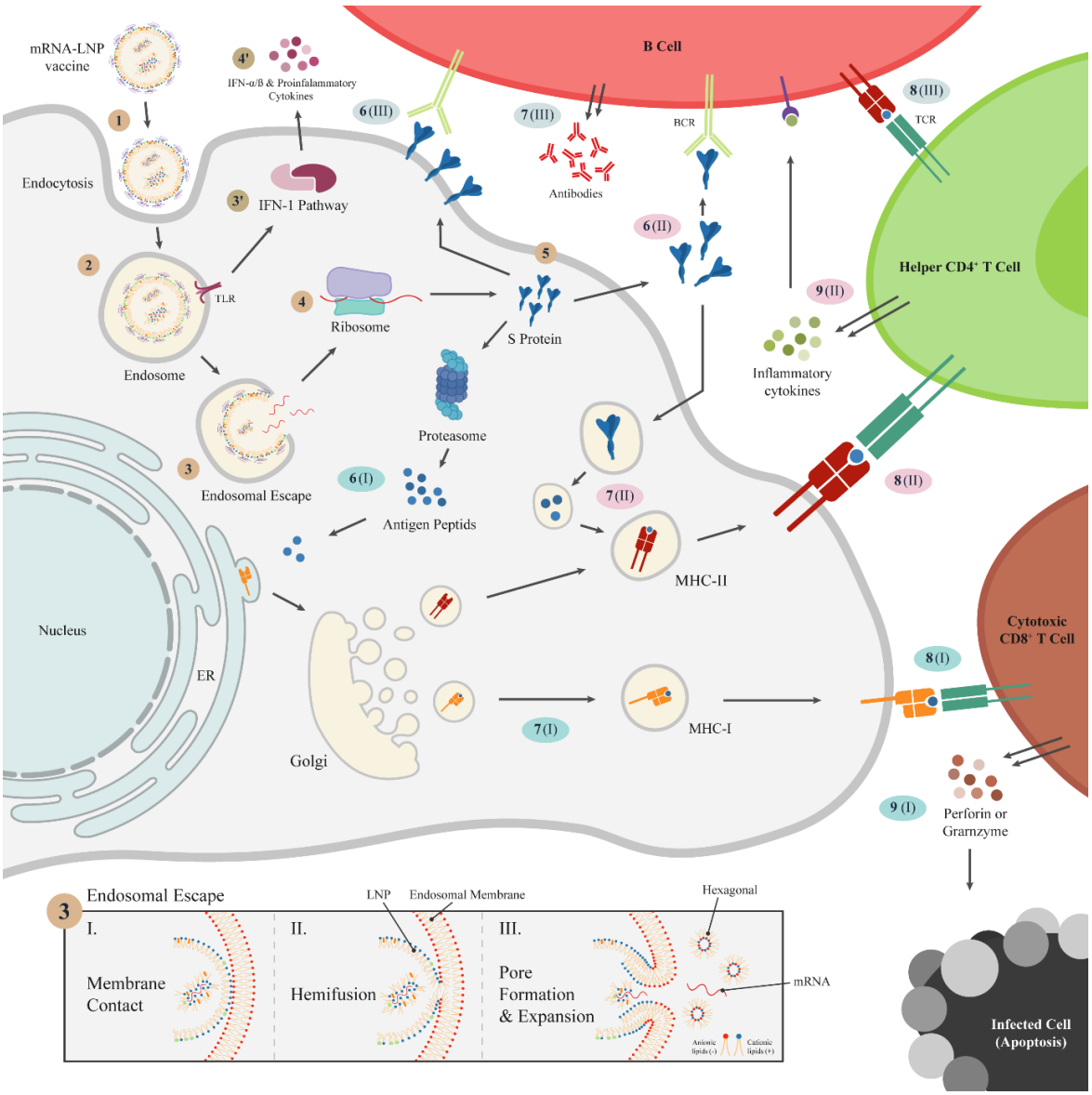


Fig. 4. Immune responses induced by BNT162b2 mRNA vaccine. Following intramuscular administration, the vaccine is taken up by endocytosis (1-2). After release from the endosome (3), an mRNA vaccine is translated into protein by ribosomes (4-5). The translated protein can activate the immune system in two ways (I) and (II). mRNA can also bind to Toll-like receptors (TLR) in the endosome, and activates the innate immune response by the production of Type-I interferon (IFN-I) (3'-4').

including LNPs, involves several energy-dependent intermediate stages. These stages include close contact between the membranes, the creation of a hemifusion structure where the two membranes merge, the formation of a fusion pore, and, ultimately, the expansion of the pore [67] (Fig. 4.3).

However, the specific sites and processes by which lipid nanoparticles assist mRNA in escaping the endosomal compartment remain largely unknown. The efficiency of this escape likely depends on the distribution of lipid nanoparticles across various subcellular compartments [68].

8. Immune responses induced by BNT162b2 mRNA Vaccine

Although the immune systems of different individuals may respond to the same protein in a vaccine, their T cells may react to different regions of that protein. This leads to variations in the immune response to the vaccine antigen [69].

All mRNA vaccines target the same SARS-CoV-2 antigen and use mRNA to encode the spike protein [47]. Once released from the endosomes, the mRNA is translated into protein by ribosomes [70]. The translated protein can then activate the immune system in two ways, as described below in points (I) and (II).

(I) Intracellular proteins are broken down into peptide fragments by the ubiquitin-proteasome system. These fragments are then presented on the cell surface as antigens by major histocompatibility complex (MHC) class I molecules [71], which bind to the T cell receptor to activate CD8+ T cells [64] in a process called cross-presentation. This mechanism enables CD8+ T cells to identify and destroy infected cells [72]. CD8+ T cells release cytotoxic molecules, such as perforin and granzyme, to kill virally infected cells [73] (Fig. 4).

(II) Proteins secreted extracellularly are engulfed by APCs [65]. These antigenic particles are internalized through endocytosis or phagocytosis, processed into small

peptides, and then inserted into the MHC-II cleft for activating Th (CD4+) cells [74]. This activation can trigger a cellular immune response by secreting inflammatory cytokines, as well as a humoral immune response by activating B cells [70]. The activation of the B cells begins with the recognition of the S protein, and the S1 subunit — dissociated from the S2 subunit — that binds to the B cell receptor (BCR) [75] (Fig. 4).

The recognition of the spike protein by B cells leads to the production of neutralizing antibodies, accompanied by a robust germinal center reaction [76]. This process provides long-term protection by generating long-lived plasma cells and memory B cells, which refine and expand the B cell repertoire, allowing for more effective responses upon subsequent exposures [77]. Strategies aimed at stabilizing the spike protein in its prefusion state and enhancing its expression are believed to improve both the quality and quantity of vaccine-induced antibodies [78].

Research has primarily focused on the humoral immune response to BNT162b2 in healthy, uninfected individuals, demonstrating that two doses are required to achieve an effective and strong IgG antibody response, with limited involvement of IgM and IgA in serum [79].

Additionally, mRNA vaccines deliver both single-stranded and double-stranded RNA, which bind to Toll-like receptors in the endosome. This binding activates the innate immune response through the production of Type-I interferon (IFN-I). As a result, several IFN-I-stimulated genes involved in antiviral innate immunity are induced through a mechanism known as the self-adjuvant effect of sequence-engineered mRNA [64] (Fig. 4).

9. Approval of the BIONTECH-PFIZER vaccine

On December 11, 2020, the U.S. Food and Drug Administration (FDA) issued the first emergency use authorization (EUA) for a BioNTech COVID-19 vaccine to prevent COVID-19 in individuals aged 16 years and older [80]. This approval followed approximately 11 months after the launch of 'Project Lightspeed', a collaborative effort between BioNTech and Pfizer to develop an RNA vaccine for COVID-19 [81].

Subsequently, on August 23, 2021, the FDA officially approved the BNT162b2 vaccine for individuals aged 16 and older for the prevention of COVID-19. The vaccine remains available under EUA for individuals aged 12 to 15 years, and for the administration of a third dose in certain immunocompromised individuals [82].

On October 29, 2021, the FDA expanded emergency use authorization of the Pfizer-BioNTech COVID-19 vaccine to include children aged 5-11 years. The vaccine is administered as two-dose primary series, three weeks apart, but with a reduced dose of 10 µg [83].

The rapid approval of the BNT162b2 vaccine, which occurred just 221 days after the start of the First-in-Human (FIH) studies, compared to the industry median of 9 years and 4 months for other vaccines. This accelerated approval was due to several factors, including:

Scientific factors: preclinical studies were conducted alongside clinical development, allowing for a faster selection of the most promising vaccine candidate for Phase 3 trials.

Operational factors: i) selecting a suitable partner, ii) adopting a 'one team mindset' with decisive leadership, iii) employing a parallel rather than sequential R&D process and making substantial at-risk investments in R&D and manufacturing despite limited scientific data, iv) financing a comprehensive program budget [84].

10. Efficacy and safety of the BNT162b2 mRNA vaccine

Non-Clinical Phase: Four mRNA vaccine candidates were assessed in nonclinical studies, with two encoding the RBD (BNT162b1 and BNT162b3), and two encoding the full-length S protein in its pre-fusion conformation (BNT162b2 [V8] and BNT162b2 [V9]). These studies showed safety and tolerability in rats. Rats were chosen as the test subjects due to their extensive historical data and ability to generate antigen-specific immune responses to the S protein [85]. The two vaccine candidates, BNT162b1 and BNT162b2, induced strong antigen-specific immune responses in both mice and macaques, providing protection against SARS-CoV-2 in macaques. Notably, BNT162b2 protected the lower respiratory tract from the presence of viral RNA without any signs of disease enhancement [86].

Phase 1: The 10-µg, 20-µg, and 30-µg dose levels of BNT162b1 and BNT162b2 were evaluated in adults aged 18 to 55 years and 65 to 85 years. A total of 195 participants were randomly assigned for this assessment [87].

The safety and immunogenicity profiles of both vaccine candidates supported the progression of BNT162b2 to a critical Phase 2-3 safety and efficacy evaluation (NCT04368728) [87-88].

Phase 2/3: An ongoing multinational, placebo-controlled, observer-blinded trial was performed with 43,548 participants aged 16 years or older. A two-dose regimen of BNT162b2 (30 µg per dose) demonstrated a remarkable 95% protection against Covid-19. The safety profile observed over a median of two months was similar to that of other viral vaccines, paving the way for the progression of the BNT162b2 vaccine candidate into phase 3 [89] (NCT04368728).

However, according to the U. S. National Library of Medicine (Clinicaltrials.gov), there have been a total of 1,314 registered clinical trials worldwide on COVID-19 mRNA vaccines till 30/5/2024, with 135 trials specifically focusing on BNT162b2 vaccine. The majority of which are in phase 2 (evaluating efficacy in human patients), and phase 3.

Many clinical trials have shown the effectiveness of COVID-19 BNT162b2 vaccine, and safety with commonly reported symptoms including fatigue, myalgia, nausea, headache, soreness, chills, joint pain, muscle spasm, fever, feelings of relief, sweating, flushing, dizziness, brain fogging, anorexia, localized swelling, decreased sleep quality, itching, diarrhoea, nasal stuffiness, tingling, and palpitations [90]. No serious side effects have been detected in most of the trials (Table 1). The vaccine has shown an efficacy of 91.3% against COVID-19 after 6 months of follow-up among participants without prior SARS-CoV-2 infection [91].

Notably, individuals previously infected with COVID-19 may require only a single dose for an effective immune response [92]. The BNT162b2 vaccine in participants within the age of (12-15 years) had a favorable safety profile,

producing a stronger immune response compared to young adults, with 100% efficacy against COVID-19 (95% CI, 75.3 to 100) [93]. Two doses of BNT162b2 (10-µg) administered 21 days apart was found to be safe, immunogenic, and effective in children (5-11 years) (90.7% efficacy; 95% CI, 67.7 to 98.3) [94].

Table 1. Clinical trials of BNT162b2 mRNA vaccine for the prevention of COVID-19.

Clinical Trial Identifier (phase)	Study Design	No. of Participants (Country)	Participants Characteristics	Follow-up	AEs and SAEs	Key results	Reference
NCT04955626 (phase 3)	A randomized trial (3rd dose of the BNT162b2 vaccine or placebo)	10,136 participants (5081 in the BNT162b2 group) (South Africa, United States, and Brazil)	Median age was 53 years (1% were 16 or 17 years of age), almost half the participants had coexisting conditions, (obesity, chronic pulmonary disease or diabetes without chronic complications)	2.5 months	AEs: Injection-site pain. SAEs included tachycardia and increased hepatic enzyme levels	The trial underscores the benefit of a third dose of BNT162b2, providing 95.3% efficacy against Covid-19	95
NCT04368728 (phase 1)	An ongoing, observer-blinded, dose-escalation, trial (10, 20, 30) µg, two doses, at 21 days apart, of BNT162b1 and BNT162b2; and one dose of 100 µg	195 participants (United States)	healthy individuals (18 – 55 years) and (65- 85 years)	6 months	AEs: mild to moderate local reactions, mainly pain at injection site. Systemic events: (muscle pain, joint pain, fatigue, headache, chills, and fever). No serious adverse events were reported	Results supported BNT162b2 vaccine for progression to a crucial Phase 2-3 safety and effectiveness assessment	87
NCT04368728 (phase 2/3)	A randomized, observer-blinded, multinational, pivotal efficacy trial (two doses (30-µg) of BNT162b2 or placebo, at 21 days apart	43,548 participants (22,030 in the BNT162b2 group) (Argentina, United States, Turkey, South Africa, Germany, and Brazil)	16 years or older, (2264 participants 12-15 years), 49% female	6 months	AEs: pain at the injection site, lethargy, asthenia, decreased appetite, malaise, hyperhidrosis, night sweats, and fatigue Few participants had SAEs that led to trial withdrawal	BNT162b2 exhibited a favorable safety profile, and high efficiency	91
NCT04368728 (phase 3)	placebo-controlled, observer-blinded trial (2-dose BNT162b2 and placebo)	3813 participants (152 sites in 6 countries)	Participants (≥ 16 years) for safety; and (≥ 12 years) for efficacy. Participants had a history of neoplasm; most common malignancies were melanoma, prostate, and breast	Up to 6 months	AEs: injection-site pain, fatigue, and pyrexia. Three individuals who received the BNT162b2 vaccine and one who received a placebo withdrew from the study due to vaccine-related adverse events. There were no reported deaths related to the vaccine.	The vaccine's efficacy and safety in individuals with a history of or active neoplasms were similar to those observed in the general trial population	96
Eudra-CT: 2021-002030-16 (phase 4)	An open-label, observational trial (two doses of BNT162b2)	2760 participants (Tyrol, Austria)	Participants ≥16 years, mean age 47·4 years, 60.9% female, 39.1% male, 712 of participants had a previous SARS-CoV-2 infection	5-9 months	AEs: pain at injection site, headache and fatigue within one week after vaccination	In contrast to the T-cell response, higher levels of binding and neutralizing antibodies following BNT162b2 vaccination were associated with a reduced risk of breakthrough SARS-CoV-2 infections	97

NCT04750720 NCT05315583	A cohort study (2 or 3 doses of BNT162b2)	26 participants (Orléans, France)	mean age was 59 years [33; 95], men (54%)	Mean of Follow-up duration after first-dose 368 (days) [168; 483]	Not Available	The administration of a third vaccine dose notably enhanced the neutralization capacity against various SARS-CoV-2 variants of concern, despite variability between individuals. The average duration of detectable neutralization capacity against non-Omicron variants of concern is estimated to range from 348 to 587 days, while for Omicron variants, it is projected to be between 173 and 256 days following the third dose.	98
NCT04887948 (phase 3)	A randomized, double-blind, multicenter study (BNT162b2 and PCV20 coadministered, or BNT162b2 or PCV20 only)	570 participants (United States)	aged ≥65 years, Female (41.6%), Hispanic/Latino (13.5%), Mean age 71.8, Never smoked (56.2%)	6 months	AEs: injection-site pain and fatigue. AEs and SAEs were infrequent and comparable across groups, with no SAEs attributed to vaccination	Strong immune responses were observed, and the safety and immunogenicity of the co-administration of BNT162b2 and PCV20 were comparable to those of each vaccine administered individually, suggesting the potential for their combined use	99
TCTR20220125 002	An open-label, randomized study (BNT162b2 (two doses) with an 8-week (extended dosing) vs. 3-week interval. The third dose was offered to participants who had surrogate virus NT <68% inhibition	382 children (Thailand)	median age of 8.4 years, and body mass index of 16.5 kg/m2	3 months	AE: pain at injection site. Systemic reactions were reported including myalgia, fatigue, headache, and fever. No SAEs were reported	The reactogenicity following BNT162b2 vaccination was mild. The BNT162b2 booster elicited a strong neutralizing antibody response against the Omicron variant. Additionally, the extended dosing of BNT162b2 resulted in higher levels of neutralizing antibodies against the Omicron variant	100
NCT04816643 (phase 2/3)	An ongoing study, open-label third (booster) dose of (10-µg) BNT162b2 at least six months after dose 2	401 participants	5 to 11 years old	after dose 3: 1.3 (1.0–1.8) months	Mild to moderate reactogenicity events. There were no severe grade 4 events or fevers exceeding 40 °C reported. As with the first and second doses, some AEs were reported such as injection-site pain, fatigue and headache	A 3rd dose of the BNT162b2 (10 µg) vaccine boosted neutralizing titers, including those against the Omicron BA.1 and BA.4/BA.5 strains. The safety and tolerability profile of this dose was deemed acceptable	101

Eudra-CT: 2021-005094-28	An ongoing single-centre, open-label, clinical cohort study (co-administration of a BNT162b2 booster with tetravalent influenza vaccine, as well as the administration of BNT162b2 and the influenza vaccine individually)	838 participants (Vienna, Austria)	healthcare workers with median age of 43 (32-54), men (38.1%)	4 weeks	AEs were categorized into local reactions (pain at the injection site, swelling, redness, and itching) and systemic reactions (myalgia, arthralgia, nausea, fatigue, vomiting, fever and headache)	The co-administration of BNT162b2 and the tetravalent influenza vaccine was found to be safe. However, a decrease in immune response was observed when the BNT162b2 booster was given in combination with the tetravalent influenza vaccine	102
NCT04816643 (phase 1/2/3)	A Phase 1, open-label, dose-finding study was conducted with two BNT162b2 doses: a 10-µg dose for children aged 2 to 4 years and a 3-µg dose for children aged 6 months to less than 2 years	64 participants	The study included 16 children aged 6 months to less than 2 years, and 48 children aged 2 to 4 years, with 59% male participants	1 week	The majority of systemic events were mild to moderate in severity, with no reports of grade 4 systemic events. Severe events were rare, occurring in ≤1.1% of participants	A three-dose primary series of 3-µg BNT162b2 was shown to be safe, immunogenic, and effective in children between 6 months and 4 years of age	103
	Phase 2/3 Trial of the Selected Dose, underwent randomization (two doses of 3-µg BNT162b2 or saline placebo, and third dose)	4526 participants (Brazil, Finland, Poland, Spain, and the United States)	1776 children (6 months to < 2 years), and 2750 children (2 - 4 years). (male 50%) (white 79.3%)	1.3- 1.4 months			
ISRCTN-73765130, (phase 2)	A multicentre, blinded, randomized trial (two doses of BNT162b2 or ChAdOx1 nCoV-19, third dose of BNT162b2, and fourth dose of either BNT162b2 (full dose) or mRNA-1273 (half dose) (1:1)	166 participants (18 sites in the UK)	Full BNT162b2 as fourth dose (n=83), median age was 70.1 years, female (52%)	3 months	The most frequent AEs following BNT162b2 or mRNA-1273 booster doses were pain and fatigue. There were no SAEs related to the study vaccine reported after a fourth dose of BNT162b2	The fourth dose of the COVID-19 mRNA booster vaccines was well tolerated and improved both cellular and humoral immunity. The peak response following the fourth dose was comparable to, and potentially even greater than, the peak response observed after the 3rd dose	104

EudraCT: 2021-000175-37 and NCT04780659 (Phase 4)	open-label, non-randomized prospective clinical trial, (two doses of BNT162b2)	539 participants (Karolinska University Hospital, Stockholm, Sweden)	Individuals aged 18 and older, with no history of coronavirus infection, and who have primary immunodeficiency diseases, or secondary immunodeficiency diseases due to human immunodeficiency virus (HIV) infection, hematopoietic stem cell transplantation (HSCT)/chimeric antigen receptor T cell therapy (90 participants), solid organ transplantation (89 participants), or chronic lymphocytic leukemia (90 participants)	6 weeks	AEs observed after 6 weeks were generally mild, although one case of a fatal suspected unexpected SAEs was reported	The mRNA BNT162b2 vaccine was considered safe for immunocompromised patients. However, the rate of seroconversion was significantly lower compared to healthy individuals, with varying rates and antibody titers observed across the different patient groups and subgroups	105
NCT05472038 (phase 2/3)	Cohort, ongoing, randomized trial (fourth dose (second booster) of 30-µg bivalent original/Omicron -BA.4/BA.5-adapted BNT162b2)	939 participants (30 US sites)	12-17 years old (n = 108), 18-55 years old (n = 313), and >55- years old (n = 306), previously received BNT162b2 (3 original doses)	6 months	AEs: injection-site pain and fatigue. No grade 4 events were reported, and most reactogenicity events were mild to moderate in severity	The 30-µg BNT162b2-Omi.BA.4/BA.5 booster demonstrates a favorable benefit-risk profile	106

11. Conclusions

The safety, efficacy, and overall trustworthiness of the Pfizer vaccine continue to be subjects of debate even today. While numerous studies and health organizations have endorsed its effectiveness in preventing severe illness from COVID-19, concerns persist regarding potential side effects, particularly among specific demographics such as younger individuals and those with pre-existing conditions. The vaccine may also exhibit different protective efficacy among different population groups, such as immunocompromised populations, children, and women, suggest the need for long-term evaluations. Discussions have also emerged around the vaccine's long-term effects, the necessity of booster shots, and the speed at which it was developed and authorized for emergency use. Additionally, misinformation and varying public perceptions have fueled further controversy, leading to polarized opinions about vaccination mandates and public health policies. As a result, ongoing research and transparent communication are crucial to address these concerns and provide a more thorough understanding of the vaccine's performance.

We hope that this study will contribute to enriching the scientific discussion surrounding the Pfizer vaccine and enhance collective understanding of the importance of vaccination as a fundamental means of combating the pandemic. We believe that this information will benefit both the academic community and the general public, making it a valuable addition to the available scientific content.

Author Contributions: Conceptualization, E.A, B.J., T.S, W.A, D.J.; methodology, E.A, B.J., T.S, W.A.; validation, E.A, B.J., T.S, W.A, D.J.; resources, E.A, B.J., T.S, W.A.; data curation, B.J.; writing—original draft preparation, E.A, B.J., T.S, W.A.; writing—review and editing, E.A, B.J., T.S, W.A, D.J.; visualization, B.J.; supervision; project administration; investigation, D.J. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: This manuscript is supported by University of Aleppo, Syria.

Conflicts of Interest: The authors have no conflicts of interest, financial or otherwise, to declare.

References

1. World Health Organization (March 2020). ‘’WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020”. Available online: https://www.who.int/director-general/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020. (accessed on 17-10-2024)
2. World Health Organization. WHO COVID-19 dashboard. “COVID-19 Global circulation.” Available online: https://data.who.int/dashboards/covid19. (accessed on 17-10-2024)
3. Trojánek, M.; Grebenyuk, V.; Herrmannová, K.; Nečas, T.; Gregorová, J.; Kucbel, M.; et al. Novel coronavirus (SARS-CoV-2) and COVID-19. *Cas. Lek. Cesk.* **2020**, *159(2)*, 55-66. Available online: https://www.prolekare.cz/casopisy/casopis-lekaru-ceskych/2020-2-6/novy-koronavirus-sars-cov-2-a-onemocneni-covid-19-122272
4. Lamer, M.; Haagmans, B. SARS-CoV-2 pathogenesis. *Nat. Rev. Microbiol.* **2022**, *20*, 270-284. DOI: 10.1038/s41579-022-00713-0
5. Wang, Y.; Grunewald, M.; Perlman, S. Coronaviruses: An Updated Overview of Their Replication and Pathogenesis. In: *Coronaviruses. Methods Mol Bio,*. Maier, H., Bickerton, E. (eds). Humana, New York, NY. **2020**, *vol 2203*, 1-29. DOI: 10.1007/978-1-0716-0900-2_1

6. Li, F. Structure, Function, and Evolution of Coronavirus Spike Proteins. *Ann. Rev. Virol*. **2016**, *3(1)*, 237-261. DOI: 10.1146/annurev-virology-110615-042301
7. Sharma, B.; Ravindra, P. Immune responses to infection and covid-19 vaccines. *Explor. Immunol*. **2022**, *2*, 648-664. DOI: 10.37349/ei.2022.00074
8. Farhud, D.; Zokaei, S. A Brief Overview of COVID-19 Vaccines. *Iran. J. Public Health* **2021**, *50(7)*, 1-6. DOI: 10.18502/ijph.v50i7.6656
9. Chung, V.; Beiss, V.; Fiering, S.; Steinmetz, N. COVID-19 Vaccine Frontrunners and Their Nanotechnology Design. *ACS. Nano* **2020**, *14*, 12522−12537. DOI: 10.1021/acsnano.0c07197
10. Schlake, T.; Thess, A.; Fotin-Mleczek, M.; Kallen, K. Developing mRNA-vaccine technologies. *RNA. Biol*. **2012**, *9(11)*, 1319–1330. DOI: 10.4161/rna.22269 9
11. Szabó, G.; Mahiny, A.; Vlatkovic, I. COVID-19 mRNA vaccines: Platforms and current developments. *Mol. Ther*. **2022**, *30(5)*, 1850-1868. DOI: 10.1016/j.ymthe.2022.02.016
12. Naqvia, A.; Fatimab, K.; Mohammada, T.; Fatimac, U.; Singhd, I.; Singhe, A.; et al. Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: Structural genomics approach. *BBA Mol. Basis Dis*. **2020**, *1866 (10)*, Art. No: 165878. DOI: 10.1016/j.bbadis.2020.165878
13. Young, M.; Crook, H.; Scott, J.; Edison, P. Covid-19: virology, variants, and vaccines. *Bmj Med*. **2022**,*1(1)*, Art. No: e000040. DOI: 10.1136/bmjmed-2021-000040
14. Masters, P. The molecular biology of coronaviruses. *Adv. Virus Res*. **2006**, *66*, 193–292. DOI: 10.1016%2FS0065-3527(06)66005-3.
15. Jungreis, I.; Sealfon, R.; Kellis, M. SARS-CoV-2 gene content and COVID-19 mutation Impact by comparing 44 Sarbecovirus genomes. *Nat. Commun*. **2021**,*12*, 1-20. DOI: 10.1038/s41467-021-22905- 7
16. Ke, Z.; Oton, J.; Qu, K.; Cortese, M.; Zila, V.; McKeane, L.; et al. Structures and distributions of SARS-CoV-2 spike proteins on intact virions. *Nature* **2020**, *588*, 498–502. DOI: 10.1038/s41586-020-2665-2
17. Huang, Y.; Yang, C.; Xu, W.; Liu, S. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacol. Sin*. **2020**, *41*, 1141–1149. DOI: 10.1038/s41401-020-0485-4
18. Yan, R.; Zhang, Y.; Li, Y.; Xia, L.; Guo, Y.; Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* **2020**, *367(6485)*, 1444-1448. DOI: 10.1126/science.abb2762
19. Abdulla, A.; Al-Bashir, S.; Alzoubi, H.; Al-Salih, N.; Aldamen, A.; Abdulazeez, A. The Role of Immunity in the Pathogenesis of SARS-CoV-2 Infection and in the Protection Generated by COVID-19 Vaccines in Different Age Groups. *Pathogens* **2023**, *12(2)*, Art. No: 329. DOI: 10.3390/pathogens12020329 .
20. Szymczak, A.; Jędruchniewicz, N.; Torelli, A.; Kaczmarzyk-Radka, A.; Coluccio, R.; Kłak, M.; et al. Antibodies specific to SARS-CoV-2 proteins N, S and E in COVID-19 patients in the normal population and in historical samples. *J.Gen. Virol*. **2021**,*102(11)*, Art. No: 001692. DOI: 10.1099/jgv.0.001692
21. Primorac, D.; Vrdoljak, K.; Brlek, p.; Pavelic, E.; Molnar, V.; Matisˇic', V.; et al. Adaptive Immune Responses and Immunity to SARS-CoV-2. *Front. Immunol*. **2022**, *13*, Art. No: 848582. DOI: 10.3389/fimmu.2022.848582
22. Manna, P.; Reddy, P. Healthy immunity on preventive medicine for combating covid-19. *Nutrients* **2022**, *14(5)*, Art. No: 1004. DOI: 10.3390/nu14051004
23. Silva, M.; Ribeiro, L.; Lima, K.; Lirr, L. Adaptive immunity to SARS-CoV-2 infection: A systematic review. *Front. Immunol*. **2022**, *13*, Art. No: 1001198. DOI: 10.3389/fimmu.2022.1001198
24. Fenwick, C.; Croxatto, A.; Coste, A.; Pojer, F.; André, C.; Pellaton, C.; et al. Changes in SARS-CoV-2 Spike versus Nucleoprotein Antibody Responses Impact the Estimates of Infections in Population Based Seroprevalence Studies. *J. Virol*. **2021**, *95(3)*, e01828-20. DOI: 10.1128/jvi.01828-20
25. Krammer, F. SARS-CoV-2 vaccines in development. *Nature* **2020**, *586*, 516-527. DOI: 10.1038/s41586-020-2798-3
26. Gote, V.; Bolla, P.; Kommineni, N.; Butreddy, A.; Nukala, P.; Palakurthi, S. A Comprehensive Review of mRNA Vaccines. *Int. J. Mol. Sci*. **2023**, *24*, Art. No: 2700. DOI: 10.3390/ijms24032700
27. Karam, M.; Daoud, G. mRNA vaccine: Past, present, future. *AJPS* **2022**, *17(4)*, 491-522. DOI: 10.1016/j.ajps.2022.05.003
28. Rosa, S.; Prazeres, M.; Azevedo, M.; Marques, P. mRNA vaccines manufacturing: Challenges and bottlenecks. *Vaccine* **2021**, *39(16)*, 2190–2200. DOI: 10.1016/j.vaccine.2021.03.038.
29. Kwon, H.; Kim, M.; Seo, Y.; Moon, Y. S.; Lee, H. J.; Lee, K.; et al. Emergence of synthetic mRNA: In vitro synthesis of mRNA and its application in regenerative medicine. *Biomaterials* **2018**, *156*, 172-193. DOI: 10.1016/j.biomaterials.2017.11.034
30. Whitley, J.; Zwolinskiz, C.; Dwnis, C.; Maughan, M.; Hayles, L.; Clarke, D.; et al. Development of mRNA manufacturing for vaccines and therapeutics: mRNA platform requirements and development of a scalable production process to support early phase clinical trials. *Transl. Res*. **2021**, *242*, 38-55. DOI: 10.1016/j.trsl.2021.11.009
31. Henderson, J.; Ujita, A.; Hill, E.; Yousif-Rosales, S.; Smith, C.; Ko, N.; et al. Cap 1 Messenger RNA Synthesis with Co-transcriptional CleanCap® Analog by In Vitro Transcription. *Curr. Protoc*. **2021**, *1(2)*, Art. No: e39. DOI: 10.1002/cpz1.39
32. Jalkanen, A.; Coleman, S.; Wilusz, J. Determinants and implications of mRNA poly(A) tail size – Dose this protein make my tail look big?. *Semin. Cell Dev. Biol*. **2014**, *34*, 24-32. DOI: 10.1016/j.semcdb.2014.05.018
33. Chaudhary, N.; Weissman, D.; Whitehead, K. mRNA vaccines for infectious diseases: principles, delivery and clinical translation. *Nat. Rev. Drug Discov*. **2021**, *20*, 817–838. DOI: 10.1038/s41573-021-00283-5
34. Thorn, C.; Sharma, D.; Combs, R.; Bhujbal, S.; Romine, J.; Zheng, X.; et al. The journey of a lifetime —

development of Pfizer's COVID-19 vaccine. *Curr. Opin. Biotechnol.* **2022**, *78*, Art. No: 102803. DOI: 10.1016/j.copbio.2022.102803

35. Tossberg, T.; Esmond, T.; Aune, T. A simplified method to produce mRNAs and functional proteins from synthetic double-stranded DNA templates. *Biotechniques* **2020**, *69(4)*, 281–288. DOI: 10.2144/btn-2020-0037.
36. Mey, W.; Schrijver, P.; Autaers, D.; Pfitzer, L.; Fant, B.; Locy, H.; et al. A synthetic DNA template for fast manufacturing of versatile single epitope mRNA. *Mol. Ther. Nucleic Acids* **2022**, *17(29)*, 943-954. DOI: 10.1016/j.omtn.2022.08.021
37. Zhang, F.; Wang, W.; Wang, X.; Dong, H.; Chen, M.; Du, N. RT-IVT method allows multiplex real-time quantification of in vitro transcriptional mRNA production. *Commun. Biol.* **2023**, *6*, Art. No: 453. DOI: 10.1038/s42003-023-04830-1
38. Kang, D.; Li, H.; Dong, Y. Advancements of in vitro transcribed mRNA (IVT mRNA) to enable translation into the clinics Advanced Drug Delivery Reviews. *Adv. Drug Deliv. Rev.* **2023**, *199*, Art. No: 114961. DOI: 10.1016/j.addr.2023.114961
39. Zhang, J.; Liu, Y.; Li, C.; Xiao, Q.; Zhang, D.; Chen, y.; et al. Recent Advances and Innovations in the Preparation and Purification of In Vitro-Transcribed-mRNA-Based Molecules. *Pharmaceutics* **2023**, *15(9)*, Art. No: 2182. DOI: 10.3390/pharmaceutics15092182
40. Wang, Y.; Kumari, M.; Chen, G.; Hong, M.; Yuanb, J.; Tsai, J.; Wu, H. mRNA-based vaccines and therapeutics: an in-depth survey of current and upcoming clinical applications. *J. Biomed. Sci.* **2023**, *30(1)*, Art. No: 84. DOI: 10.1186/s12929-023-00977-5
41. Wolf, J.; Dai, N.; Chan, S.; Ivan, R.; Jr. Selective Characterization of mRNA 5′ End-Capping by DNA ProbeDirected Enrichment with Site-Specific Endoribonucleases. *ACS Pharmacol. Transl. Sci.* **2023**, *6(11)*, 1692-1702. DOI: 10.1021/acsptsci.3c00157.
42. Granados-Riveron, J.; and Aquino-Jarquin, G. Engineering of the current nucleoside-modified mRNA-LNP vaccines against SARS-CoV-2. *Biomed. Pharmacother.* **2021**, *142*, Art. No: 111953. DOI: 10.1016/j.biopha.2021.111953
43. Nance, K.; Meier, J. L. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent. Sci.* **2021**, *7(5)*, 748-756. DOI: 10.1021/acscentsci.1c00197.
44. Xia, X. Detailed Dissection and Critical Evaluation of the Pfizer/BioNTech and Moderna mRNA Vaccines. *Vaccines (Basel)* **2021**, *9(7)*, Art. No: 734. DOI: 10.3390/vaccines9070734.
45. World Health Organization (September 2020). "Messenger RNA encoding the full length SARS-CoV-2 spike glycoprotein" (DOC). (accessed on 17-10-2024)
46. Morais, P.; Adachi, H.; Yu, YT. The Critical Contribution of Pseudouridine to mRNA COVID-19 Vaccines. *Front. Cell Dev. Biol.* **2021**, *9*, Art. No: 789427. DOI: 10.3389/fcell.2021.789427
47. Verbeke, R.; Lentacker, I.; Smedt, S.; Dewitte, H. The dawn of mRNA vaccines: The COVID-19 case. *J. Control. Rel.* **2021**, *333*, 511–520. DOI: 10.1016/j.jconrel.2021.03.0433
48. Lewis, L.; Badkar, A.; Cirelli, D.; Combs, R.; Lerch, T. The Race to Develop the Pfizer-BioNTech COVID-19 Vaccine: From the Pharmaceutical Scientists' Perspective. *J. Pharm. Sci.* **2022**, *112(3)*, 640-647. DOI: 10.1016/j.xphs.2022.09.014
49. Kim, S.; Sekhon, S.; Shin, W.; Ahn, G.; Cho, B.; Ahn, J.; et al. Modifications of mRNA vaccine structural elements for improving mRNA stability and translation efficiency. *Mol. Cell Toxicol.* **2022**, *18(1)*, 1-8. DOI: 10.1007/s13273-021-00171-4
50. Feng, X.; Su, Z.; Cheng, Y.; Ma, G.; Zhang, S. Messenger RNA chromatographic purification: advances and challenges. *J. Chrom.* **2023**, *1707*, Art. No: 464321. DOI: 10.1016/j.chroma.2023.464321
51. Kariko, K.; Muramatsu, H.; Ludwig, J.; Weissman, D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res.* **2011**, *39(21), Art. No:* e142. DOI: 10.1093/nar/gkr695
52. Baronti, L.; Karlsson, H.; Marušič, M.; Petzold, K. A guide to large-scale RNA sample preparation. *Anal. Bioanal. Chem.* **2018**, *410(14)*, 3239-3252. DOI: 10.1007/s00216-018-0943-8
53. Wallace, R.; Rochfort, K. Ion-Exchange Chromatography: Basic Principles and Application. *Methods Mol. Biol.* **2023**, *2699*, 161-177. DOI: 10.1007/978-1-0716-3362-5_9
54. Grinsted, J.; Liddell, J.; Bouleghlimat, E.; Kwok, K.; Taylor, D.; Marques, M.; et al. Purification of therapeutic & prophylactic mRNA by affinity chromatography. *Cell Gene Ther. Insights* **2022**, *8(2)*, 335–349. DOI: 10.18609/cgti.2022.049
55. Mascellino, M.; Timoteo, F.; Angelis, M.; Oliva, A. Overview of the Main Anti-SARS-CoV-2 Vaccines: Mechanism of Action, Efficacy and Safety. *Infect. Drug Resist.* **2021**, *14*, 3459–3476. DOI: 10.2147/IDR.S315727
56. Hengelbrock, A.; Schmidt, A.; Strube, J. Formulation of Nucleic Acids by Encapsulation in Lipid Nanoparticles for Continuous Production of mRNA. *Processes* **2023**, *11*, Art. No: 1718. DOI: 10.3390/pr11061718
57. Park, J.; Lagniton, P.; Liu, Y.; Xu, R. mRNA vaccines for COVID-19: what, why and how. *Int. J. Biol. Sci.* **2021**, *17*, 1446-1460. DOI: 10.7150/ijbs.59233
58. Wilson, B.; Geetha, K. Lipid nanoparticles in the development of mRNA vaccines for COVID-19. *J. Drug Deliv. Sci.* Technol. **2022**, *74*, 2-7. DOI: 10.1016/j.jddst.2022.103553
59. Semple, S.; Leone, R. Barbose C, Tam Y, Lin P. Lipid Nanoparticle Delivery Systems to Enable mRNA-Based Therapeutics. *Pharmaceutics* **2022**, *14(2)*, Art. No: 398. DOI: 10.3390/pharmaceutics14020398.
60. Chen, K.; Fan, N.; Huang, H.; Jiang, X.; Qin, S.; Wen, Xiao. W.; et al. mRNA Vaccines Against SARS-CoV-2 Variants Delivered by Lipid Nanoparticles Based on Novel Ionizable Lipids. *Adv. Funct. Mater.* **2022**, *32(39)*, Art. No: 2204692. DOI: 10.1002/adfm.202204692
61. Lamb, Y. BNT162b2 mRNA COVID 19 Vaccine: First Approval. *Drugs.* **2021**, *81(4)*, 495–501. DOI: 10.1007/s40265-021-01480-7
62. Zeng, C.; Zhang, C.; Walker, G.; Dong, Y. Formulation

and Delivery Technologies for mRNA Vaccines. *Curr. Top. Microbiol. Immunol.* **2022**, *440*, 71–110. DOI: 10.1007/82_2020_217

63. Dowdy, S. Endosomal escape of RNA therapeutics: How do we solve this rate-limiting problem? RNA **2023**, *29(4),* 396-401. DOI: 10.1261/rna.079507.122

64. Fang, E.; Liu, X.; Li, M.; Zhang, Z.; Song, L.; Zhu, B.; et al. Advances in COVID-19 mRNA vaccine development. Signal Transduct. *Target. Ther.* **2022**, *7(94),* Art. No: 94. DOI: 10.1038/s41392-022-00950-y

65. Chavda, V.; Jogi, G.; Dave, S.; Patel, B.; Vineela, Nalla. L.; Koradia, K. mRNA-Based Vaccine for COVID-19: They Are New but Not Unknown! *Vaccines* **2023**, *11(3),* Art. No: 507. DOI: 10.3390/vaccines11030507

66. Varkouhi, A.; Scholte, M.; Storm, G.; Haisma, H. Endosomal escape pathway for delivery of biologicals. *J. Control Rel.* **2011**, *151(3),* 220-228. DOI: 10.1016/j.jconrel.2010.11.004

67. Schlich, M.; Palomba, R.; Costabile, G.; Shoshy, Mizrahy S.; Pannuzzo, M.; Peer, D.; et al. Cytosolic delivery of nucleic acids: The case of ionizable lipid nanoparticles. *Bioeng. Transl. Med.* **2021**, *6(2),* Art. No: e10213. DOI: 10.1002/btm2.10213

68. Paramasivam, P.; Franke, C.; Stoter, M.; Hoijer, A.; Stefano, Bartesaghi. S.; Sabirsh, A.; et al. Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. *J. Cell Biol.* **2022**, *221(2),* Art. No: e202110137. DOI: 10.1083/jcb.202110137

69. Institute of Medicine (US) Board on Health Promotion and Disease Prevention. Immune Response to Vaccine Antigens, in: *Vaccine Safety Forum: Summaries of Two Workshops*. Washington (DC): National Academies Press (US) **1997**. Available from: https://www.ncbi.nlm.nih.gov/books/NBK233000/

70. Al Fayez, N.; Nassar, M.; Alshehri, A.; Alnefaie, M.; Almughem, F.; Alshehri, B.; et al. Recent Advancement in mRNA Vaccine Development And Applications. *Pharmaceutics* **2023**, *15(7),* Art. No: 1972. DOI: 10.3390/pharmaceutics15071972

71. Sijts, E.; Kloetzel, P. The role of the proteasome in the generation of MHC class I ligands and immune responses. *Cell. Mol. Life Sci.* **2011**, *68(9),* 1491–1502. DOI: 10.1007/s00018-011-0657-y

72. Cruz, F.; Chan, A.; Rock, K.; Pathways of MHC I cross-presentation of exogenous antigens. *Semin. Immunol.* **2023**, *66*, Art. No: 101729. DOI: 10.1016/j.smim.2023.101729

73. Vojdani, A.; Koksoy, S.; Vojdani, E.; Engelman, M.; Benzvi, M.; Lerner, A. Natural Killer Cells and Cytotoxic T Cells: Complementary Partners against Microorganisms and Cancer. *Microorganisms* **2024**, *12(1),* Art. No: 230. DOI: 10.3390/microorganisms12010230.

74. Bellavite, P.; Alessandra Ferraresi, A.; Isidoro, C. Immune Response and Molecular Mechanisms of Cardiovascular Adverse Effects of Spike Proteins from SARS-CoV-2 and mRNA Vaccines. *Biomedicines* **2023**, *11(2),* Art. No: 451. DOI: 10.3390/biomedicines11020451.

75. Gohda, E. Effectiveness of and Immune Responses to SARS-CoV-2 mRNA Vaccines and Their Mechanisms. *J. Disaster. Res.* **2022**, *17(1),* 7-20. DOI: 10.20965/jdr.2022.p0007

76. Lee, J.; Woodruff, M.; Kim, E.; Nam, J. Knife's edge: Balancing immunogenicity and reactogenicity in mRNA vaccines. *Exp. Mol. Med.* **2023**, *55(7)*, 1305-1313. DOI: 10.1038/s12276-023-00999-x

77. Lam, J.; Smith, F.; Baumgarth, N. B Cell Activation and Response Regulation During Viral Infections. *Viral Immunol.* **2020**, *33(4),* 294-306. DOI: 10.1089/vim.2019.0207

78. Dai, L.; Gao, G. Viral targets for vaccines against COVID-19. *Nat. Rev. Immunol.* **2021**, *21, 73-82*. DOI: 10.1038/s41577-020-00480-0

79. Mancuso, R.; Agostini, S.; Citterio, L.; Chiarini, D.; Santangelo, M.; Clerici, M. Systemic and Mucosal Humoral Immune Response Induced by Three Doses of the BNT162b2 SARS-CoV-2 mRNA Vaccines. *Vaccines* **2022**, *10(10),* Art. No: 1649. DOI: 10.3390/vaccines10101649

80. FDA Takes Key Action in Fight Against COVID-19 By Issuing Emergency Use Authorization for First COVID-19 Vaccine. Action Follows Thorough Evaluation of Available Safety, Effectiveness, and Manufacturing Quality Information by FDA Career Scientists, Input from Independent Experts. Administration FaD; **2020**. Available online: https://www.fda.gov/news-events/press-announcements/fda-takes-key-action-fight-against-covid-19-issuing-emergency-use-authorization-first-covid-19 (accessed on 17-10-2024)

81. Sahin, U.; Muik, A.; Vogler, I.; Derhovanessian, E.; Kranz, L.; Vormehr, M.; et al. BNT162b2 vaccine induces neutralizing antibodies and poly-specific T cells in humans. *Nature* **2021**, *595*, 572-577. DOI: 10.1038/s41586-021-03653-6.

82. FDA Approves First COVID-19 Vaccine. Approval Signifies Key Achievement for Public Health. Administration FaD; **2021**. Available online: https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine (accessed on17-10-2024)

83. FDA Authorizes Pfizer-BioNTech COVID-19 Vaccine for Emergency Use in Children 5 through 11 Years of Age. Administration FaD; **2021**. Available online: https://www.fda.gov/news-events/press-announcements/fda-authorizes-pfizer-biontech-covid-19-vaccine-emergency-use-children-5-through-11-years-age (accessed on 17-10-2024)

84. Fernando, k.; Menon, S.; Jansen, k.; Naik, P.; Nucci, G.; Roberts, J.; et al. Achieving end-to-end success in the clinic: Pfizer's learnings on R&D productivity. *Drug Discov. Today* **2022**, *27(3),* 697-704. DOI: 10.1016/j.drudis.2021.2.010

85. Rohde, C.; Lindemann, C.; Giovanelli, M.; Sellers, R.; Diekmann, J.; Choudhary, S.; et al. Toxicological Assessments of a Pandemic COVID-19 Vaccine—Demonstrating the Suitability of a Platform Approach for mRNA Vaccines. *Vaccines* **2023**, *11(2),* Art. No: 417. DOI: 10.3390/vaccines11020417

86. Vogel, A.; Kanevsky, I.; Che, Y.; Swanson, K.; Muik, A.; Vormehr, M. BNT162b vaccines protect rhesus macaques from SARS-CoV-2. *Nature* **2021**, *592*, 283-289. DOI: 10.1101/2020.09.08.280818.

87. Walsh, E.; Frenck, R.; Falsey, A.; Kitchin, N.; Absalon, J.; Gurtman, A.; et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. *N. Engl. J. Med.* **2020**, *383*, 2439-2450. DOI: 10.1056/NEJMoa2027906.

88. Mulligan, M.; Lyke, K.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* **2020**, *586*, 589-593. DOI: 10.1038/s41586-020-2639-4.

89. Polack, F.; Thomas, S.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N. Engl. J. Med.* **2020**, *383*, 2603-2615. DOI: 10.1056/NEJMoa2034577.

90. Kadalia, R.; Janagamac, R.; Perurud, S.; Malayalae, S. Side effects of BNT162b2 mRNA COVID-19 vaccine: A randomized, cross-sectional study with detailed self-reported symptoms from healthcare workers. *IJID*. **2021**, *106*, 376–381. DOI: 10.1016/j.ijid.2021.04.047

91. Thomas, S.; Moreira, E.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine through 6 Months. *N. Engl. J. Med.* **2021**, *385(10)*, 1761-1773. DOI: 10.1056/NEJMoa2110345

92. Gobbi, F.; Buonfrate, D.; Moro, L.; Rodari, P.; Piubelli, C.; Caldrer, S.; et al. Antibody Response to the BNT162b2 mRNA COVID-19 Vaccine In Subjects with Prior SARS-CoV-2 Infection. *Viruses* **2021**, *13(3)*, Art. No: 422. DOI: 10.3390/v13030422

93. Frenck, R.; Klein, N.; Kitchin, N.; Gurtman, A.; Absalon, J.; Lockhart, S.; et al. Safety, Immunogenicity, and Efficacy of the BNT162b2 Covid-19 Vaccine in Adolescents. *N. Engl. J. Med.* **2021**, *385*, 239-250. DOI: 10.1056/NEJMoa2107456

94. Walter, E.; Talaat, K.; Sabharwal, C.; Gurtman, A.; Lockhart, S.; Paulsen, G.; et al. Evaluation of the BNT162b2 Covid-19 Vaccine in Children 5 to 11 Years of Age. *N. Engl. J. Med.* **2022**, *386*, 35-46. DOI: 10.1056/NEJMoa2116298

95. Moreira, E.; Kitchin, N.; Xu, X.; Dychter, S.; Lockhart, S.; Gurtman, A.; et al. Safety and Efficacy of a Third Dose of BNT162b2 Covid-19 Vaccine. *N. Engl. J. Med.* **2022**, *386(20)*, 1910-1921. DOI: 10.1056/NEJMoa2200674

96. Thomas, S.; Perez, J.; Lockhart, S.; Hariharan, S.; Kitchin, N.; Bailey, R.; et al. Efficacy and safety of the BNT162b2 mRNA COVID-19 vaccine in participants with a history of cancer: subgroup analysis of a global phase 3 randomized clinical trial. *Vaccine* **2022**, *40*, 1483-1492. DOI: 10.1016/j.vaccine.2021.12.046

97. Seekircher, L.; Bánki, Z.; Kimpel, J.; Rössler, A.; Schäfer, H.; Falkensammer, B.; et al. Immune response after two doses of the BNT162b2 COVID-19 vaccine and risk of SARS-CoV-2 breakthrough infection in Tyrol, Austria: an open-label, observational phase 4 trial. *Lancet Microb.* **2023**, *4(8)*, e612-e621. DOI: 10.1016/s2666-5247(23)00107-6

98. Clairon, Q.; Prague, M.; Planas, D.; Bruel, T.; Hocqueloux, L.; Prazuck, T.; et al. Modeling the kinetics of the neutralizing antibody response against SARS-CoV-2 variants after several administrations of Bnt162b2. *PloS Comput. Biol.* **2023**, *19(8)*, Art. No: e1011282. DOI: 10.1371/journal.pcbi.1011282

99. Fitz-Patrick, D.; Young, M.; Yacisin, K.; McElwee, K.; Belanger, T.; Belanger, K.; et al. Randomized trial to evaluate the safety, tolerability, and immunogenicity of a booster (third dose) of BNT162b2 COVID-19 vaccine coadministered with 20-valent pneumococcal conjugate vaccine in adults ≥65 years old. *Vaccine* **2023**, *41*, 4190-4198. DOI: 10.1016/j.vaccine.2023.05.002.

100. Chantasrisawad, N.; Techasaensiri, C.; Kosalarksa, P.; Phongsamart, W.; Tangsathapronpong, A.; Ampornpan, J.; et al. The immunogenicity of an extended dosing interval of BNT162b2 against SARS-CoV-2 Omicron variant among healthy school-aged children, a randomized controlled trial. *Int. J. Infect. Dis.* **2023**, *130*, 52-59. DOI: 10.1016/j.ijid.2023.02.017

101. Simões, E.; Klein, P.; Sabharwa, C.; Gurtman, A.; Kitchin, N.; Ukkonen, B.; et al. Immunogenicity and Safety of a Third COVID-19 BNT162b2 mRNA Vaccine Dose in 5- to 11-Year Olds. *J. Pediatric. Infect. Dis. Soc.* **2023**, *12(4)*, 234-238. DOI: 10.1093/jpids/piad015

102. Radner, H.; Sieghart, D.; Jorda, A.; Fedrizzi, C.; Hasenöhrl, T.; Zdravkovic, A.; et al. Reduced immunogenicity of BNT162b2 booster vaccination in combination with a tetravalent influenza vaccination: results of a prospective cohort study in 838 health workers. *Clin. Microbiol. Infect.* **2022**, *29(5)*, 635-641. DOI: 10.1016/j.cmi.2022.12.008

103. Muñoz, F.; Sher, L.; Sabharwal, C.; Gurtman, A.; Xu, X.; Kitchin, N.; Lockhart, S.; et al. Evaluation of BNT162b2 Covid-19 Vaccine in Children Younger than 5 Years of Age. *N. Engl. J. Med.* **2023**, *388(7)*, 621-634. DOI: 10.1056/NEJMoa2211031

104. Munro, A.; Feng, S.; Janani, L.; Cornelius, V.; Aley, P.; Babbage, G.; et al. Safety, immunogenicity, and reactogenicity of BNT162b2 and mRNA-1273 COVID-19 vaccines given as fourth-dose boosters following two doses of ChAdOx1 nCoV-19 or BNT162b2 and a third dose of BNT162b2 (COV-BOOST): a multicentre, blinded, phase 2, randomised trial. *Lancet Infect. Dis.* **2022**, *22(8)*, 1131-1141. DOI: 10.1016/S1473-3099(22)00271-7

105. Bergman, P.; Blennow, O.; Hansson, L.; Mielke, S.; Nowak, P.; Chen, p.; et al. Safety and efficacy of the mRNA BNT162b2 vaccine against SARS-CoV-2 in five groups of immunocompromised patients and healthy controls in a prospective open-label clinical trial. *EBioMed.* **2021**, *74*, Art. No: 103705. DOI: 10.1016/j.ebiom.2021.103705

106. Usdan, L.; Patel, S.; Rodriguez, H.; Xu, X.; Lee, D.; Finn, D.; et al. A Bivalent Omicron-BA.4/BA.5-Adapted BNT162b2 Booster in ≥12-Year-Olds. *Clin. Infect. Dis.* **2024**, *78(5)*, 1194-1203. DOI: 10.1093/cid/ciad718