RESEARCH ARTICLE

BioNTech RNA-Based COVID-19 Injections Contain Large Amounts Of Residual DNA Including An SV40 Promoter/Enhancer Sequence

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Abstract

Background: BNT162b2 RNA-based COVID-19 injections are specified to transfect human cells to efficiently produce spike proteins for an immune response.

Methods: We analyzed four German BNT162b2 lots applying HEK293 cell culture, immunohistochemistry, ELISA, PCR, and mass spectrometry.

Results: We demonstrate successful transfection of nucleoside-modified mRNA (modRNA) biologicals into HEK293 cells and show robust levels of spike proteins over several days of cell culture. Secretion into cell supernatants occurred predominantly via extracellular vesicles enriched for exosome markers. We further analyzed RNA and DNA contents of these vials and identified large amounts of DNA after RNase A digestion in all lots with concentrations ranging from 32.7 ng to 43.4 ng per clinical dose. This far exceeds the maximal acceptable concentration of [illegible] per clinical dose that has been set by international regulatory authorities. C[illegible]lyses with selected PCR primer pairs proved that residual DNA represents not only fragments of the DNA matrices coding for the spike gene, but of all genes

from the plasmid including the SV40 promoter/enhancer and the antibiotic resistance gene.

Conclusion: Our results raise grave concerns regarding the safety of the BNT162b2 vaccine and call for an immediate halt of all RNA biologicals unless these concerns can be dispelled.

Keywords

BNT162b2, Cell Transfection, Comirnaty, COVID-19, Plasmid, RNA-Vaccine, SV40 Promoter/Enchancer

Introduction

In 2020, politically promoted campaigns like "Operation Warp Speed" [1,2] and "Project Lightspeed" [3,4] pushed the development of a completely new class of drugs finally aiming at vaccinating seven billion people worldwide against COVID-19 [5]. These so-called "mRNA-vaccines" – hereinafter referred to as RNA injections or RNA biologicals – consist of nucleoside-modified mRNA (modRNA) packaged in transfection-competent lipid nanoparticles (LNP). According to the underlying idea, modRNA, once in the cell, forces this cell to produce SARS-CoV-2 spike proteins and present it on the cell surface, subsequently resulting in the stimulation of the immune system to generate specific antibodies against the presented spike antigen [6,7]. The "speed of science" [8] and the demand of the governments worldwide faced the manufacturers with the challenge to produce large amounts of modRNA within a very short time. Thus, the initial PCR-based process for the generation of the DNA matrices (process-1) for modRNA production, which received authorization for use in the phase-3 clinical trial, very soon reached its limits and the companies switched to a large-scale production of DNA matrices via cloned shuttle vectors, which can be easily multiplied in bacterial cell culture systems (process-2) [9]. Starting with the governmental vaccine roll-out, this process-2 product was employed instead of the original product.

Already in 2021, it has been reported that the modRNA-induced spike proteins could be found circulating in the blood of vaccinees weeks after the injections [10]. In 2022, the first detailed post-mortem investigation revealed the presence of

vaccine-induced spike proteins at multiple locations in vessel walls and different tissues weeks after the last BNT162b2 injection [11]. Recently, vaccine-induced spike proteins were identified in placentas of women injected with RNA biologicals during pregnancy [12]. Dhuli and colleagues reported the presence of a sequence corresponding to a fragment of the modRNA in blood cells of long-COVID patients with a history of two doses of the BioNTech/Pfizer vaccine [13]. Importantly, the production of spike proteins by the body cells is not restricted to the injection area and did not terminate within a few days as had been proclaimed by the manufacturers and the responsible authorities. Several mechanisms have been suggested so far that could contribute to the remarkable long-lasting expression of spike proteins in vaccinated individuals.

First, biologicals contain nucleoside-modified mRNA (modRNA) to extend its lifetime [14], to reduce its destruction by turning off toll-like receptor detection [15], and to maximize its translation. This was achieved by replacing natural uridines with synthetic N1-methyl-pseudouridines (mPsi) and by increasing the content in guanine and cytosine (known as codon optimization) [6,14,16].

Second, transfected modRNA may be reverse transcribed into DNA and integrated into the cell´s genome via a LINE1 (Long Interspersed Nuclear Element-1) mediated mechanism, as data from transfection experiments in human cell lines HEK293T [17] and Huh7 [18] suggested.

Third, lipid nanoparticles (LNP) delivering modRNA to the cells may also contain DNA, which originated from the production process, where spike-coding DNA was used as a template for the in-vitro transcription of modRNA. Remaining DNA may not completely be separated from modRNA and degraded by deoxyribonuclease-I (DNase-I) digestion and, subsequently, be packaged in the LNP together with the modRNA. It is well known that DNase-I can adhere to the surfaces of reaction vessels and can exhibit reduced efficiency in the presence of hybrids of DNA and RNA [19]. According to a manufacturer, it is “probably impossible to remove every single strand of DNA in an RNA preparation” [20]. Given the fact that the European Medicines Agency and the German Paul Ehrlich Institute fixed a residual DNA of 10 ng per injected clinical dose as acceptable (and indeed DNA up to this margin was shown in the registration documents [9]), it m[illegible]ackaging of this DNA into the lipid nanoparticles highly likely.

This possibility emerged on the scene in February 2023, when McKernan and

colleagues announced the discovery of large amounts of both spike-coding DNA and residual plasmid-DNA derived from the expression vector system in BioNTech/Pfizer and Moderna vaccine lots [21,22]. The bulk was represented by fragmented, linearized DNA, but also intact plasmids being able to successfully transfect E. coli cells [21,22]. Assuming that these intact plasmids were packaged in the LNP together with the modRNA, stable expression vectors could enter the cells and thus provide a rich source of long-lasting spike production in the case that the cells are able to transcribe the coded spike region. Incomprehensibly, plasmids from BioNTech/Pfizer, but not from Moderna, do not only contain the bacterial T7 promoter system, but also the mammalian Simian Virus 40 (SV40) promoter/enhancer sequence [23-25]. This gives cause for concern, as already in 1999, Dean and colleagues demonstrated that nuclear entry of plasmid-DNA, especially in non-dividing cells, requires a 72 bp sequence of the SV40 promoter/enhancer [23]. Of note, neither the promoter, nor the origin of replication are needed for nuclear localization of plasmid-DNA [23]. Meanwhile, the results of the McKernan team have been confirmed and extended [26]. Recently, König and Kirchner published data on large amounts of residual DNA within several BNT162b2 lots [27].

Against this background, we performed a series of experiments to answer the following urgent questions. First, can the large amount of residual DNA in BioNTech lots [27] and even plasmids identified in Pfizer lots [21,22] be confirmed on BioNTech only lots (BNT162b2, Comirnaty) distributed in Germany by different comparable DNA detection methods? Second, can residual plasmids or DNA fragments, if present, be efficiently transfected into human cells together with the coding modRNA? Third, can these biologicals induce continued cellular expression of spike protein thus creating long-term foci for immune attack? To answer these questions, we applied an in-vitro cell culture model using HEK293 cells, as these cells simulate dividing human cells and, therefore, are not only a suitable target for protein production but are also most susceptible for a potential interaction of the transfected foreign nucleic acids and the cell´s genome. The fact that we obtained positive results on all issues raises the strongest concerns on the safety of the BNT162b2 vaccine.

Materials and Methods

Vaccine Lots

The following original and unopened BNT162b2 vaccine lots were used: FD7958 (monovalent Wuhan; expiry date October 2021), FE6975 (monovalent Wuhan; expiry date October 2021), EX8679 (monovalent Wuhan; expiry date August 2021), HD9869 (bivalent Wuhan/Omicron XBB1.5; expiry date October 2024). As positive control for the PCR reaction and mass spectrometry analysis, lot GH9715 (bivalent Wuhan/Omicron BA.4 and BA.5; expiry date June 2023) was used, because its contamination with the SV40 containing plasmid was proven already [25]. The vials were provided to us by the pharmacy in the manufacturer's refrigerated state. They were unopened and always refrigerated during transport and storage.

Cell Line Experiments and ELISA

HEK293 cells were grown in a humidified incubator at 37 °C, 5% CO_2. The cells used were from original stocks (Cell Lines Service GmbH, Eppenheim, Germany) regularly tested negative for mycoplasma and stored in aliquots in liquid nitrogen. Cells were thawed fresh prior to transfection experiments, cultured in 10 cm well dishes with DMEM with 10% fetal calf serum supplemented with 1% penicillin/streptomycin until confluency and transferred after trypsinization (0.05% trypsin/EDTA (Gibco #11500636; 3 min 37 °C) to new wells, according to the experimental set up described below.

For all transfection experiments, the vials with the monovalent mRNA were pre-diluted 1:5 with sterile RNase-free phosphate-buffered saline (PBS) to the clinical concentration, according to the manufacturer. The bivalent vials were not pre-diluted since the clinical concentration was already present. For ELISA, 12-well plates with 80% cell density and a medium volume of 1 ml each were transfected with 1/12 (25 µl) of a clinical dose of a vial and cells and media were harvested at time points day 1, day 3, day 5, and day 7. Untransfected cells at day 7 served as negative control. For protein analysis, cells were washed twice in sterile phosphate-buffered saline (PBS) after the respective incubation time (see above), lysed in lysis buffer (25 mM Tris, 150 mM NaCl, 1% Triton-X-100, 1% NP40, pH 7.6) and the spike protein level was determined in the protein supernatant and the media using a commercially available high-sensitive S-Plex SARS-CoV-2 Spike ELISA assay (Mesoscale Discovery K150ADJS).

Immunohistochemistry

Immunohistochemistry was performed using primary antibodies against SV40 large T antigen (Abcam, ab16879) and secondary antibodies conjugated to horseradish peroxidase (HRP). Detection was carried out using a DAB substrate kit (Vector Laboratories, SK-4100), and slides were counterstained with hematoxylin. Negative controls included omission of primary antibodies and staining of tissues known to be SV40-negative.

DNA and RNA Quantification and Sequencing

Genomic DNA was extracted from tissue samples using the DNeasy Blood & Tissue Kit (Qiagen, 69504). Quantification was performed using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Q32854). Sequencing libraries were prepared using the Illumina DNA Prep Kit (Illumina, 20018705) and sequenced on the Illumina NovaSeq 6000 platform. Bioinformatic analysis was conducted using custom pipelines in Python and R, with alignment to the human reference genome (GRCh38) and the SV40 genome (NC_001669.1).

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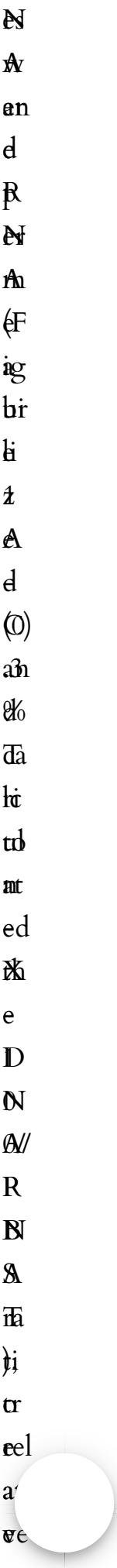

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Results

Successful Transfection Of Cells Results In The Production of Spike Protein

The modRNA-containing lipid nanoparticles represent a powerful tool for the transfection of mammalian cells [33]. To test for transfection effectiveness, we analyzed the expression pattern of spike proteins after transfecting human embryonic kidney cells (HEK293) with four different BioNTech (Comirnaty) vaccine lots, namely the monovalent lots FD7958, FE6975, EX8679, and the bivalent lot HD9869. All four lots successfully transfected the HEK293 cells, as demonstrated by a strong spike protein immunohistochemical signal (Figure 1A). The transfection efficiency as rated by spike-positive cells was 90.5%, 74.6%, 76.4%, 80.7%, and 0% for lots FD7958, FE6975, EX8679, HD9869 and untreated cells, respectively. In addition, transfected cells showed clear signs of a cytopathic effect compared to the non-transfected (control) cell line, as evidenced by the formation of large vacuoles and detached cells (Figure 1B). To quantify the spike protein expression stability over time, we measured the amount of spike protein in cell lysates after 1, 3, 5, and 7 days using a commercially available ELISA. All four lots followed the same expression pattern with already clear expression of spike protein after day 1, increasing production until day 5, and still higher spike c[illegible]tion on day 7 than on day 1 (Figure 1C).

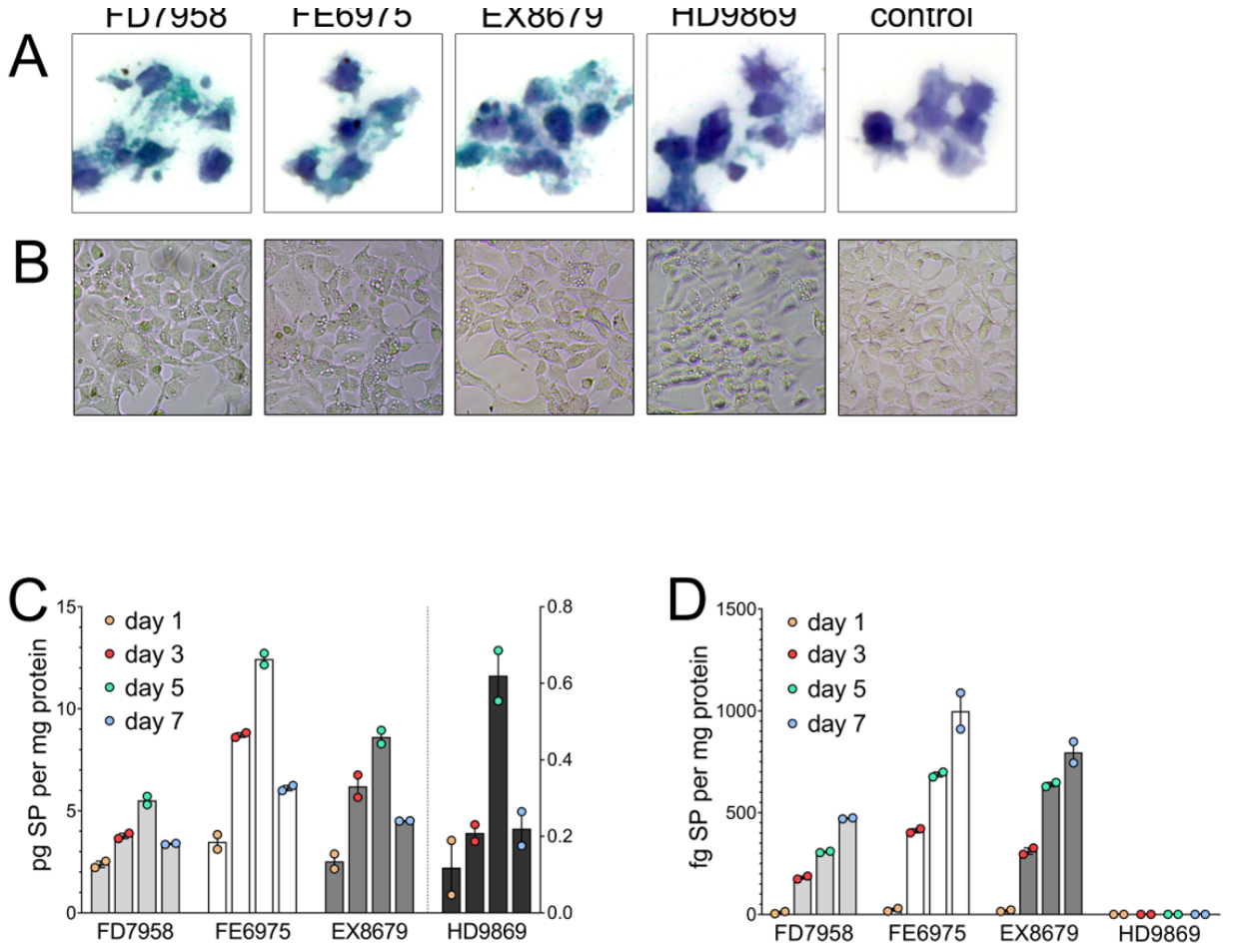

Figure 1.

Spike protein expression in HEK293 cells after transfection with BNT162b2 biologicals. A: Staining of spike proteins in cells transfected with different lots visualized in green color by immunohistochemistry. Non-transfected cells (control) show no staining. Hematoxylin serves as nuclei counterstain. B: Brightfield microscopy of transfected HEK293 cells with different lots show an accumulation of intracellular vesicle formation. C: Quantification of intracellular spike protein (SP) concentrations over time measured by ELISA. n=2; mean+SEM. D: Quantification of secreted spike protein (SP) levels over time measured by ELISA. For lot HD9869, secreted spike protein concentration was below the detection limit. n=2; mean+SEM. The negative control showed no spike protein content in the cell lysate and cell supernatant. SEM, standard error of the mean.

Spike Protein Is Released Into The Supernatant Of Transfected Cell

To determine whether spike proteins are presented on the cells only or can either shed off or be secreted from the transfected cells, we analyzed the cell-free culture supernatant for spike proteins applying ELISA. The measured values are c[illegible]ve quantities from day 0 to the respective indicated time point. We o[illegible] a clear increase in the amount of spike protein in the media over time in

Spike Protein is Mainly Released Via Extracellular Vesicles

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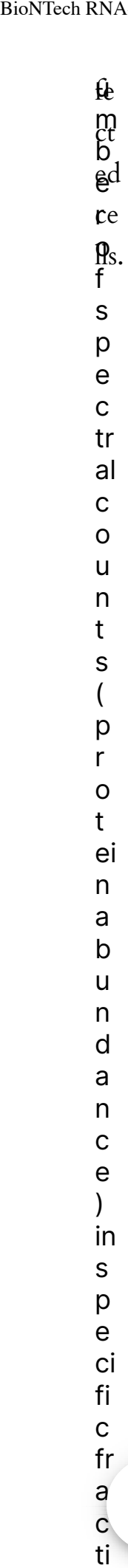
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Figure 2: Residual DNA from

dsDNA Contaminant Integrity Differences Between BioNTech and Moderna Products

Kevin McKernan, Ph.D.

Abstract

Introduction: Recent studies have identified significant levels of double-stranded DNA (dsDNA) contamination in mRNA-based COVID-19 vaccines, raising concerns about potential genotoxicity and long-term health effects. This study aimed to quantify and characterize the dsDNA contamination in BioNTech and Moderna vaccine products.

Methods: We employed a novel gel electrophoresis technique to analyze the integrity of dsDNA contaminants in BioNTech and Moderna vaccine samples. The method allowed for the visualization of DNA fragments ranging from 100 to 10,000 base pairs.

Results: Our findings revealed substantial differences in dsDNA integrity between the two vaccine products. BioNTech samples exhibited a predominant band at approximately 2,000 base pairs, while Moderna samples showed a smear pattern indicating more fragmented DNA. Quantification using a standard curve method estimated the BioNTech dsDNA concentration at 1.1 ng/µL, with a total yield of 110 ng in the 100 µL sample.

Discussion: The presence of intact dsDNA fragments in BioNTech vaccines, as opposed to the fragmented DNA in Moderna vaccines, may have significant implications for potential integration into host genomes and subsequent biological effects. These findings underscore the importance of rigorous quality control and characterization of mRNA vaccine products.

Conclusion: This study provides evidence of substantial dsDNA contamination in BioNTech COVID-19 vaccines and highlights the need for further investigation into the potential risks associated with these findings. Our results call for increased transparency and standardization in vaccine manufacturing processes.

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Figure 1. Agarose gel electrophoresis of BioNTech RNA-based COVID-19 injections (Comirnaty) and Moderna RNA-based COVID-19 injections (Spikevax).

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Table 3. DNA levels in the individual vials as compared with the theoretical value (which is zero)

from BioNTech's own Technical Information sheet for the Pfizer-BioNTech COVID-19 mRNA vaccine, which is also known as BNT162b2. As the sheet shows, the vaccine contains a number of ingredients, including mRNA, lipids, salts, sugar, and buffers. The mRNA is encoded with the genetic information for the SARS-CoV-2 spike protein, which is the protein that the virus uses to enter human cells. The lipids are used to encapsulate the mRNA and help it to enter cells. The salts, sugar, and buffers are used to maintain the pH and osmolarity of the vaccine.

Triton-X-100 treatment. Figure 1. Fragment size distribution of DNA contaminants in the BioNTech RNA-based COVID-19 injections before and after Triton-X-100 treatment.

Upon release of nucleic acids from the Triton-treated lysis buffer, the nucleic acids bind to the magnetic beads. The beads are then separated from the solution using a magnetic field, and the supernatant is discarded. The beads are washed with ethanol to remove any remaining impurities, and the nucleic acids are eluted from the beads using a low-salt buffer. The purified nucleic acids are then ready for downstream applications such as PCR or sequencing.

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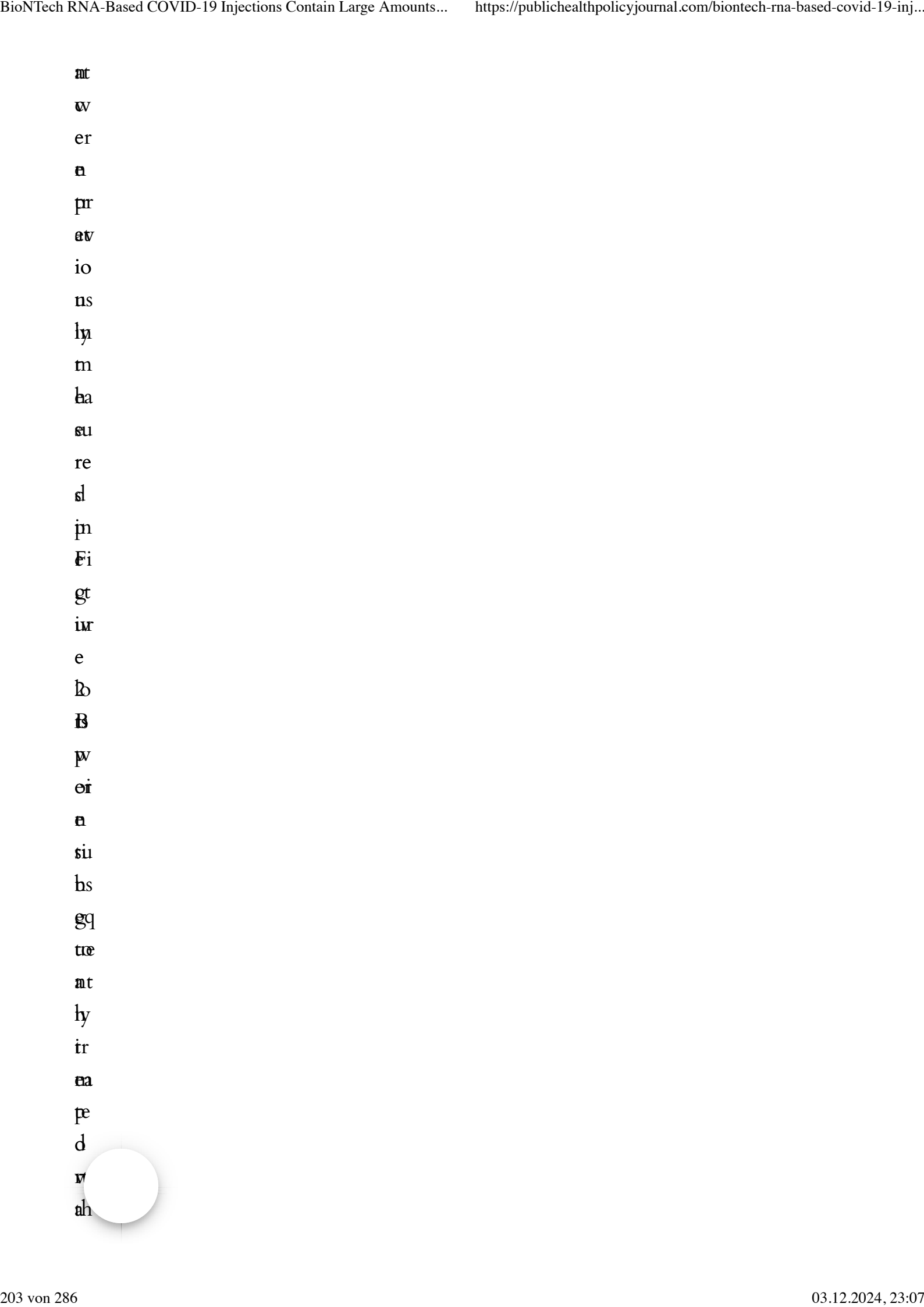

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Figure 1. Dual DNA Contamination Steps in mRNA Production Process Requiring Plasmid Elements And Lipid Nanoparticle Encapsulation

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Interestingly, all four vials tested at a dilution of 1:10 showed strong signals

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Figure 3. A & B.

Polymerase Chain Reaction (PCR) amplification of residual DNA fragments

Plasmid DNA is the template used to produce mRNA. The mRNA is then purified to remove undesirable components, leaving behind the desired mRNA strand. Lipid nanoparticles are then added to encapsulate the mRNA, forming the final product. Each step in the process is crucial to ensure the quality and safety of the vaccine.

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Discussion

W zed the nucleic acid content of four BNT162b2 (Comirnaty) vials d ed in Germany and found that the RNA concentration fits with the manufacturer´s specification of 30 µg per clinical dose demonstrating the accuracy

of our analysis. In addition, we identified residual DNA including plasmid-DNA in amounts similar to that reported by McKernan et al. [21,22] for US-Pfizer lots. DNA concentrations in the vials analyzed in this study ranged from 32.7 to 43.4 ng per clinical dose after eliminating RNA via RNase digestion. This far exceeds the maximal upper limit of 10 ng per clinical dose, which the WHO has declared to be tolerable in injectable biologicals [35].

In order to allow the analysis of the total DNA content present in the vials, we performed Triton-X-100 treatment to open the LNP. Comparison of the untreated vaccine solutions with the Triton-X-100 treated paired aliquots exhibited a 1.6-fold to 6.7-fold increase of the DNA content, which probably reflects a high variability in the LNP packing of the DNA leftovers during the manufacturing process. Our results are in accordance with data from Raoult [36], who recently reported a clear increase of the DNA content after LNP opening in one Comirnaty batch. The amount of residual DNA, released from the LNP by treatment with Triton-X-100, analyzed in this study is less than that reported recently [27]. We suspect that this may be due to cross-interaction of RNA with intercalating dyes [37]. Indeed, when samples were treated with RNase, DNA concentrations were markedly reduced, albeit to levels that still remained manyfold above the limits that have been deemed acceptable for naked DNA. In a recent preprint [38], product-related DNA impurities in BNT162b2 have been reported to coincide with the approved mRNA specifications. However, authors performed an additional ethanol precipitation step prior to Qubit analysis, which is known to inefficiently precipitate short DNA sequences representing the majority of reminiscent DNA after DNase digestion during the manufacturing process. This approach resulted in a high intra-batch variability of the calculated RNA amount in two Comirnaty batches. While our results (obtained without ethanol precipitation) showed an only low scattering between batches and an RNA content nearly identical and next to the 30 µg content specified per dose, the calculated RNA content by Kaiser et al. [38] was much lower with a maximum of 20 µg per dose. This suggests that they probably lost a considerable amount of nucleic acids during sample preparation. As is the case for the low resulting RNA concentration, also their DNA analysis resulted in far too low concentrations and do not represent the real residual DNA content when compared with the initial DNA concentration in the vials (free and p[illegible] LNP) before the precipitation, washing, and resolution step losses.

Of note, official limit values for residual DNA in biologicals are defined for

antibodies, attenuated vaccines, and protein solutions, but not for RNA injections and – even more important – for nucleic acids packaged in transfection reagents like lipid nanoparticles, which were used for the first time in the COVID-19 injections [39]. In fact, no scientific evidence exists that would permit a safety level of residual DNA to be defined in such injectables whatsoever.

Yet more concerning is the identification of gene sequences that disclose residuals of the BNT162b2 lots with plasmid-DNA that was employed as the expression system in the manufacturing process [9]. Originally, the presence of intact plasmids was demonstrated by McKernan and colleagues [21,22] in transformation experiments, whereby plasmid-encoded kanamycin resistance was conferred on to recipient E. coli. However, the complementary question of whether transfection of human cells might also occur has not yet been addressed. After transfection of cell cultures without any additional transfection reagents, plasmid-derived sequences like the kanamycin gene and SV40 promoter/enhancer could subsequently be re-isolated from transfected cells, suggesting that packaging into lipid nanoparticles and transfection into the cells had occurred, since free, unpackaged DNA would not be taken up by the cells.Cellular uptake is thus not followed by rapid degradation and disposal of plasmid-DNA. While further investigations need to clarify whether the residual plasmid-DNA also can serve as a template for the generation of functional proteins in the cell, our results leave no room for doubt that residual plasmid-DNA and fragments derived thereof that are contained in the RNA biologicals will enter myriads of cells in the human recipients. We could not demonstrate, whether some of the transfected cells contained the intact plasmids, however, the presence of the identified SV40 promoter/enhancer region is highly worrying regardless of the presence of the plasmid [23,40,41].

Our molecular analyses of the plasmid components confirmed data reported by McKernan and colleagues [21,22], namely the presence of a DNA sequence of the SV40 promoter/enhancer. This sequence was not declared in the plasmid map that BioNTech/Pfizer submitted in the approval procedure [42, page 24]. This finding is very surprising and raises the legitimate question: Why did BioNTech/Pfizer apply this totally unnecessary but highly dangerous element in their plasmids and use it as a template for the production of modRNA? In our opinion, BioNTech/Pfizer must be held accountable for incorporating this highly dangerous element in the plasmids.

By design, the plasmids used by BioNTech and Pfizer are so-called shuttle vectors. They contain cloning sites and polyadenylation signals, as well as elements that are necessary for replication and translation in bacterial systems, like the T7 phage promoter, and in addition a promoter/enhancer element in order to permit transcription initiation in eukaryotic cells, which usually derives from viruses like CMV or SV40. One of the first shuttle vectors which served as a kind of prototype for the current BioNTech/Pfizer plasmid was established as early as 1988 [43]. A similar SV40 component containing mammalian expression vectors [Addgene: pcDNA3.1 SARS-CoV-2 S D614, however, without the bacterial components], were used already in 2020 for the analysis of spike protein functions in human cell lines [44].

According to the official generation process of the RNA coding for the spike protein, no eukaryotic promoter/enhancer is necessary, since the whole process-2 is performed in-vitro in the bacterial E. coli system. In addition, amongst available strong viral promoter/enhancer elements, like CMV, Bakulovirus, and RSV, the SV40 element is the most dangerous for the integrity of the target cells since long before the development of RNA biologicals, it was shown that the 72 bp SV40 promoter/enhancer fragment facilitates maximal transport of plasmid-DNA into the nucleus of transfected cells [23,41], a feature not found in CMV and RSV elements. Explicitly, one aspect should raise the alarm bell which is expressed by Dean et al. [23]: “The inclusion of this SV40 sequence in non-viral vectors may greatly increase their ability to be transported into the nucleus especially in non-dividing cells.” This transport of plasmids containing the SV40 promoter/enhancer element into the nucleus was found for a broad variety of cell types tested [45] leading to the promotion of the SV40 promoter/enhancer element for highly effective gene therapy approaches. The detection of the SV40 promotor/enhancer sequences begs the question what intention the manufacturers had when selecting an expression system containing this mammalian cell active element instead of selecting a pure prokaryotic expression system for the manufacturing process.

Finally, we demonstrate that transfected cells are able to produce and secrete spike proteins. We did not use any other transfection-enhancing substances in any of the transfections. This enabled us to analyze the direct gene transfer of the pure substance from the lipid nanoparticles as a “transfection reagent” into the host cells simulating the situation in the vaccinated individuals. It is known that an additional administration of transfection enhancers, such as apolipoprotein E3 (ApoE3),

which binds to the cholesterol of the lipid envelope of the LNP and thus facilitates the uptake of the LNP into the cells via ApoE3 receptors, would have led to a higher transfection efficiency. The transfection efficiency can also be increased by changing the target cell line or increasing the administration of LNP. Our measured values of intracellular and extracellular spike concentrations therefore only refer to the HEK293 cells under the specified parameters, which are described in detail in the methods section. After transfection, our cells exhibited large intracellular vesicles representing a clear feature of cell health impairment after uptake of the modRNA containing LNP. The envelope of the latter consists of four lipid components. In particular, the group of cationic (ionizable) lipids is well-known for its toxic and pro-inflammatory effect on cells both in-vitro and in-vivo [46]. It is currently unknown how many and which cell types in the body are affected, but it has been reported that the LNP spread throughout the body and the modRNA is found in all organs examined [9]. We discerned that the production of spike proteins lasts for many days. After one week, even more spike proteins are present in the cells than after 24 hours, although the highest modRNA level is expected after 24 hours, before it is degraded. The amount of spike proteins produced varied between lots, but the progression over time was identical for all, with a peak on day 5 after transfection. We also showed that the level of production is related to the amount of injected modRNA, as lot HD9869 reveals a significantly lower spike protein level than the other three lots. This was due to the fact that HD9869 is a bivalent vaccine consisting of two different modRNAs for two different spike protein variants, namely the Wuhan variant and the Omicron variant. As the ELISA applied in this study detects only the Wuhan variant, the spike proteins produced by the Omicron variant are not detectable and we obtain lower values than for the three monovalent vaccines with only the Wuhan variant.

Using mass spectrometry, we were able to demonstrate that the spike proteins are almost exclusively released into the medium via exosomes. In case of an in-vivo situation, this would mean that the spike proteins are transported within exosomes to other tissues and organs via the blood stream and, consequently, taken up by the target cells. In fact, it has already been reported that spike proteins can be found in exosomes of vaccinated individuals [10]. The functions of exosomes are manifold. They mainly serve as a communication platform between cells of the same tissue and between cells of different organs. The exosomes are easily taken up by the target cells through various mechanisms and the content thus enters the target cell and induces a structural and functional response [47]. In the case of BNT162b2, it

can be assumed that spike proteins can be transferred and taken up from one tissue to the next in this way regardless of the presence of LNP or modRNA. Whether the target cells are damaged by the uptake of spike protein-containing exosomes has not yet been investigated. However, this potent transfection ability raises alarm clocks in light of the background of the co-transfected residual DNA and especially the SV40 promoter/enhancer elements of the primary material, the plasmid. In preliminary experiments, another lot of BNT162b2 was able to transfect ovarian cancer cells and here, parts of the transfected nucleic acid material were indeed found by whole genome sequencing to be integrated into chromosomes 9 and 12 of the cells [48].

The sequence of the modRNA shows that the leader sequence for the translation of the spike protein into the lumen of the endoplasmic reticulum and the sequence for the membrane anchor were not removed from the modRNA. As a consequence, the produced spike proteins are predominantly expressed on the cell surface. According to BioNTech, the modRNA is translated in the cytosol [49]. This would mean that the spike proteins remain inside the cells and are not presented as full-length proteins on the cell surface. However, this contradicts the function of a leader and an anchor sequence within RNA in other membrane proteins. The possibility of shedding of the spike proteins was also not considered by BioNTech [49].

Limitation Of The Study and Variability Of The Data

We used technical and biological replicates (where appropriate) to reproduce our results and determine variability. As the variability for each experiment was small within a group, we can rule out technical artifacts. We only had access to a few lots, but we considered them sufficient to demonstrate the fundamental problem of residual DNA and cell transfection by "RNA vaccines." When experiments were carried out (2023), the expiration date of the three monovalent vaccine lots specified by the manufacturer had already passed. Due to the fact that the vials were consistently stored unopened at -80 °C and that the expiration date was extended officially several times by the German Paul Ehrlich Institute (PEI) [50: 10. Sept 2021 from 6 to 9 months; 51: 24. March / 4. April 2022 from 9 to 12 months; 52: 2. Dec 2022 from 12 to 18 months], we do not expect any negative effects on our re[illegible]. We cannot rule out that the vaccines also contain and transfect stable de[illegible]tranded RNA (dsRNA) and RNA:DNA hybrids in parallel to the single-

stranded modRNA and the vector-based DNA. We are also unable to fully map the toxicity of LNP on different cell types. We used a relatively robust human embryonic kidney cell line for our experiments that is immortalized and can therefore withstand toxic substances up to a certain level. Further studies are necessary to test, for example primary cell cultures such as a primary neuronal cells or immune cells, which would react much more sensitively to LNP. Since it is known that LNP are distributed throughout the body and thus probably affect all cell types, further in vitro studies on toxicity, expression behavior, and proteome analysis must be carried out.

Conclusion

We demonstrated that transfection of the human cell line HEK293 with four different BNT162b2 lots results in the production of spike proteins over several days, which are released into the cell supernatant via exosomes. We detected residual plasmid-DNA in all vials at concentrations far exceeding the allowed EMA limit of 0.33 ng dsDNA per 1 mg RNA. We identified all plasmid genes as well as the two copies of the SV40 promoter/enhancer element. The DNA was shown to enter and persist in the cells.

Already before the start of the governmental vaccination campaign, physicians and scientists pointed out that serious adverse events would be triggered by the gene-based agents. In the meantime, the spectrum of adverse side events has become so multifaceted that the term “spikeopathy” has been created to denote the new disease complex [53]. The eternal dangers of all RNA biologicals are 4-fold: First, modRNA encoding any foreign protein will trigger detrimental autoimmune reactions [54]. Second, the lipid nanoparticles are themselves highly toxic [55]. Third, residual plasmid-DNA and reverse transcribed mRNA will genetically modify cells. Fourth, replacement of uridine in natural mRNA by N1-methyl-pseudouridine in synthetic modRNA causes +1 ribosomal frameshifting resulting in haphazard production of utterly alien proteins [56].

Our results confirm and extend published reports and raise grave concerns regarding the safety of the BNT162b2 vaccine. We call for an immediate halt of all [illegible]ed biologicals until these concerns are scientifically addressed and co[illegible]ingly dispelled.

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Author Contributions

Conceptualization, U.K., V.S. and K.S.; methodology, U.K and V.S.; software, V.S.; validation, U.K. and V.S.; formal analysis, U.K., V.S. and K.S.; investigation, U.K. and V.S.; resources, U.K. and V.S.; data curation, U.K. and V.S.; writing—original draft preparation, U.K., V.S. and K.S.; writing—review and editing, U.K., V.S. and K.S.; visualization, U.K. and V.S.; supervision, U.K., V.S. and K.S.; project administration, V.S. and K.S.; All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

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Sections

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