Expertise on the genotoxic risks of the Pfizer COVID-19 vaccine

Michael Palmer, MD, Sucharit Bhakdi, MD, and Wolfgang Wodarg, MD

June 27, 2022

Abstract

In early 2021, the European Commission authorized the use of the Pfizer COVID-19 vaccine, based on an assessment report by the EMA. This report dismissed the vaccine's inherent risk of genotoxicity. In the meantime, new evidence has come to light which unambiguously proves the genotoxic risk to be real and urgent. The vaccine's ongoing use must therefore be halted immediately.

1 EMA dismissed the genotoxicity risks of the Pfizer COVID-19 vaccine based on outdated science

In the EMA assessment report on the Pfizer COVID-19 vaccine, we find the following succinct statement [1, p. 50]:

> *No genotoxicity studies have been provided. This is acceptable as the components of the vaccine formulation are lipids and RNA that are not expected to have genotoxic potential.*

Apparently, EMA's experts were assuming that RNA in general will not affect the integrity of the host cell genome. The first exception to this rule has been known since 1970, when oncogenic retroviruses were found to carry a reverse transcriptase activity that could copy the viral RNA genome into DNA, which could then insert into the host genome [2, 3]. The realization that eukaryotic cells themselves have similar reverse transcriptase activities came one and a half decades later [4], but it could hardly be considered a novelty in 2020.

1.1 Genomic insertion of RNA viruses through cellular reverse transcriptase activities. The first studies to demonstrate the existence of mammalian (mouse) DNA sequences that were derived from an RNA virus which was *not* a retrovirus were reported by Klenerman et al. [5] in 1997. The virus in question was Lymphocytic Choriomeningitis Virus. Since this virus does not itself encode a reverse transcriptase enzyme, it followed that the observed partial DNA copies of the viral RNA genome had to have been created through reverse transcription by cellular enzymes. The molecular mechanism was later elucidated in detail by scientists from the same laboratory [6]. It turned out that a *retrotransposon* had accomplished both the reverse transcription of the viral RNA and the insertion of the DNA copy into the cellular genome.

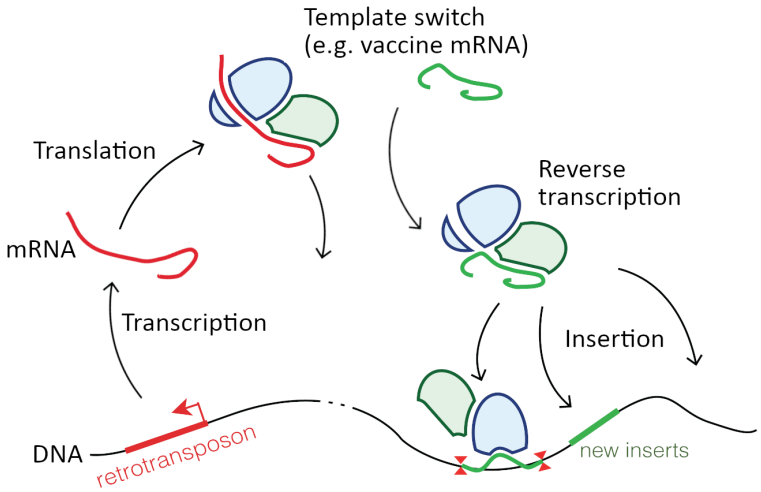


Figure 1 Reverse transcription and genomic insertion of an mRNA molecule by a retrotransposon (graphic adapted from Wikipedia). A retrotransposon, which is part of the cellular DNA, is initially transcribed and translated by the cellular machinery. It encodes two proteins that usually mediate the reverse transcription of the transposon's own mRNA into DNA, and the subsequent insertion of this DNA copy into the cellular DNA. However, occasionally the mRNA template may be replaced by another RNA molecule, e.g. an mRNA vaccine, which will then end up as a DNA copy in the genome.

1.2 The biological role of cellular retrotransposons. Retrotransposons are mobile genetic elements in the cellular genome that encode the protein apparatus for generating additional copies of themselves. Most of the time, it is the mRNA of the retrotransposon itself that ends up being copied back into DNA and inserted. However, the retrotransposon proteins may occasionally 'lose' their own mRNA template and pick up another RNA molecule instead, which will then undergo reverse transcription into DNA and insertion into the cellular genome (Figure 1).

There are several homologous families of retrotransposons, of which in humans the most active and important one is the LINE-1 family [7–9]. Since the location of new insertions within the genome is largely random [10], the biological outcomes are quite varied. If the insertion occurs within a functional gene, that gene may be disrupted; if insertion occurs in the vicinity of a functional gene, then the activity of the latter may be regulated upward or downward. Depending on the specific role of the affected gene, the behaviour of the cell may be changed, and cancer or other diseases may result [11, 12].

While retrotransposon activity differs between the types and functional states of our body cells, it is noteworthy that retrotransposons are active in both dividing and non-dividing cells [13] and also in oocytes [14]. We must therefore expect that viral or other foreign RNAs may be inserted by retrotransposons not only into somatic cells, and thereby potentially cause cancer, but also into germline cells, and therefore propagate within the human population.

1.3 Genomic DNA sequences derived from non-retroviral RNA viruses. A multitude of RNA viruses other than retroviruses have given rise to partial copies inserted into the genomes of mammals and other vertebrates [15–18]. Similar findings have been made in other eukaryotic organisms such as fungi, plants and protozoa [19–21]. All of these virus-derived sequences must have arisen through some kind of retrotransposition mechanism, which clearly substantiates the above point that retrotransposition can occur in the germline cells of all these species.

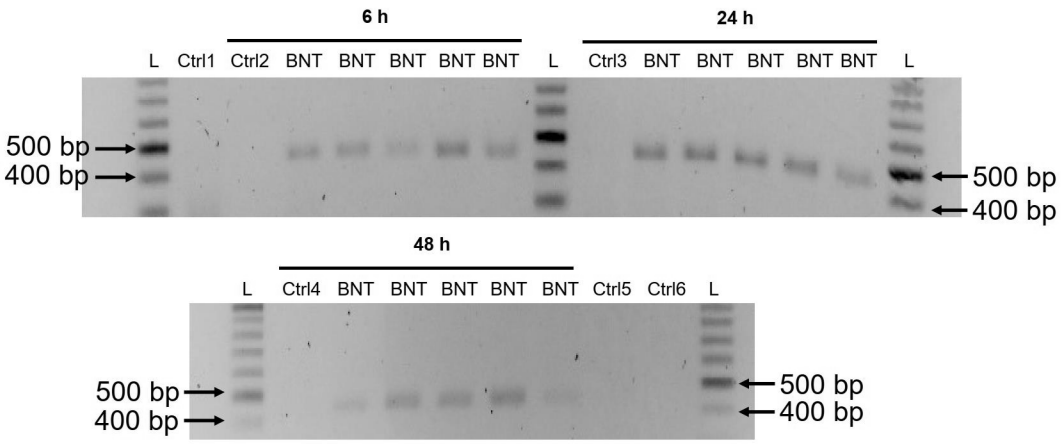


Figure 2 Detection of copies of the Pfizer COVID-19 vaccine mRNA within the cellular DNA of a human liver cell line (taken from Figure 5 in [23]). The cells were exposed to the vaccine for the lengths of time indicated. Cellular DNA was then isolated, and inserted DNA copies of the vaccine mRNA detected by PCR amplification of a fragment 444 base pairs (bp) in length. All samples labelled with 'BNT' had been treated with the vaccine, and they all show a PCR product of the expected length, as is evident from comparison to a DNA fragment length standard ('L'). Samples labelled with 'Ctrl *n*' were controls: Ctrl 1–4 contained DNA from cells not incubated with vaccine, Ctrl 5 contained RNA (not DNA) from vaccine-treated cells, and Ctrl 6 the same but additionally treated with RNAse, which step was also performed in the purification of DNA samples. As expected, none of the control samples contain the PCR product.

While all of the observations cited here pertain to sequences derived from RNA viruses, retrotransposition by LINE-1 is not sequence-specific [22], and there is no reason to exclude the possibility that other RNA sequences, such as for example those of the Pfizer or Moderna mRNA vaccines, would be subject to the same mechanism.

1.4 Summary. Even though this had not yet been experimentally demonstrated when EMA released its assessment report [1], there was ample precedent to suggest the *strong possibility* that DNA copies of the vaccine mRNA would be produced and inserted into the cellular genome. Rather than waving away this risk as it did, EMA should have obligated Pfizer to carry out the necessary studies for excluding this risk *before* green-lighting authorization.

2 The current state of the evidence

As of this writing, substantial new evidence has accumulated regarding the genetic risks posed by the Pfizer COVID-19 vaccine.

2.1 DNA copies of the Pfizer COVID-19 vaccine mRNA are inserted into the host cell genome. Already in 2021, it was demonstrated that partial DNA copies of the genomic RNA of the SARS-CoV-2 virus can insert into the cellular DNA of infected cells [24]. Even though this does not directly relate to the mRNA vaccines, it does show that SARS-CoV-2-derived RNA sequences are not exempt from the general mechanism. Moreover, this study demonstrated that the insertion was mediated LINE-1 retrotransposons.

Of even greater and more immediate relevance is the recent demonstration that the mRNA contained in the Pfizer COVID-19 vaccine itself can integrate into the cells of a human-derived

liver cell line [23]. Even though in this initial study the participation of LINE-1 was not rigorously demonstrated, the evidence of the vaccine mRNA's integration into the DNA as such is solid (see Figure 2).

2.2 Long-term expression of the spike protein. While it had initially been assumed that expression of the spike protein after vaccination would be of short duration and largely limited to the injection site, it has since become clear that it is neither. A recent study by Röltgen et al. [25] detected both the spike protein and mRNA encoding it within lymph nodes of vaccinated people at 60 days after the most recent injection. Bansal et al. [26] detected the spike protein on exosomes (small cell-derived membrane vesicles) in the circulation even at four months after the most recent injection.

This surprisingly long persistence is difficult to reconcile with the notion that the expression is only driven directly by the injected recombinant mRNA. Of note, the Pfizer COVID-19 vaccine mRNA is modified with 1-methylpseudouridine [1]. It is sometimes asserted that RNA containing 1-methylpseudouridine will be more stable than that which contains regular uridine [27]. However, while the substitution very strongly increases the level of protein expression from the mRNA, its effect on RNA lifetime is rather modest, so that the half-life of both the modified and the unmodified mRNA is on the order of no more than a few days [28, 29]. We must therefore take the possibility very seriously that the gene encoding the spike protein is perpetuated and continuously expressed in vivo by way of DNA insertion.

2.3 Distribution of injected vaccine to interior organs via the bloodstream. While the pharmacokinetics of the vaccine was covered only rather briefly in the EMA assessment report [1, p. 46-47], a fuller and more illuminating description of Pfizer's own animal studies on this question was obtained and shared by the Japanese regulators [30].[1] The key data on the distribution of the vaccine in vivo are shown in Figure 3. The experiments used a model vaccine which had the same lipid composition as the Pfizer COVID-19 vaccine but contained a different mRNA. It is likely that the distribution in the organism is mostly controlled by the lipid composition, although we cannot rule out the possibility that expression of the spike protein may modify the distribution, for example by way of increasing capillary leakiness.

We note that the vaccine appears in the blood plasma after a very short time. The highest plasma level is reached at two hours after the injection; however, already at 15 minutes the level already reaches almost half of that maximal value. As the blood plasma level drops off, the concentration rises in several other organs. The fastest and highest rise is observed in the liver and the spleen. Both of these organs are rich in *phagocytes*, that is, cells that are in charge of clearing particles such as microbes or the fragments of decayed body cells from the bloodstream. Phagocytes are also numerous in the bone marrow, where the vaccine reaches somewhat lower but still substantial levels (not shown).

While the phagocytes are likely responsible for most of the uptake in the spleen, this may not be the case in the liver. Here, the vaccine likely ends up mostly in the organ-specific epithelial cells, which are in direct contact with the blood plasma and very rich in receptors for *lipoproteins*, that is, carrier particles that mediate the transport of lipids (fats and fat-like molecules) between organs. Artificial lipid nanoparticles (LNPs) such as those used in the Pfizer

[1] The text of the cited document is in Japanese, but an English translation is available [31].

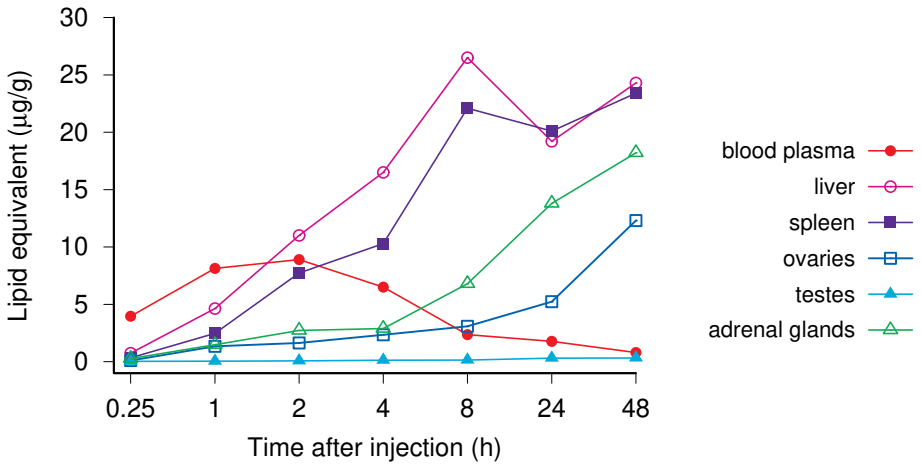


Figure 3 Organ distribution in rats of a model mRNA vaccine with the same lipid composition as the Pfizer COVID-19 vaccine. Plot generated from data in Table 2.6.5.5B of [30]. The blood level rises quickly and then falls as the vaccine accumulates in various organs. The vaccine was measured using a radioactively labelled cholesterol derivative (unlabelled cholesterol is a regular ingredient of the vaccine lipid nanoparticles). The data represent vaccine content in µg of vaccine lipid per gram of tissue or blood plasma. Note the high concentrations in liver, spleen, adrenal glands, and ovaries.

COVID-19 vaccine are known to acquire a shell—a "corona"—of *apolipoprotein* molecules, which normally decorate the body's own lipoproteins [32]. It is this effect that directs such synthetic LNPs to the same cell types that can take up regular lipoproteins.

As with the liver, uptake into the ovaries and into the adrenal glands is likely also mediated by lipoprotein receptors. Both organs normally take up lipoproteins to obtain cholesterol, which they use as a precursor for producing steroid hormones—corticosteroids in the adrenal glands, and female sexual hormones (estrogens and progestins) in the ovaries. The testes, too, produce sexual hormones (in particular testosterone) from cholesterol, but here the accumulation of vaccine lipid is remarkably much lower. The scientific literature does not offer a full, straightforward explanation for the restricted uptake into the testes, but it may be related to the so-called *blood-testes-barrier*. Another organ that synthesizes steroid hormones (progestins) in large amounts is the placenta. Here, too, we must expect substantial accumulation of the vaccine particles, even though the Pfizer study does not report any data on this question.

In most other organs examined the levels were similarly low as in the testes. We note, however, that at least the blood vessels will be exposed to the circulating vaccine in every organ and in every tissue.

2.4 Summary. The reverse transcription of the Pfizer COVID-19 vaccine mRNA into DNA and the integration of the DNA copy into the genome of host cells has been directly demonstrated in vitro, and the spike protein's documented long-term persistence in the bodies of vaccinated persons suggests that DNA integration may occur in vivo and perpetuate the expression of the spike protein. Moreover, the ovaries accumulate high levels of the vaccine, which implies that oocytes may be exposed to significant amounts of the recombinant mRNA. Accumulation in the ovaries and likely also the placenta raises concerns regarding female fertility.

3 Known and plausible risks that arise from the recently established genomic insertion of Pfizer COVID-19 vaccine

The results reported by Aldén et al. [23], even though preliminary in some respects, pose some very serious questions that can no longer be ignored by the EMA and other regulatory authorities.

3.1 Likelihood of DNA insertion occurring in vivo. One remarkable feature in Figure 2 is that the PCR product which signals genomic insertion is observed in each of the DNA samples isolated from vaccine-treated cells. This indicates that one or more insertion events have occurred in each experiment. As noted above, the Pfizer COVID-19 vaccine mRNA is modified with 1-methylpseudouridine, which will protect the mRNA from certain degradative pathways [27, 33–35], which may conceivably increase the likelihood of reverse transcription and insertion. This question has apparently not been experimentally elucidated; not having compelled Pfizer to carry out such experiments is another glaring oversight committed by the EMA.

In the depicted experiments, the concentration of vaccine was higher than that which can be expected to occur in vivo. However, in the absence of evidence to the contrary, it is reasonable to surmise that the likelihood of insertion will be the same for each individual mRNA molecule and independent of the number of such molecules within a given cell. Thus, the number of insertion events in vivo would be limited simply by the total amount of mRNA injected; and that amount exceeds the combined amount used in all samples shown in Figure 2.

Even though we do not know how the efficiency of genomic insertion compares between the particular human cell line used by Alden et al. and the various cell types found in the human body, we must expect, at least until proof positive of the opposite is obtained, that some insertion events will occur in many or even all vaccinated persons.

Retrotransposition is particularly common in actively dividing cells, because during cell division the membrane barrier which separates the nucleus from the cytoplasm transiently breaks down; this facilitates the entry of the DNA copy that was generated from the mRNA in question into the nucleus. While most tissues inside the body have lower proliferation rates than cell cultures in vitro, some, e.g. the bone marrow and the intestinal mucous membranes, do proliferate at comparable rates. Moreover, we reiterate that retrotransposition (i.e., genomic insertion) events may occur in non-dividing cells also [13].

3.2 Biological consequences of DNA insertion. With the LINE-1 retrotransposon at least, DNA insertions are apparently distributed in a random fashion [10], but they will occur preferentially within or near transcriptionally active genes, since the DNA of inactive genes will be tightly packed into complexes with histone proteins and therefore poorly accessible. The genotoxic effect of an insertion on an active gene can be manifested in several ways.

3.2.1 Gene inactivation. Insertion may occur within a gene and disrupt it. This can lead to the loss of important cellular gene products (i.e., proteins) and thus, potentially, to the development of disease including cancer [11, 12]. Insertion may be accompanied by the deletion of large gene fragments [36].

3.2.2 Gene regulation. Transcriptional and epigenetic regulation mechanisms may be affected, thus modulating protein expression levels upward or downward with unpredictable and un-

desirable results. Indirect regulatory effects may affect even distant genes located on other chromosomes.

3.2.3 Activation of oncogenes. This is a special case of the preceding point, but it is important enough to be highlighted separately. The occurrence of malignancies through DNA integration and activation of cancer-promoting genes (oncogenes) has been demonstrated in clinical trials with a retroviral vector for the genetic treatment of children with SCID-X1 (severe combined immune deficiency) [37]. These malignancies will typically become manifest only several years after the completion of treatment [38]. Therefore, thorough long-term investigations concerning possible genotoxic effects of chromosomal integration are absolutely necessary, in both the pre-clinical and the clinical trial stages, for a valid benefit-risk analysis. This does not apply just with retroviral vectors, but with any recombinant nucleic acid that can end up inserting into the chromosomes of the cell. With both the adenovector- and the mRNA-based COVID-19 vaccines, the risk of insertion into the chromosomal DNA must be taken seriously [39].

3.2.4 Autoimmune-like disease. Integration of the spike protein gene into the host cell could lead to permanent expression of this antigen and thus induce chronic autoimmune-like disease.

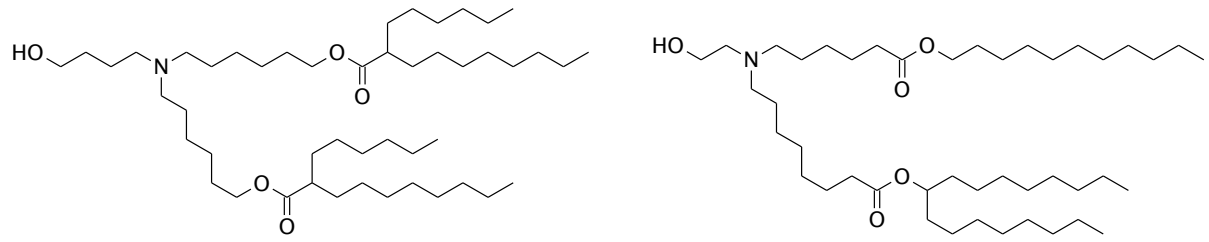
3.2.5 Germline integration. We noticed above that Pfizer's own experiments indicate a high level of vaccine accumulation in the ovaries. Furthermore, LINE-1 and other retrotransposons are active and cause genomic insertion events in human oocytes [14]. In combination, these findings indicate that the Pfizer COVID-19 vaccine gene sequence may be integrated into the DNA of oocytes, and hence into the human germline. Insertion into male germline cells cannot be ruled out either, even though according to the animal studies the tissue levels of the Pfizer COVID-19 vaccine in the testes are significantly lower than in the ovaries.

Should this indeed come to pass—should the germline cells of vaccinated individuals be rendered transgenic—then the risk of spawning or conceiving transgenic children will not be limited to these individuals only, but it will necessarily be shared by their current or future spouses. In effect, an entire generation of future parents will be exposed to this risk.

3.3 Summary. Integration of the mRNA sequences into somatic cells is likely and implies a risk of cancer and of autoimmune disease. Moreover, the risk of germline integration, resulting in transgenic offspring, cannot be denied. These risks must urgently be addressed through in-depth animal studies. Meanwhile, the authorizations based on EMA's demonstrably inadequate scientific assessment must urgently be revoked.

4 Genotoxic potential of lipid nanoparticles

While any adverse consequences of genomic insertion would take some time to become manifest as clinical disease, very many severe adverse events have occurred shortly after vaccination and must therefore be ascribed to other pathogenetic mechanisms. The toxicity of the spike protein, as well as the consequences of immune attack on the cells producing it, were already discussed in detail in our previous submission to the court, and they will therefore not be reiterated here. We will only note that the death toll continues to mount, with the latest figures from EudraVigilance having surpassed 43,000 dead [40]. In the following, we will briefly discuss the potential toxicity of the major synthetic lipid contained in the Pfizer COVID-19 vaccine.



ALC-315 (Pfizer) SM-102 (Moderna)

Figure 4 Molecular structures of the proprietary cationic lipids contained in the mRNA vaccines produced by Pfizer and by Moderna, respectively. The nitrogen (N) atoms will be partially protonated under intracellular conditions and thereby acquire a positive charge. Oxygen (O) atoms are indicated; unlabelled atoms are carbon, saturated with hydrogen.

The quote from the EMA assessment report given at the beginning of Section 1 dismissed not only RNA, but also lipids as possible causes of genotoxicity. With respect to the latter, such a statement is rather curious, since lipids are a very large and somewhat vaguely defined class of compounds, which invalidates any general claims as to their toxicity or the absence thereof. But since we do know the specific lipids contained in the lipid nanoparticles of the Pfizer COVID-19 vaccine, we can consider whether or not they pose a risk of genotoxicity. There are four lipids overall, two of which occur naturally (cholesterol and distearoyl-phosphatidylcholine), whereas the other two are synthetic and had not previously been approved for use in humans. We will focus here on the more abundant one of these synthetic lipids, which is known by the short name ALC-315 (see Figure 4).

4.1 Cytotoxic effect of cationic lipids. The first step in the process of vaccine particle uptake is *endocytosis*—the particle enters the cell, but it is initially still trapped within a membrane vesicle that budded off the cell membrane. The crucial step of releasing the mRNA from this vesicle (the endosome) into the cytosol is mediated by a synthetic cationic (positively charged) lipid. In the Pfizer vaccine, that lipid is ALC-315. After their escape into the cytosol, the cationic lipid molecules will continue to disrupt intracellular membranes, including those of the *mitochondria*. These are little organelles within our cells that carry out *cell respiration*—they generate hydrogen (in the form of NADH) and react it with molecular oxygen in order to produce ATP, the most important energy-rich metabolite of the cell. Disruption of mitochondrial metabolism will cause *reactive oxygen species* (ROS) to form. These ROS, in turn, can wreak all kinds havoc inside the cell, including damage to the DNA—hence, they cause genotoxicity.

It should be noted that with any agent that causes genetic damage—this includes ionizing radiation, but also cytotoxic anticancer drugs—there is a risk of cancer and leukæmia, and moreover there is a lifetime limit on the overall dose that can be tolerated. Thus, the prospect of frequently repeated COVID "booster shots," and also that of extending mRNA technology to vaccines against other pathogens or non-infectious diseases, conjures up a very grave public health risk.

4.2 Indications of genetic damage due to cationic lipids in Moderna's mRNA vaccine. According to the EMA assessment report on the Pfizer COVID-19 vaccine, this manufacturer did not provide any experimental data on the potential cytotoxicity of their lipid mixture (and the

EMA committed a grave error in letting them get away with it). In contrast, Moderna, in its own application to the EMA, did supply some experimental data. While Moderna uses a different proprietary cationic lipid named SMA-102, the two lipids are very similar in structure (see Figure 4), and there is no reason to expect a major difference in cytotoxic activity.

In Moderna's animal experiments, *polychromatic* erythrocytes (red blood cells, RBC) were counted, as were those with *micronuclei.* Polychromatic RBC are those which have only just finished their differentiation inside the bone marrow and disposed of their nuclei. At this stage, they still retain their ribosomal RNA, which causes them to appear bluish rather than red in the Giemsa stain. Changes in the percentage of RBC with this characteristic indicate changes in erythrocyte maturation kinetics. Genotoxic agents can cause both decreases [41] and increases [42] in this parameter. Differences between sexes are expected to be small. Using a luciferase-encoding mRNA packaged into a lipid mixture which contained SM-102, Moderna found a significantly decreased level of erythrocyte polychromasia, but only in male rats. The reported gender difference raises questions about the statistical power of this study.

Using another model mRNA and again a lipid mixture containing SM-102, Moderna found "statistically significant increases in micronucleated erythrocytes ... in both sexes." A so-called micronucleus is a chromosome fragment which was produced by chromosome damage [42, 43] and then left behind in the cytoplasm when the main nucleus was expelled. The micronucleus assay is widely used to assess genotoxicity in vivo [43].

The EMA report on the Moderna vaccine [44] quotes a study done by the company to the effect that the increased abundance of micronucleated RBC might have been due not to genotoxicity, but rather to the impeded clearance of these cells from the bloodstream as a consequence of the vaccine's spleen toxicity. However, no proof of this contention is shown; and the EMA report further states that "a strong increase in Molecular initiating event (MIE) was observed 48 hours after the final administration in the highest dose group in male rats." While no details are given as to the exact nature of the observed MIE, an "increase in molecular initiating events" clearly suggests an actual increase in the rate of formation of genetically damaged cells rather than merely a decrease in their clearance. Certainly, a report as sketchy and opaque as this one does not provide a sound basis for dismissing the risk of genotoxicity and proceeding with approval.

In conclusion, while the data provided by Moderna are incomplete, they strongly suggest that their SM-102 lipid is indeed genotoxic. This agrees with prior observations of genotoxicity associated with similar cationic lipids in liposomes, reviewed for example by Inglut et al. [45]. Unless proof positive to the opposite is provided, it must be assumed that the same also applies to Pfizer's ALC-315 lipid.

4.3 Sensitivity of lymphocytes to cytotoxic agents. As noted above, reactive oxygen species also mediate to a large extent the cytotoxic effects of ionizing radiation. A cell type that is particularly sensitive to radiation, but also to metabolically inflicted genetic damage, are the lymphocytes.[2] Since the lymphocytes are the backbone of the adaptive immune system, we must expect that cationic lipid toxicity will cause immunosuppression.

[2]See in particular the example of adenosine deaminase deficiency, a metabolic disease that causes genotoxic stress to all body cells yet selectively eradicates the lymphocytes, which causes severe combined immunodeficiency (SCID) [46].

4.4 Summary. Apart from the mRNA, the cationic lipid contained in the Pfizer COVID-19 vaccine also poses a risk of genotoxicity. The EMA erred in neglecting this risk and not insisting on its rigorous experimental assessment by the manufacturer.

5 EMA's evaluation of the Pfizer COVID-19 vaccine did not comply with EU regulations

EMA's evaluation process, as documented in the EMA assessment report [1], fell short of the rules set out in various EU directives and regulations.

5.1 Failure to enforce the submission of mutagenicity studies. The most comprehensive EU directive on the evaluation and approval of new medicines is Directive 2001/83/EC of the European Parliament and of the Council of 2001 [47]. While it has been superseded in parts by later directives, most of its provisions remain in force. This includes in particular Part 3 on toxicological and pharmacological tests. Under the subheading *II. Performance of Tests*, paragraph *D. Mutagenic potential* specifies that studies on mutagenicity are obligatory with any new substance. This provision is general and not limited to any particular category of medicines.

Both of the synthetic lipids and the mRNA contained in the Pfizer COVID-19 vaccine are novel compounds that so far had not been approved for use as part of any other medicine. Thus, in waiving Pfizer's obligation to subject such studies, the EMA failed to enforce compliance with this binding and specific regulation.

5.2 Gene-based vaccines are a form of "advanced therapy." The above-mentioned directive was updated and partly superseded by EC regulation No 1394 in 2007 [48]. This regulation introduces the concept of "advanced therapies" (emphasis added):

> *(1) New scientific progress in cellular and* ***molecular biotechnology*** *has led to the development of* ***advanced therapies****, such as gene therapy . . .*
>
> *(2) Insofar as advanced therapy products are presented as having properties for treating or* ***preventing diseases*** *in human beings, or that they may be used in or administered to human beings with a view to restoring, correcting or modifying physiological functions by* ***exerting principally a*** *pharmacological,* ***immunological or metabolic action*** *. . .*

The relevance of this definition of "advanced therapies" is that, by virtue of the terms highlighted in the quote, it unequivocally includes gene-based vaccines, even though the subsequently issued directive 2009/120/EC [49] explicitly excludes "vaccines against infectious diseases" from its definition of "gene therapy medicinal products."

5.3 Failure to enforce evaluation of risk of genome integration. Another Commission Directive, 2009/120/EC, is concerned entirely with "advanced therapy medicinal products," which as just shown includes the gene-based vaccines. It states that a risk-based approach "may be applied" to determine what kind of studies will be required for approval. In this context, the "level of integration of nucleic acid sequences" into the genome and the risk of oncogenicity are explicitly mentioned.

Section 4 on *specific requirements regarding Module 4*, subheading 4.1 on *all advanced therapy medicinal products,* states that with pharmacological and toxicological testing, rationales

for the choice of models and experiments must be given. This arguably implies that rationales for *not* performing certain studies must also be provided—as indeed was done by the EMA in its barren statement that "the components of the vaccine formulation are lipids and RNA that are not expected to have genotoxic potential." Of course, as is clear from both earlier evidence and the experimental demonstration of the vaccine's genome integration, the EMA's reasoning was flawed.

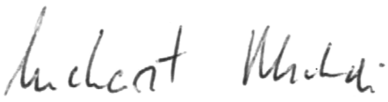
5.4 Summary. The EMA has failed in its duty to protect the EU population from the inherent genotoxic risks of the Pfizer COVID-19 vaccine. Even without understanding the relevant science at the depth we should expect of it, the EMA could easily have avoided this grave mistake by adhering to the letter of existing EU regulations on medicinal products in general and on "advanced therapies" in particular.

Signatures

SIGNED AT Waterloo, Ontario, Canada, on June 27, 2022

Dr. Michael Palmer

SIGNED AT Martinsrade, Schleswig-Holstein, Germany, on June 27, 2022



Prof. Dr. Sucharit Bhakdi

SIGNED AT Warder, Schleswig-Holstein, Germany, on June 27, 2022

Dr. Wolfgang Wodarg

Short biographies of the authors

Sucharit Bhakdi, MD, is a Professor Emeritus of Medical Microbiology and Immunology and Former Chair, Institute of Medical Microbiology and Hygiene, Johannes Gutenberg University of Mainz. Dr. Bhakdi has conducted experimental research on numerous topics including the complement system, bacterial toxins, malaria, and atherosclerosis.

Michael Palmer, MD, was until March 2022 an Associate Professor of Biochemistry in the Department of Chemistry at the University of Waterloo, Ontario, Canada. He obtained a board certification in Medical Microbiology and Infectious Disease Epidemiology from the German province of Rhenania-Palatinate while working with Dr. Sucharit Bhakdi at the University of Mainz, Germany. His research has focused on bacterial toxins and lipopeptide antibiotics, and his teaching experience includes medical microbiology, metabolism, and pharmacology.

Wolfgang Wodarg, MD, is a specialist in pulmonary and bronchial internal medicine, hygiene and environmental medicine, epidemiology, and public health; Honorary Member of the Parliamentary Assembly of the Council of Europe and former Head of the Health Committee of the Parliamentary Assembly of the Council of Europe; former Member of the German federal parliament (the Bundestag); initiator and spokesman for the study commission 'Ethics and Law in Modern Medicine;' author and university lecturer.

References

[1] Anonymous: *EMA Assessment report: Comirnaty*. 2021. URL: https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf.

[2] D. Baltimore: RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 226 (1970), 1209–11. PMID: 4316300.

[3] S. Spiegelman et al.: Characterization of the products of RNA-directed DNA polymerases in oncogenic RNA viruses. *Nature* 227 (1970), 563–7. PMID: 4317039.

[4] Y. Sakaki et al.: The LINE-1 family of primates may encode a reverse transcriptase-like protein. *Cold Spring Harb. Symp. Quant. Biol.* 51 Pt 1 (1986), 465–9. PMID: 2438081.

[5] P. Klenerman et al.: A non-retroviral RNA virus persists in DNA form. *Nature* 390 (1997), 298–301. PMID: 9384383.

[6] M. B. Geuking et al.: Recombination of retrotransposon and exogenous RNA virus results in non-retroviral cDNA integration. *Science* 323 (2009), 393–6. PMID: 19150848.

[7] C. Esnault et al.: Human LINE retrotransposons generate processed pseudogenes. *Nat. Genet.* 24 (2000), 363–7. PMID: 10742098.

[8] R. Cordaux and M. A. Batzer: The impact of retrotransposons on human genome evolution. *Nature reviews. Genetics* 10 (2009), 691–703. PMID: 19763152.

[9] W. Ding et al.: L1 elements, processed pseudogenes and retrogenes in mammalian genomes. *IUBMB Life* 58 (2006), 677–85. PMID: 17424906.

[10] I. Ovchinnikov et al.: Genomic characterization of recent human LINE-1 insertions: evidence supporting random insertion. *Genome Res.* 11 (2001), 2050–8. PMID: 11731495.

[11] C. R. Beck et al.: LINE-1 Elements in Structural Variation and Disease. *Annu. Rev. Genomics Hum. Genet.* 12 (2011), 187–215. PMID: 21801021.

[12] J. R. Kemp and M. S. Longworth: Crossing the LINE Toward Genomic Instability: LINE-1 Retrotransposition in Cancer. *Front. Chem.* 3 (2015), 68. PMID: 26734601.

[13] S. Kubo et al.: L1 retrotransposition in nondividing and primary human somatic cells. *Proc. Natl. Acad. Sci. U. S. A.* 103 (2006), 8036–41. PMID: 16698926.

[14] I. Georgiou et al.: Retrotransposon RNA expression and evidence for retrotransposition events in human oocytes. *Hum. Mol. Genet.* 18 (2009), 1221–8. PMID: 19147684.

[15] V. A. Belyi et al.: Unexpected inheritance: multiple integrations of ancient bornavirus and ebolavirus/marburgvirus sequences in vertebrate genomes. *PLoS Pathog.* 6 (2010), e1001030. PMID: 20686665.

[16] M. Horie et al.: Endogenous non-retroviral RNA virus elements in mammalian genomes. *Nature* 463 (2010), 84–7. PMID: 20054395.

[17] M. Horie and K. Tomonaga: Non-retroviral fossils in vertebrate genomes. *Viruses* 3 (2011), 1836–48. PMID: 22069518.

[18] A. Katzourakis and R. J. Gifford: Endogenous viral elements in animal genomes. *PLoS Genet.* 6 (2010), e1001191. PMID: 21124940.

[19] S. Chiba et al.: Widespread endogenization of genome sequences of non-retroviral RNA viruses into plant genomes. *PLoS Pathog.* 7 (2011), e1002146. PMID: 21779172.

[20] E. V. Koonin: Taming of the shrewd: novel eukaryotic genes from RNA viruses. *BMC Biol.* 8 (2010), 2. PMID: 20067611.

[21] H. Liu et al.: Widespread horizontal gene transfer from double-stranded RNA viruses to eukaryotic nuclear genomes. *J. Virol.* 84 (2010), 11876–87. PMID: 20810725.

[22] O. Dhellin et al.: Functional differences between the human LINE retrotransposon and retroviral reverse transcriptases for in vivo mRNA reverse transcription. *EMBO J.* 16 (1997), 6590–602. PMID: 9351839.

[23] M. Aldén et al.: Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Current issues in molecular biology* 44 (2022), 1115–1126. DOI: 10.3390/cimb44030073.

[24] L. Zhang et al.: Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proc. Natl. Acad. Sci. U. S. A.* 118 (2021). PMID: 33958444.

[25] K. Röltgen et al.: Immune imprinting, breadth of variant recognition and germinal center response in human SARS-CoV-2 infection and vaccination. *Cell* (2022). PMID: 35148837.

[26] S. Bansal et al.: Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines. *J. Immunol.* 207 (2021), 2405–2410. PMID: 34654691.

[27] U. Sahin et al.: mRNA-based therapeutics—developing a new class of drugs. *Nat. Rev. Drug Discov.* 13 (2014), 759–780. PMID: 25233993.

[28] O. Andries et al.: N^1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J. Control. Release* 217 (2015), 337–344. DOI: 10.1016/j.jconrel.2015.08.051.

[29] N. Pardi et al.: Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. *J. Exp. Med.* 215 (2018), 1571–1588. PMID: 29739835.

[30] Anonymous: *SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048) 2.6.4 [Summary statement of the pharmacokinetic study] (Japanese).* 2020. URL: https://www.pmda.go.jp/drugs/2021/P20210212001/672212000_30300AMX00231_I100_1.pdf.

[31] Anonymous: *SARS-CoV-2 mRNA Vaccine (BNT162, PF-07302048) 2.6.4 Summary statement of the pharmacokinetic study [English translation]*. 2020. URL: https://archive.org/details/pfizer-confidential-translated.

[32] V. Francia et al.: The Biomolecular Corona of Lipid Nanoparticles for Gene Therapy. *Bioconjug. Chem.* 31 (2020), 2046–2059. PMID: 32786370.

[33] B. R. Anderson et al.: Incorporation of pseudouridine into mRNA enhances translation by diminishing PKR activation. *Nucleic Acids Res.* 38 (2010), 5884–92. PMID: 20457754.

[34] B. R. Anderson et al.: Nucleoside modifications in RNA limit activation of 2'-5'-oligoadenylate synthetase and increase resistance to cleavage by RNase L. *Nucleic Acids Res.* 39 (2011), 9329–9338. DOI: 10.1093/nar/gkr586.

[35] K. Karikó et al.: Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. *Mol. Ther.* 16 (2008), 1833–40. PMID: 18797453.

[36] N. Van de Water et al.: A 20.7 kb deletion within the factor VIII gene associated with LINE-1 element insertion. *Thromb. Haemost.* 79 (1998), 938–42. PMID: 9609225.

[37] F. J. T. Staal et al.: Sola dosis facit venenum. Leukemia in gene therapy trials: a question of vectors, inserts and dosage? *Leukemia* 22 (2008), 1849–1852. PMID: 18769449.

[38] S. Hacein-Bey-Abina et al.: Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. *J. Clin. Invest.* 118 (2008), 3132–42. PMID: 18688285.

[39] W. Doerfler: Adenoviral Vector DNA- and SARS-CoV-2 mRNA-Based Covid-19 Vaccines: Possible Integration into the Human Genome—Are Adenoviral Genes Expressed in Vector-based Vaccines? *Virus Res.* 302 (2021), 198466. PMID: 34087261.

[40] B. Shilhavy: *43,898 Dead, 4,190,493 Injured Following COVID Vaccines in European Database of Adverse Reactions*. 2022. URL: https://healthimpactnews.com/2022/43898-dead-4190493-injured-following-covid-19-vaccines-in-european-database-of-adverse-reactions/.

[41] Y. Suzuki et al.: The micronucleus test and erythropoiesis. Effects of erythropoietin and a mutagen on the ratio of polychromatic to normochromatic erythrocytes (P/N ratio). *Mutagenesis* 4 (1989), 420–4. PMID: 2516221.

[42] J. A. Heddle et al.: The induction of micronuclei as a measure of genotoxicity. A report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 123 (1983), 61–118. PMID: 6888413.

[43] S. Sommer et al.: Micronucleus Assay: The State of Art, and Future Directions. *Int. J. Mol. Sci.* 21 (2020). PMID: 32102335.

[44] Anonymous: *EMA Assessment report: COVID-19 Vaccine Moderna*. 2021. URL: https://www.ema.europa.eu/en/documents/assessment-report/spikevax-previously-covid-19-vaccine-moderna-epar-public-assessment-report_en.pdf.

[45] C. T. Inglut et al.: Immunological and Toxicological Considerations for the Design of Liposomes. *Nanomaterials* 10 (2020). PMID: 31978968.

[46] W. L. Nyhan: Disorders of purine and pyrimidine metabolism. *Mol. Genet. Metab.* 86 (2005), 25–33. PMID: 16176880.

[47] Anonymous: *Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use*. 2001. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32001L0083&from=EN.

[48] Anonymous: *Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004*. 2007. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32007R1394&from=EN.

[49] Anonymous: *Commission Directive 2009/120/EC of 14 September 2009 amending Directive 2001/83/EC of the European Parliament and of the Council on the Community code relating to medicinal products for human use as regards advanced therapy medicinal products*. 2009. URL: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32009L0120.