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Adjuvant Activity and Toxicological Risks of Lipid Nanoparticles Contained in the COVID-19 "mRNA Vaccines"

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Abstract

The LNPs reportedly used as the platform by Pfizer/BioNTech for its SARS-CoV-2 "mRNA vaccines" allegedly consist of a mixture of phospholipids, cholesterol, PEGylated lipids, and an ionizable cationic lipid. This study reviews some of the main toxicological risks and immunostimulatory properties of such nanomaterials, with particular attention to the ionizable LNPs and their adjuvant properties, inflammatory responses, stimulation of immune cells, and formation of ROS inside transfected cells. The decision not to carry out safety pharmacology, carcinogenicity, and genotoxicity tests on these nanomaterials appears unjustifiable and in contradiction with the international policies provided for *novel adjuvants*. Important gaps are highlighted on the activities by the relevant regulatory bodies, related to the scientific evaluation, risk management, and pharmacovigilance for new medicinal products in the EU. Given the findings discussed here, it is strongly urged that the mRNA-LNP-based "vaccines" and their boosters should be removed from the worldwide market because of unacceptable and potentially fatal safety risks.

Keywords: *COVID-19 mRNA vaccine, LNP, lipidnanoparticle, nanotechnology, ROS, adjuvant, novel adjuvant*

Introduction

The ionizable lipidnanoparticles (LNPs) in the two COVID-19 mRNA-LNP-based vaccines (Comirnaty by Pfizer/BioNTech and Spikevax by Moderna) are allegedly, according to documentation from the manufacturers, formed by four different types of lipids: an ionizable cationic lipid whose positive charge binds to the negatively charged backbone of the modRNA[1], a polyethylene glycol (PEG)-linked lipid that is supposed to help prolong the half-life of the composition, a phospholipid (DSPC) to facilitate the formation of a two-layer structure, and

[1] A nucleoside-*modified* messenger RNA (modRNA) is a synthetic messenger RNA, in which some nucleosides are replaced by other synthetically modified nucleosides or analogues, to induce cells to make proteins that they do not normally produce. In other words, it's a kind of blueprint for foreign proteins "smuggled" into the cells.

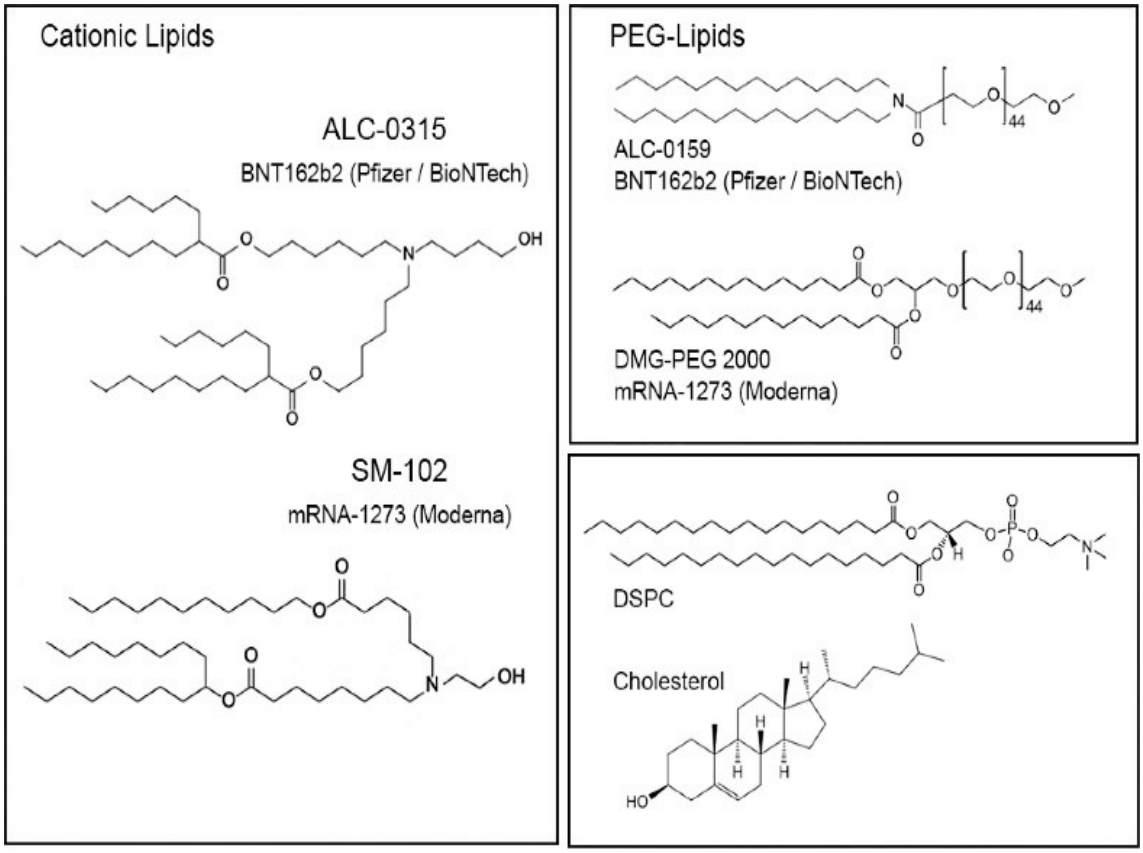


Figure 1. "Structures of the lipid constituents of the LNPs of the COVID-19 mRNA vaccines" reprinted from Figure 8, page 16989 from the article by Tenchov, R., Bird, R., Curtze, A. E., & Zhou, Q., entitled "Lipidnanoparticles—from liposomes to mRNA vaccine delivery, a landscape of research diversity and advancement" published in *ACS Nano* 2021, 15, 11, 16982–17015, 15(11), 16982–17015, https://pubs.acs.org/doi/full/10.1021/acsnano.1c04996. Copyright © by the authors 2021 and licensed under CC-BY 4.0.

cholesterol intended to function as a membrane fluidity modulator/stabilizer (Figure 1).

These nanoparticles are supposed to have the primary function of encapsulating the "experimental" modRNA, protecting it from enzymatic degradation and assisting its penetration into the cells of the host organism, after intramuscular injection (Nance & Meier, 2021).

It is likely that these RNA's modifications are partly, maybe wholly, responsible for the unnatural clots found in living and dead recipients of these experimental injectables, and that these injectables are increasing all-cause mortality across the globe (Santiago, 2022a, 2022b; Santiago & Oller, 2023).

In the case of the medicinal product Comirnaty by Pfizer/BioNTech, placed on the market in Europe, with conditional marketing authorization dated December 21, 2020, the modified RNA (mRNA BNT162b2), which is expected to encode the viral Spike protein inside the host cell, is encapsulated in lipidnanoparticles formed by the two functional lipids ALC-0315 [2] and ALC-0159 [3], and the two structural lipids DSPC [4] and cholesterol.

[2] ALC-0315: ((4-Hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate)
[3] ALC-0159: 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide
[4] DSPC: 1,2-Distearoyl-sn-glycero-3-phosphocholine

Regulatory non-compliances and absence of toxicological studies regarding the novel LNP components of the mRNA "vaccines" have already been discussed in recent studies (Segalla, 2023a; Banoun, 2023).

In this review, the focus is on some of the main toxicological and immunological concerns presented by these lipid nanomaterials, with particular attention to ionizable LNPs used by Pfizer/BioNTech. These concerns involve manifest lapses and contradictions that emerge from a detailed comparative analysis of the official safety documentation offered by both the manufacturer and the regulatory body responsible for the scientific evaluation, supervision, risk management plan approval, and pharmacovigilance of medicinal products in the European Community.

Reactive Oxygen Species and Nanoparticle Toxicity

Numerous studies have confirmed that the toxic effects produced by nanoparticles in biological systems are mainly and substantially due to the formation of reactive oxygen species (ROS) inside cells. ROS are particles that contain oxygen, among which the most relevant are *hydrogen peroxide* (H_2O_2), *superoxide anion radical* (O_2-) and *hydroxyl radicals* (•OH). See discussion in Segalla (2023a).

In scientific studies on the subject, it is generally noted that, despite the benefits and progress made in the use of nanomaterials in the biomedical field, concerns remain about the potential toxicological effects of nanoparticles, especially in relation to their tendency to generate ROS. Due to their strong oxidation potential, excess ROS induced by nanoparticles can cause damage to biomolecules and cell organelle structures. They can produce oxidative carbonylation of proteins, lipid peroxidation, DNA/RNA breakdown, and destruction of cell membranes — factors that can induce a complex of pathophysiological effects, such as genotoxicity, necrosis, apoptosis, cytokine inflammation, fibrosis, metaplasia, hypertrophy, carcinogenicity, or even mutagenesis impacting future generations. The extreme penetration and mobility of nanoparticles within the body account for their easy entry into the systemic circulation and accumulation in organs such as kidneys, liver, heart, brain, intestinal tract, and lungs, causing dysfunctions and abnormalities (Yu et al., 2020).

There is overwhelming evidence that overproduction of ROS is the main cause of nanoparticle biotoxicity. By concentrating mainly in lysosomes, mitochondria, and the nucleus of the cell, and generating ROS at those sites, nanoparticles can cause devastating consequences. Numerous studies irrefutably confirm that nucleotide components of cellular DNA and RNA constitute a significantly vulnerable target to the aggression of ROS generated by nanomaterials (Imlay et al., 1988; Maki et al., 1992; Demple et al., 1994).

The foregoing consequences can result in irreparable genetic damage, resulting in the development of *genotoxicity* (Kang et al., 2008; Singh et al., 2009; Chompoosor et al., 2010; Di Bucchianico et al., 2014; Proquin et al., 2017), *mutagenicity* (Kirsch-Volders et al., 2002; Mateuca et al., 2006; Dufour et al., 2006; Levine et al., 2017; Jena, 2012), and *carcinogenicity* (Rusyn et al., 2004; Nel et al., 2006; Liou et al., 2010; Tretyakova et al., 2015). The rationale behind this sequential order of toxicological events is that one of the fundamental mechanisms that may lead to carcinogenesis is damage to DNA (Poirier, 2004).

The accumulation of nanoparticles in the body can further induce *inflammation and immune responses*, which in turn can cause cell injury or apoptosis (cell death), dysfunction of vital organs and, finally,

stimulate the onset of numerous diseases, such as Alzheimer's, Parkinson's, inflammation of the liver, and dysembryoplasia (Yu et al., 2020).

Furthermore, as reported even by Moderna's researchers (Packer et al., 2021), a new class of impurities (lipid-mRNA adducts), formed through lipid-mRNA reactions, have been identified only through new sophisticated HPLC and Mass Spectrometry techniques, as such reactions are typically undetectable by traditional mRNA purity analytical techniques. These newly identified modifications can render the cellular mRNA untranslatable, leading to loss of protein expression, and can also result in the formation of electrophilic (genotoxic) reactive compounds or metabolites by a nucleophilic substitution mechanism (Martella et al., 2023).

The intrinsic pKa value (9.6) of the ionizable lipid ALC-0315 is too high, which makes it a stronger base than ammonia itself (pKa 9.25) in aqueous solution, and therefore it becomes completely protonated once released into the cytosol of the host cell, at physiological pH. Such elevated cationic charge, acquired by ALC-0315 after its endosomal escape, can stimulate the formation of pro-inflammatory cytokines and ROS, that can disrupt the mitochondrial membrane and release its content, cause RNA mistranslation, polymerization of proteins and DNA, DNA mutations, destruction of the nuclear membrane and consequent release of its content (Yu et al., 2020; Segalla, 2023b).

Undoubtedly, among the greatest risks to human health caused by the exceptional penetrability, mobility, chemical reactivity and systemic accumulation of uncontrollable cationic lipidnanoparticles within biological systems, those related to *genotoxicity* and *carcinogenicity*, must *always* be identified and evaluated. In vitro assays are considered an extremely important, if not indispensable, tool for a thorough understanding of the toxicity mechanisms and an adequate assessment of the health risks caused by cationic nanomaterials, especially in the medium to long term (Barone et al., 2017).

Genetic toxicity evaluation of new chemicals is a high priority in safety risk management and evaluations that focus on whether a new chemical may induce mutagenicity and/or carcinogenicity are required as part of hazard identification and risk characterization (Cimino, 2006; Petkov et al., 2015; Thybaud et al., 2017).

Assaying for tumor formation after chemical administration to animals in vivo may be utilized for evaluating carcinogenicity, however high cost, long assay times and issues related to animal protection must be considered (Bourcier et al., 2015; Petkov et al., 2015). In vitro methods to evaluate genotoxicity at the early stages of chemical product development include assays such as the Ames test, micronucleus test, and the chromosomal aberration test, which taken together may be used to predict the carcinogenic potential of a chemical (Kirkland et al., 2005; Hayashi et al., 2013). Improved in vitro testing methods that may include the evaluation of DNA damage for predicting mutagenicity and carcinogenicity of chemical compounds with accuracy and at sufficiently low cost in the early stages of chemical development are sought (MacGregor et al., 2015; Petkov et al., 2015; Dertinger et al., 2019). Modern techniques based on recent DNA/RNA adductomics studies also allow good analytical results to be obtained in a relatively short time (Takeshita & Kanaly, 2019).

All tests required for the evaluation of the toxicological and ecotoxicological effects of nanomaterials are indicated and described by OECD [5] in its *Guidance Manual for the Testing of Manufactured Nanomaterials* (ENV/JM/MONO(2009)20/REV), including endpoints as oxidative

[5] OECD: Organization for Economic Co-operation and Development

stress caused by ROS, inflammatory response, carcinogenicity, reproductive toxicity, genotoxicity, and mutagenicity.

Genotoxicity and Carcinogenicity: Relevant Regulatory Policies and Guidelines

The two components of the Comirnaty, ALC-0315 and ALC-0159, are classified by EMA, in its Assessment Report of 19 February 2021, as *novel excipients*, as they have never been previously used in a medicinal product in Europe (EMA/707383).

A "novel excipient" is defined by EMA (Doc. Ref. EMEA/CHMP/QWP/396951/2006, page 8) as follows (with emphasis in italics added here and throughout the remaining quoted entries in this paper):

> A *novel excipient* is an excipient which is being *used for the first time* in a drug product, or by a new route of administration. It may be a new chemical entity or a well-established one which has not yet been used for human administration and/or for a particular human administration pathway in the EU and/or outside the EU [Figure 2].

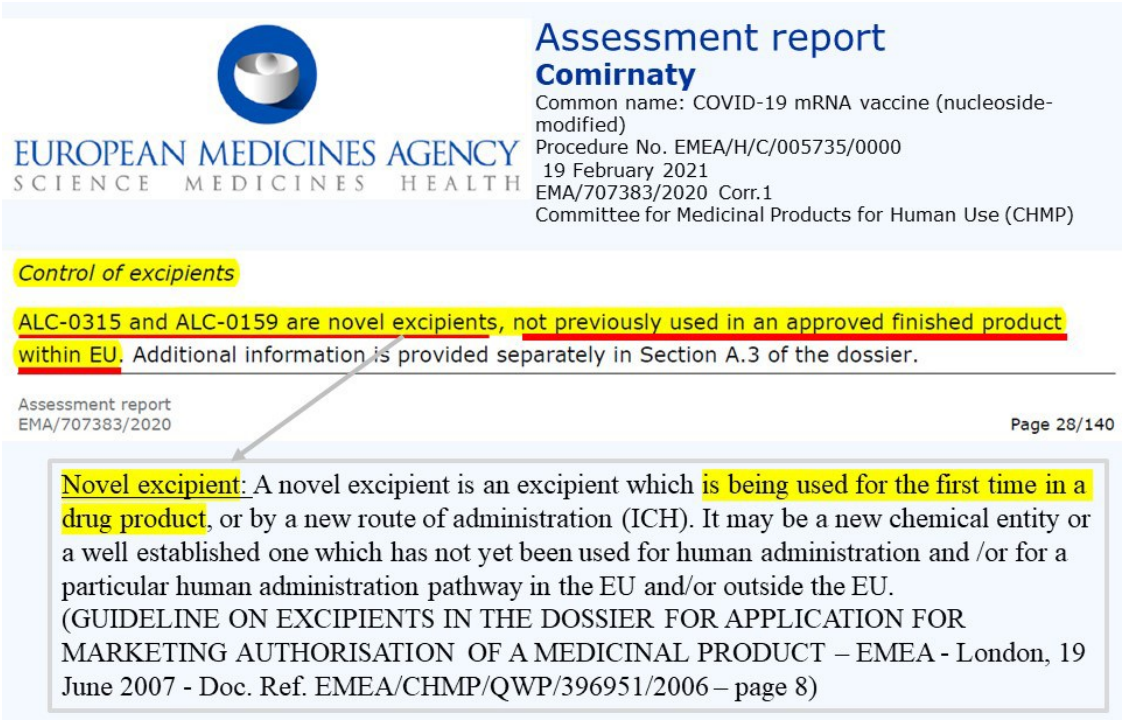


Figure 2. ALC-0315 and ALC-159 categorized by EMA as novel excipients.

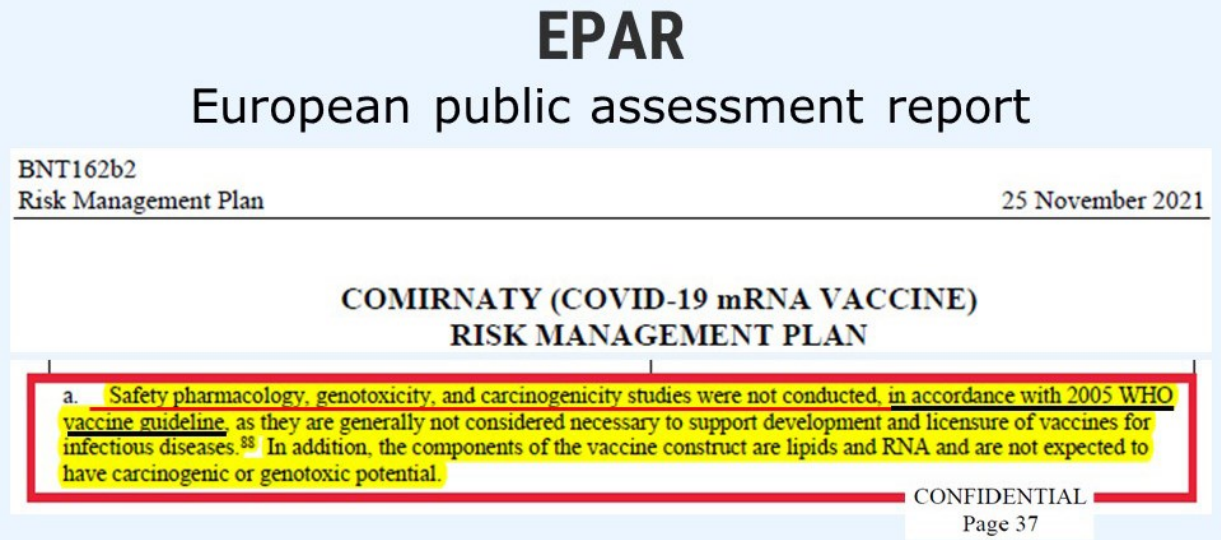
The absence of genotoxicity and carcinogenicity studies in the preclinical phase of the development of the Comirnaty vaccine is justified by EMA in its Assessment report (EMA/707383, 2021), as follows:

> *No genotoxicity nor carcinogenicity studies* have been provided. The components of the vaccine formulation are *lipids and RNA that are not expected to have genotoxic potential* [p. 55].

> As per guidance, no genotoxicity nor carcinogenicity studies were performed. The components of the vaccine (lipids and mRNA) are *not expected to have genotoxic potential*. This is acceptable to the CHMP[6] [p. 56].

Furthermore, the Pfizer/ BioNTech *Risk Management Plan*[7] dated 25 November 2021 (Figure 3), and all its subsequent updates, include the following words:

> *Safety pharmacology*, *genotoxicity*, and *carcinogenicity studies* were not conducted, *in accordance with 2005 WHO vaccine guideline*, as they are generally not considered necessary to support development and licensure of vaccines for infectious diseases. In addition, the *components of the vaccine construct are lipids and RNA and are not expected to have carcinogenic or genotoxic potential*.


EPAR

European public assessment report

BNT162b2
Risk Management Plan 25 November 2021

COMIRNATY (COVID-19 mRNA VACCINE)
RISK MANAGEMENT PLAN

a. Safety pharmacology, genotoxicity, and carcinogenicity studies were not conducted, in accordance with 2005 WHO vaccine guideline, as they are generally not considered necessary to support development and licensure of vaccines for infectious diseases.[88] In addition, the components of the vaccine construct are lipids and RNA and are not expected to have carcinogenic or genotoxic potential.

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Figure 3. Comirnaty RPM, 25 Nov 2021, asserting safety pharmacology, genotoxicity and carcinogenicity studies are not necessary, in accordance with the 2005 WHO vaccine guideline.

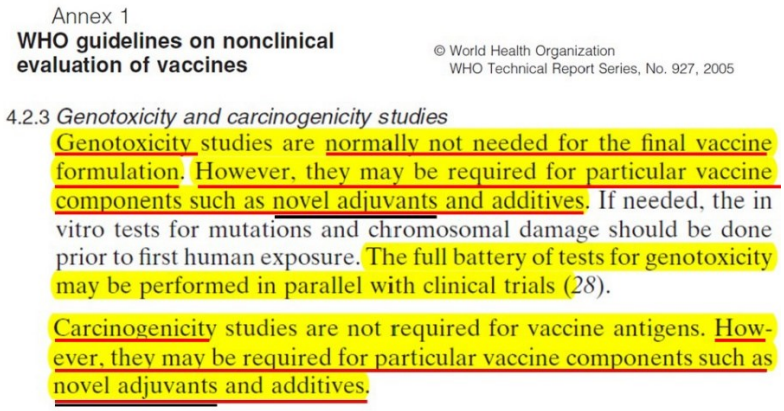
EMA, the highest agency in Europe responsible for the scientific evaluation, supervision and safety monitoring of medicines developed by pharmaceutical companies, and its Committee for Medicinal Products for Human Use (CHMP), which plays a vital role in the authorization of medicines in the European Union, have thus authorized Pfizer/BioNTech not to conduct safety pharmacology, genotoxicity and carcinogenicity tests *in accordance with* the Annex − *WHO Guidelines on non clinical evaluation of vaccines*, Technical Report Series, No. 927, 2005.

Section 4.2.3. (Figure 4) of this WHO document says:

> *Genotoxicity studies* are normally not needed for the final vaccine formulation. However, they *may be required for particular vaccine components* such as *novel adjuvants* and additives. If needed, the in vitro tests for mutations and chromosomal damage should be done *prior to first human exposure*. The full battery of tests for genotoxicity may be performed in parallel with clinical trials.

[6] CHMP: European Committee for Medicinal Products for Human Use.
[7] Companies in EU are required to submit a Risk-Management Plan (RMP) to the European Medicines Agency (EMA) when applying for a marketing authorization. RMPs include information on: a medicine's safety profile; how its risks will be prevented or minimized in patients; plans for studies and other activities to gain more knowledge about the safety and efficacy of the medicine; measuring the effectiveness of risk-minimization measures.

Carcinogenicity studies are not required for vaccine antigens. However, they *may be required for particular vaccine components* such as *novel adjuvants* and additives.



Annex 1
WHO guidelines on nonclinical evaluation of vaccines

4.2.3 *Genotoxicity and carcinogenicity studies*

Genotoxicity studies are normally not needed for the final vaccine formulation. However, they may be required for particular vaccine components such as novel adjuvants and additives. If needed, the in vitro tests for mutations and chromosomal damage should be done prior to first human exposure. The full battery of tests for genotoxicity may be performed in parallel with clinical trials (*28*).

Carcinogenicity studies are not required for vaccine antigens. However, they may be required for particular vaccine components such as novel adjuvants and additives.

Figure 4. WHO Guidelines on non clinical evaluation of vaccines, Technical Report Series, No. 927, 2005, section 4.2.3.

This 2005 WHO document clearly and unequivocally specifies that, although genotoxicity and carcinogenicity tests are not commonly required for the final formulation of a vaccine, they may nevertheless be required if *novel adjuvants* are present in the formulation.

This same WHO document provides the definition of "adjuvant":

> *Adjuvants*: Substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine.

A similar definition is contained in the European Guidelines on Excipients dated 19 June 2007:

> An *adjuvant* is a substance that helps and enhances the pharmacological effect of a drug or increases the ability of an antigen to stimulate the immune system [p. 9]

Section 5 of the WHO 2005 Guideline provides a clear explanation of why adjuvants are included in vaccine formulations:

> *Adjuvants* may be included in vaccine formulations or co-administered with vaccines to enhance the immune responses to particular antigen(s), or to target a particular immune response. *It is important that the adjuvants used comply with pharmacopeial requirements where they exist, and that they do not cause unacceptable toxicity.* Adjuvant activity is a result of many factors and the immune response obtained with one particular antigen/adjuvant formulation cannot, as a rule, be extrapolated to another antigen. Individual antigens vary in their physical and biological properties and antigens may interact differently with an adjuvant. Adjuvants must be chosen according to the type of immune response desired and *they must be formulated with the antigen in such a way that distribution of both is optimized to ensure availability to the relevant lymphatic tissues* [p. 51]. *The effect of the adjuvant should be demonstrated in preclinical immunogenicity studies. If no toxicological data exist for a new adjuvant, toxicity studies of the adjuvant alone should first be performed.* In general, *assessment of new or novel adjuvants should be undertaken as required for new chemical entity* [p. 52].

The definition of "novel" adjuvant is given in another WHO document (WHO Expert Committee on Biological Standardization – Sixty-fourth report WHO TRS N°987: 2013 − Technical Report Series 987 − Annex 2 − *Guidelines on the nonclinical evaluation of vaccine adjuvants and adjuvanted vaccines*):

> Successful preclinical evaluation of adjuvanted vaccines, including physicochemical characterization, proof-of-concept testing in animals, and toxicity testing, is an important step towards their clinical development. In addition, studies in animals are valuable tools to help select a safe dose, schedule and route of administration, and to *identify unexpected or potential adverse effects* for specific monitoring in

> clinical trials. Safety concerns include *potential inherent toxicities of the vaccine antigen and/or adjuvant*, potential toxicities of any impurities and contaminants, and *potential toxicities due to interactions of the components present in the final formulation*. The regulatory considerations for adjuvanted vaccine are similar to those for vaccines in general, with additional issues being considered that are *unique to novel adjuvants*. For the purposes of these WHO Guidelines, *a novel adjuvant is defined as an adjuvant that has not been included in a licensed vaccine* [p. 64].
>
> *Adjuvanted vaccine*: the complete formulation that includes one or more antigens, *an adjuvant(s)*, and any additives (which may include, for example, excipients or preservatives), the administration of which is *intended to stimulate the immune system* to result in *an immune response that leads to the prevention or treatment of an infection or infectious disease* [p. 65].
>
> *Novel adjuvant*: a novel adjuvant is an adjuvant that has *not been contained in a licensed vaccine* [p. 66].
>
> *Vaccine adjuvants*: substances or combinations of substances that are used in conjunction with a vaccine antigen to enhance (e.g. increase, accelerate, prolong and/or possibly target) or modulate to a different type (e.g. switch a Th1 immune response to a Th2 response or a humoral response to a cytotoxic T-cell response) the specific immune response to the vaccine antigen in order to enhance the clinical effectiveness of the vaccine. […] The term "adjuvant" is used throughout the document to *include adjuvants that exist as one individual substance as well as combination adjuvants that consist of multiple adjuvants and sometimes other additives* [p. 67].

And, with regard to Genotoxicity studies, the same document says:

> *Genotoxicity studies* are normally not needed for the final vaccine formulation. However, *a standard battery of genotoxicity studies is generally recommended for most novel adjuvants that are (or contain) new chemical entities*.
> Toxicity studies of adjuvant alone: […] Comprehensive toxicity assessment of the adjuvant alone in animals may be included as part of the study design with the adjuvanted vaccine. However, *evaluation of the adjuvant alone can be important for novel adjuvants that have not been studied previously* or will be used in multiple different vaccine formulations. In the case of a *novel adjuvant* or *combination adjuvant*, it may be advisable to include additional (lower and higher) doses of the adjuvant component(s) in order to identify a safe dose that could be used in a first-in-human clinical trial, as well as safety signals that should be monitored in the proposed clinical trial.
> Although not usually required, *safety pharmacology studies may be recommended in some cases to demonstrate that a novel adjuvant has no adverse effects on physiological functions* (e.g. on the *central nervous system*, or the *respiratory* or *cardiovascular system*, *renal function*, and *body temperature*). If needed, such evaluations could also be included as a specific arm with the adjuvant alone in the repeated-dose toxicity study of the intended final vaccine formulation. It is expected that *these studies would be conducted before initiating first-in-human clinical trials* [p. 85].

With regard to regular pharmacokinetic studies, Section 4.2.6 of the WHO 2005 Guideline says:

> *Pharmacokinetic studies* (e.g. for determining serum or tissue concentrations of vaccine components) are normally not needed. *The need for specific studies should be considered on a case-by-case basis (e.g. when using novel adjuvants* or alternative routes of administration*)* and may include local deposition studies that would assess the retention of the vaccine component at the site of injection and its further distribution (e.g. to the draining lymph nodes). *Distribution studies should be considered in the case of new formulations, novel adjuvants* or when alternative routes of administration are intended to be used (e.g. oral or intranasal) [p. 51].

Nevertheless, the EMA Assessment report on Comirnaty dated 19 February 2021, on page 55, asserts (imprudently as we will see below) that there are *no toxicological data on LNPs alone* or on its novel excipients (ALC-0315 and ALC-0159), which in any case are not to be considered "adjuvant substances" per se (Figure 5).

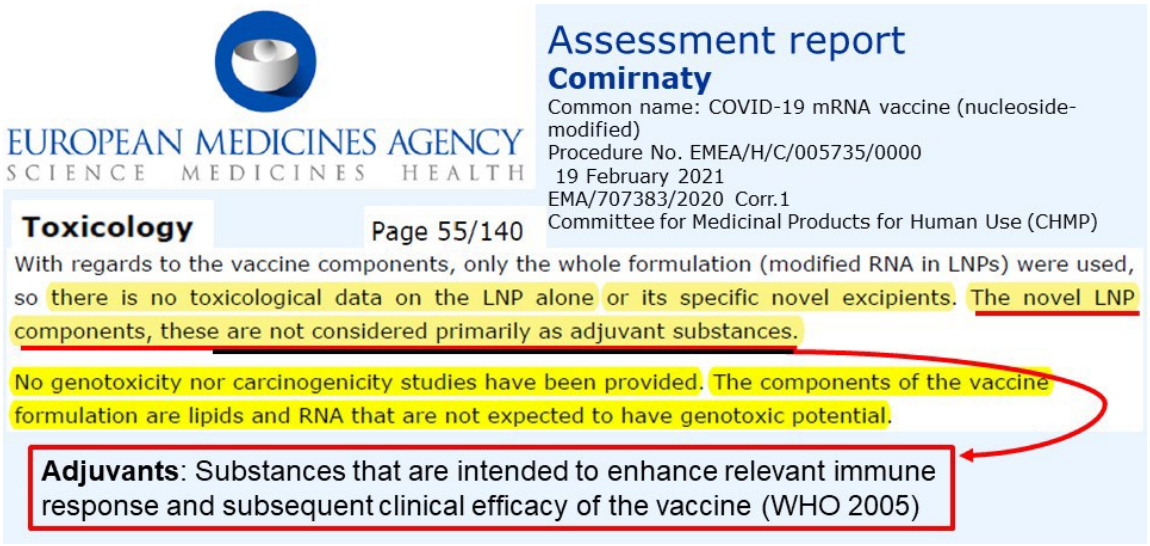


Figure 5. EMA Assessment report on Comirnaty, dated 19 February 2021, page 55, asserting that LNPs alone or its specific novel excipients are not considered primarily as adjuvant substances.

In stark contradiction to the above, just few pages earlier, the document declares that *it cannot be excluded* [*sic* that] *the LNP composition contributes to the overall immunogenicity* (Figure 6). In other words, for EMA, on page 55, section Toxicology, of its Assessment, LNPs are NOT to be considered adjuvants, but on page 42, section Pharmacology, it assumes that their adjuvanticity cannot be excluded.

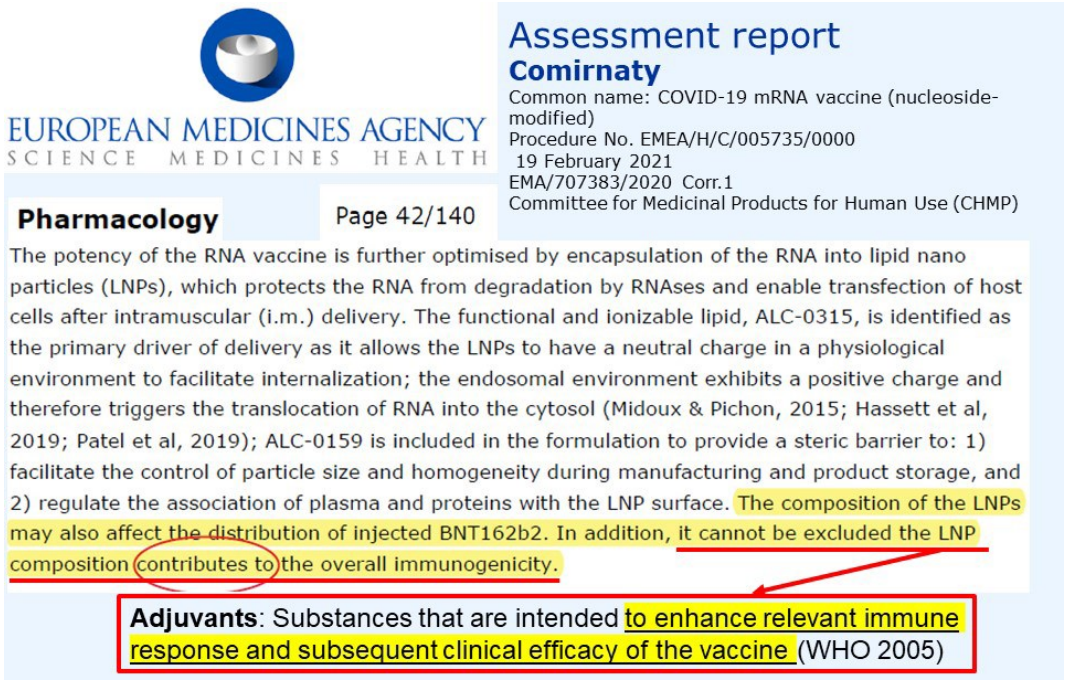
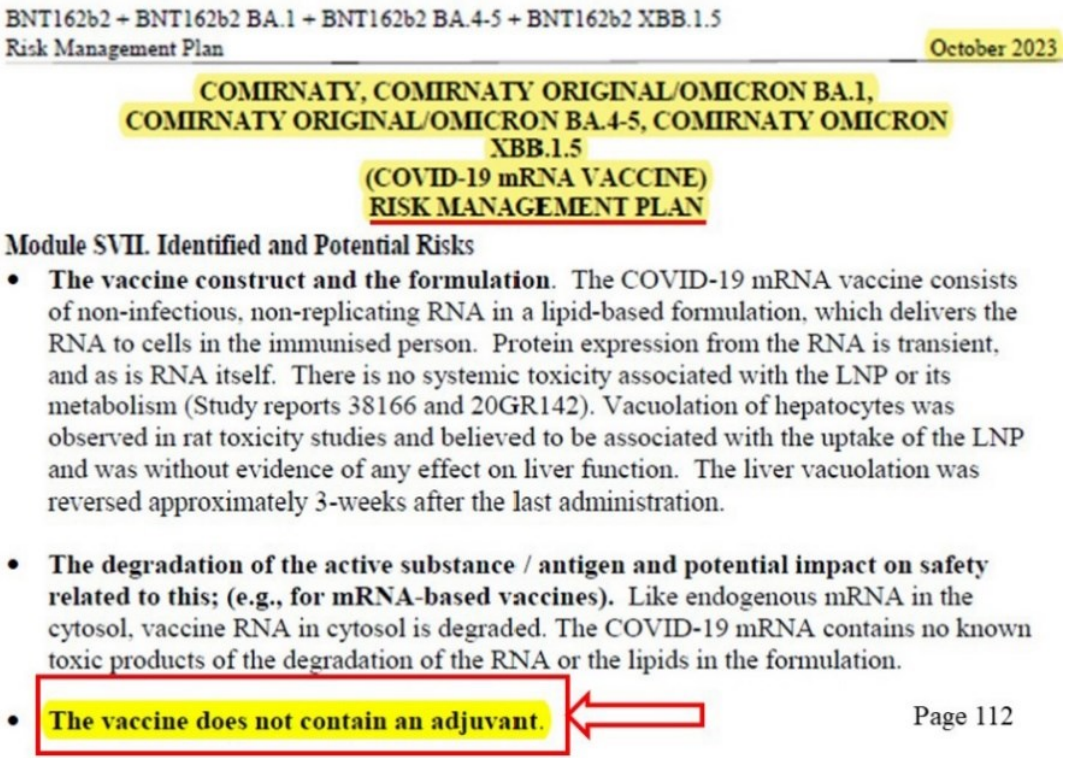


Figure 6. EMA Assessment report on Comirnaty, dated 19 February 2021, page 42, asserting that LNPs could theoretically express immunogenic adjuvanticity.

LNPs: The Adjuvant Syllogism

Section *Identified and Potential Risks* of the Risk Management Plan regarding Pfizer/BioNTech's Comirnaty vaccine peremptorily reads: "The vaccine does not contain an adjuvant" (Figure 7)



BNT162b2 + BNT162b2 BA.1 + BNT162b2 BA.4-5 + BNT162b2 XBB.1.5
Risk Management Plan October 2023

COMIRNATY, COMIRNATY ORIGINAL/OMICRON BA.1, COMIRNATY ORIGINAL/OMICRON BA.4-5, COMIRNATY OMICRON XBB.1.5
(COVID-19 mRNA VACCINE)
RISK MANAGEMENT PLAN

Module SVII. Identified and Potential Risks

- **The vaccine construct and the formulation**. The COVID-19 mRNA vaccine consists of non-infectious, non-replicating RNA in a lipid-based formulation, which delivers the RNA to cells in the immunised person. Protein expression from the RNA is transient, and as is RNA itself. There is no systemic toxicity associated with the LNP or its metabolism (Study reports 38166 and 20GR142). Vacuolation of hepatocytes was observed in rat toxicity studies and believed to be associated with the uptake of the LNP and was without evidence of any effect on liver function. The liver vacuolation was reversed approximately 3-weeks after the last administration.

- **The degradation of the active substance / antigen and potential impact on safety related to this; (e.g., for mRNA-based vaccines).** Like endogenous mRNA in the cytosol, vaccine RNA in cytosol is degraded. The COVID-19 mRNA contains no known toxic products of the degradation of the RNA or the lipids in the formulation.

- **The vaccine does not contain an adjuvant.**

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Figure 7. Pfizer/BioNTech's Comirnaty Risk Management Plan, version 11.0, page 112, October 2023

It is evident that such a statement represents the basic motivation and justification for not having implemented genotoxicity and carcinogenicity tests, in accordance with the policy indicated by the WHO 2005 Guideline. In other words, by stating in an official document, endorsed by the highest European regulatory authority on medicinal products, that the Pfizer/BioNTech Comirnaty vaccine does not contain *any* adjuvant, the basic toxicological syllogism appears evident and incontestable in all its parts:

(1) given that the 2005 WHO Guideline requires genotoxicity and carcinogenicity studies *only* if the vaccine contains *novel* adjuvants among its components, and (2) given that the Comirnaty mRNA vaccine *does not contain any* adjuvants, consequently (3) it follows that those studies are not to be considered necessary for the Comirnaty mRNA vaccine.

This entire syllogistic structure, however, collapses once the scientific unreliability and groundlessness of the above-mentioned premise (2) are carefully evaluated and demonstrated in a review of what adjuvants are and how they work.

Nanoparticle Adjuvants — Immunological Mechanisms

An extensive number of studies have reported Nanoparticles (NPs) as adjuvants. In many of these studies, Nanoparticles induced similar or higher immune responses than aluminum containing adjuvants such as Alum (Aluminum hydroxide). It is suggested that NPs can enhance antigen uptake and/or stimulate antigen-presenting cells (APC), such as dendritic cells (DC). Thanks to their peculiar physicochemical properties, such as particle size, small NPs may travel more readily through the lymphatics and accumulate in resident DCs of lymph nodes. Inherently, NPs used in vaccine formulations tend to be comparable in size to pathogens recognized by the immune system. Surface charge also plays a significant role in the induction of immune response. The uptake of cationic NPs by APC is higher because of electrostatic interactions with anionic cell membranes. To increase the persistence of NPs in the body, the surface of NPs can be modified by inclusion of hydrophilic polymers such as PEG. The primary benefit in preparing PEG-functionalized particles has been reported to improve the long-term systemic bioavailability of the NPs (Zaman et al., 2013).

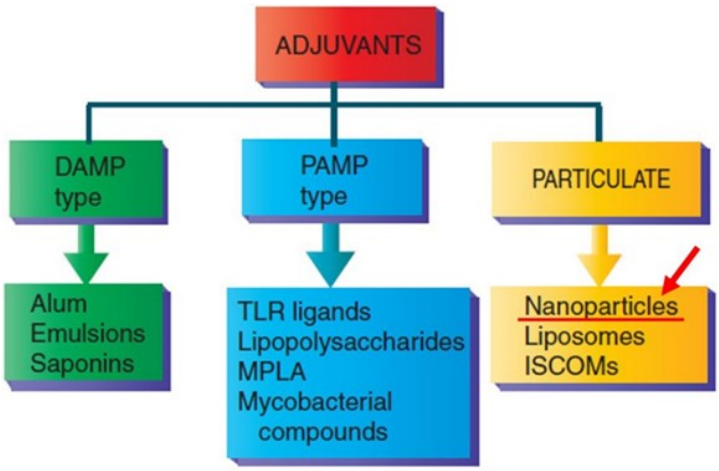


Figure 8. A classification of the different types of adjuvants. DAMPs, damage-associated molecular patterns; PAMPs, pathogen-associated molecular patterns; TLR, Toll-like receptor; MPLA, Monophosphoryl Lipid A; ISCOMs, Immune stimulating complexes. Reprinted from *Vaccines for Veterinarians*, 75-86, Tizard, 2020, Chapter 7 - *Adjuvants and adjuvanticity*, page 77. Copyright © 2021 Elsevier Inc.

A classification of the different types of adjuvants is reported in Figure 8. Each of the three major types relies on stimulation of innate immunity and the resulting enhancement of the antigen-processing step in adaptive immunity. The third category includes all the particulate type of adjuvants derived from Nanotechnology, which uses particles with an overall size range of 1 to 1000 nanometers. These nanoparticles, nanoemulsions, or nanofibers can be used as adjuvants to promote responses to vaccines. They mimic viruses and bacteria in terms of size and structure. They can also encapsulate and so protect antigens from premature degradation. Particles less than 1μm in diameter are ingested by pinocytosis; particles less than 120 nm are ingested by endocytosis. Nanoparticles under 500 nm in size traffic rapidly to draining lymph nodes whereas larger particles are retained at the injection site and are phagocytosed and carried to lymph nodes by antigen-presenting cells (APCs).

The chemistry and surface charge of the particles also affect responses. Nanoparticles have unique immunological properties that can be manipulated by altering their size, shape, charge, and hydrophobicity. They can be engineered to display a mixture of antigens and co-stimulating molecules on their surface so that the immune response is optimized (Tizard, 2020; and see Figure 9).

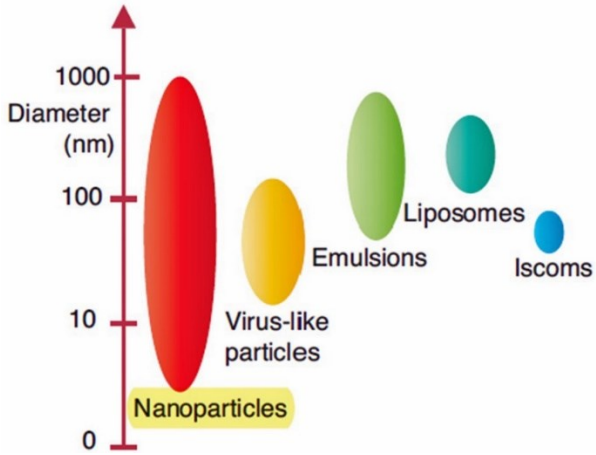


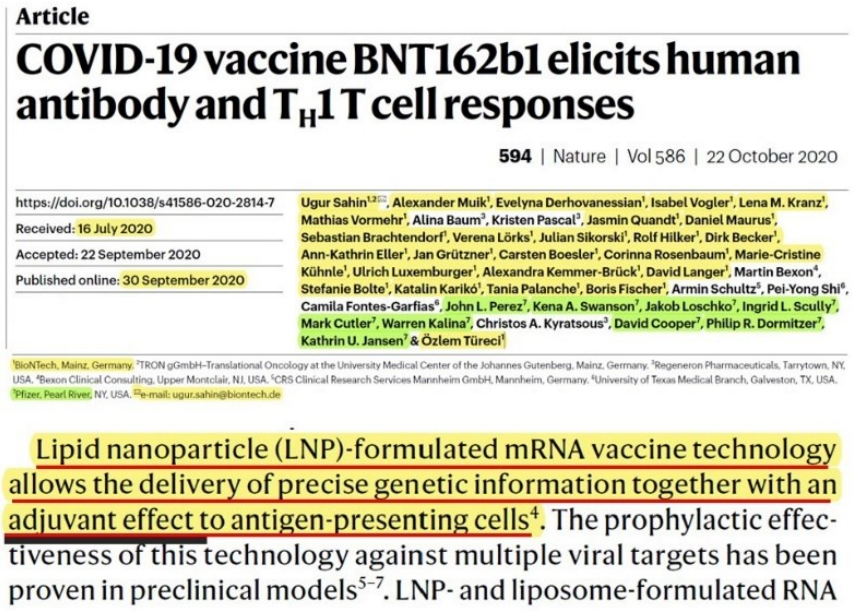
Figure 9. Nanoparticle adjuvants come in many different sizes and this profoundly influences the nature of the immune response they induce. Reprinted from *Vaccines for Veterinarians*, 75-86, Tizard, 2020, Chapter 7 — *Adjuvants and adjuvanticity*, page 84. Copyright © 2021 Elsevier Inc.

An article published in *Nature* on October 22, 2020, by 42 authors, 26 of whom were from BioNTech (including Katalin Karikó, Nobel Prize in Medicine 2023) and 9 from Pfizer (Figure 10) claims the following: "Lipidnanoparticle (LNP)-formulated mRNA vaccine technology allows the delivery of precise genetic information together with *an adjuvant effect* to antigen-presenting cells" (Sahin et al., 2020).

In their study *Innate immune mechanisms of mRNA vaccines*, Rein Verbeke et al. consider the contribution of both the mRNA and the LNP components to the immunogenicity of mRNA vaccines. The ionizable LNP (iLNP) carrier is thought to be the primary driver of the adjuvanticity and reactogenicity of mRNA-LNP vaccines. Thus, the iLNP technology not only enables the efficient delivery of mRNA into innate immune cells following vaccine administration, but also seems to play a second key role in providing a *potent adjuvant* activity to this vaccine platform. This is demonstrated by the fact that uptake of *empty* LNPs (i.e. lipidnanoparticles *not* containing any antigen or mRNA) by innate immune cells and other cell types is sufficient to induce local and systemic inflammation, characterized by the release of pro-inflammatory cytokines such as IL-1b and IL-6 (Verbeke et al., 2022).
Empty iLNPs were successfully used to adjuvant vaccines containing hepatitis B virus or dengue virus protein antigens. Remarkably, reduction in cationic lipid content of the nanoparticle dramatically reduces the LNP's ability to boost the Dengue virus specific immune response (Swaminathan et al., 2016a, 2016b).

In another study by Siri Tahtinen and 19 other authors, including BioNTech's Ugur Sahin, about the inflammatory response to mRNA vaccines, we read that RNA vaccines induce production of IL-1 cytokines, predominantly IL-1β, which is dependent on both the RNA and *lipid* formulation. RNA vaccines against COVID-19 (mRNA-1273 by Moderna and BNT162b2 by BioNTech/Pfizer) —

which use modRNA with a greatly reduced innate immunostimulatory activity — still elicit systemic adverse events in patients following initial intramuscular administration. These vaccines are formulated in LNPs which contain ionizable lipids to provide structural stability and presumably enable endosomal escape. While LNPs have been observed to have *intrinsic adjuvant activity*, demonstrated by the impressive antibody and T cell responses following vaccination, the exact mechanism by which such vaccines elicit innate immunity has not been previously characterized (Tahtinen et al., 2022).



Article

COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses

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Lipid nanoparticle (LNP)-formulated mRNA vaccine technology allows the delivery of precise genetic information together with an adjuvant effect to antigen-presenting cells[4]. The prophylactic effectiveness of this technology against multiple viral targets has been proven in preclinical models[5–7]. LNP- and liposome-formulated RNA

Figure 10. Article published in Nature, page 594, on October 22, 2020, by 42 authors, 26 of which from BioNTech (including Katalin Karikó, Nobel Prize in Medicine 2023) and 9 from Pfizer.

It has been demonstrated that empty iLNPs or iLNPs containing a non-coding RNA stimulate robust production of the same set of immune mediators in the skin of intradermally vaccinated mice, as well as a vigorous immune cell infiltrate in injected tissues; removal of the ionizable lipid component from the LNPs abolished visible skin inflammation, which clearly shows that LNPs promote robust inflammation responses at the injection site, and that such adjuvant activity primarily depends on the ionizable cationic lipid, i.e. ALC-0315 used by Pfizer/BioNTech. This could explain the potent adjuvant activity of LNPs and their superiority, compared with other adjuvants, in supporting the induction of adaptive immune responses (Ndeupen, 2021).

Alameh and 33 other authors, including BioNTech's Katalin Karikó, and Prof. Drew Weissman, both Nobel Prize winners in Medicine, in their study *Lipidnanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses*, describe how the adjuvant activity of LNPs relies on their ionizable lipid component. This study identified LNPs as a *versatile adjuvant* that enhances the efficacy of traditional and next-generation vaccine platforms; LNPs possess *strong adjuvant activity* and enhance the efficacy of protein subunit vaccines; experiments provided proof of concept that the LNP formulation used in this study has *intrinsic adjuvant property*, and is at least partially

responsible for the potency of nucleoside-modified mRNA-LNP vaccines; the ionizable lipid (such as, for instance, the ALC-0315 of Comirnaty) is responsible for the adjuvant activity of the LNP formulation and its presence is *a critical parameter for providing adjuvant activity* to LNPs (Alameh et al., 2021).

Conclusion and Outlook

Adjuvants in the COVID-19 injections are likely causes of cardiovascular disorders (Kanuri and Sirrkay, 2024) and other sequelae.

This review shows that the Comirnaty COVID-19 mRNA BNT162b2 vaccine has been evaluated, authorized, distributed, accepted worldwide, and injected into hundreds of millions of recipients on the basis of the manifest falsehood that it *does not contain novel adjuvants* (Figures 5, 7). That falsehood enabled the manufacturers to forego expensive and time-consuming safety pharmacology, genotoxicity, and carcinogenicity tests (Figure 4). It also led directly to the false conclusion that the components of the product are therefore safe and effective (Figures 3).

This self-confirming operation amounts to a sophisticated deception. It was played out in legal language, technical scientific jargon, cleverly omissive statements withholding information supposedly to protect commercial interests. All of it was predicated on appeal to authority rather than experimental facts. Could it have had some purpose other than to get ordinary people to believe in a product with no basis in any demonstrable reality? There was no effort to engage in identification, evaluation, and prioritization of risks involved in the worldwide distribution of this experimental product. The fact that it contained hazardous novel adjuvants (LNPs) was artfully camouflaged.

All the foregoing took place in violation of the requisite pharmacological studies to demonstrate that a *novel adjuvant* has no adverse effects on physiological functions (e.g., on the central nervous system, or the respiratory, or cardiovascular system, renal functions, body temperature homeostasis, etc.) which are all supposed to take place *before* initiating even the first-in-human clinical trials (WHO, 2013).

Paradoxically, there are thus incompatible interpretations of what LNPs are and more specifically of whether or not they contain — or in effect actually, are themselves — potentially dangerous, and untested, adjuvants:

> (1) According to real experts in the field, LNPs have long been known for their significant, intrinsic, robust *adjuvant* activity, and, therefore, should have been submitted to appropriate toxicological studies *before* initiating first-in-human clinical trials, as requested for *novel adjuvants*.
>
> (2) For risk managers and regulatory authorities involved in the compilation, evaluation and approval of the Management Risk Plans, LNPs contained in the Pfizer/BioNTech mRNA product were *not* to be considered *adjuvants*, and therefore toxicological studies were not deemed necessary.

Unfortunately, the inevitable result now at hand, appears to be the worst medical deception in history.

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