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Hypothesis

Regulatory Assessment Gaps and Genotoxicity Considerations in mRNA-LNP Technologies

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Abstract

Current FDA and ICH genotoxicity requirements allow pharmaceutical developers to meet safety obligations using legacy assays designed for small, cell permeable chemicals, resulting in the use of bacterial reverse-mutation tests (Ames) and in vitro micronucleus assays in human peripheral blood mononuclear cells (PBMCs) to evaluate the presumed non genotoxicity of lipid nanoparticle (LNP) based platforms, including ionizable lipids used in mRNA vaccines. However, these assays have the potential to be structurally and mechanistically incapable of detecting relevant risks. Ionizable lipids and fully formulated LNPs exceed the size exclusion limits of bacterial porins in *Escherichia coli* and *Salmonella typhimurium*, preventing intracellular entry and interaction with genomic DNA; therefore, negative Ames results do not rule out mutagenic potential. Similarly, in vitro micronucleus assays reported as negative for COVID-19 mRNA vaccines used non activated PBMCs, which largely consist of lymphocytes that do not efficiently endocytose LNPs or free ionizable lipids due to size, charge, and absence of uptake pathways; as a result, the negative findings reflect absent intracellular exposure rather than absence of risk. Ionizable lipids can form covalent adducts with chemically modified RNA in vitro and may likewise bind residual linearized plasmid DNA contaminants present in mRNA vaccine preparations and potentially with human nucleic acids, a high-risk concern that has not been tested in vivo. If plasmids reach the nucleus, they could integrate into genomic DNA, while cytosolic plasmids may activate cGAS-STING signaling. Free or LNP associated ionizable lipids can electrostatically interact with nucleic acids once intracellular, further increasing the plausibility of mutagenic or immunomodulatory effects. Because existing genotoxicity assays do not deliver ionizable lipids or LNPs into relevant human cell compartments, their negative results cannot be considered evidence of safety, yet the FDA and other relevant agencies have not addressed this concern. Updated testing frameworks must use formulated LNPs in human cell models that support endocytosis, endosomal escape, nuclear access, and analytical detection of covalent adducts using mass spectrometry, reverse-phase ion-pair HPLC, additional genomics testing, and downstream microRNA and proteomic analyses to accurately evaluate genomic and immunological risk.

Keywords: mRNA vaccines; lipid nanoparticles; genotoxicity; DNA adducts; linearized plasmid DNA; regulatory oversight

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Introduction

Messenger RNA (mRNA) therapeutics formulated in lipid nanoparticles (LNPs), including the widely deployed COVID-19 vaccines developed by Moderna and Pfizer, represent a novel drug modality with distinct physicochemical and biological properties that warrant rigorous genotoxicity assessment [1]. These formulations encapsulate chemically modified RNA within ionizable lipids and other classified products as excipients to enable cellular delivery and expression. The structural complexity of LNPs and the potential presence of linearized plasmid DNA contaminants could create exposure and reactivity pathways that differ fundamentally from those of small-molecule drugs and traditional attenuated vaccines [2,3]. Consequently, existing genotoxicity paradigms designed for

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small molecules and attenuated vaccines are not appropriate for nanoparticle encapsulated nucleic acids.

Regulatory frameworks like ICH M7 and OECD test guidelines 471 and 487 set expectations for mutagenicity testing of drug products and impurities, yet they do not explicitly address nanoparticle vehicles or nucleic acid payloads [4,5]. Conventional bacterial assays, including the Ames bacterial reverse mutation test, and in vitro micronucleus assays using isolated human peripheral blood mononuclear cells (PBMCs) were developed on assumptions of passive diffusion and small molecule bioavailability [6,7]. The ionizable lipids and assembled LNPs exceed bacterial porin size limits and cannot meaningfully access bacterial genomes. Furthermore, the unformulated ionizable lipids that are contained within the LNP the modified RNA LNP designed COVID vaccines, and other RNA LNP platforms do not reliably penetrate resting PBMCs. As a result, negative findings in these standard assays including pharmaceutical company submitted Ames and PBMC micronucleus results may reflect model inapplicability rather than true absence of intracellular or nuclear reactivity. These limitations may render any reported genotoxicity results false.

This work demonstrates the physical and biophysical exclusion of LNPs and ionizable lipids from bacterial cells and characterizes their uptake profiles in PBMCs. It reviews genotoxicity testing reported by manufacturers and regulators, evaluates mechanistic risks like covalent adduct formation with modified RNA and potential attachment to linearized plasmid DNA contaminants, and highlights the presence of linearized DNA plasmid impurities within the modified RNA and LNP manufacturing process. Finally, it proposes more relevant assays and analytical endpoints to assess the genotoxic and immunomodulatory potential of formulated LNP therapeutics.

2. Physicochemical Constraints and Exposure Relevance

2.1. Bacterial Porins versus LNP Dimensions

Gram-negative bacterial porins, including OmpF and OmpC in *Escherichia coli* and homologous channels in *Salmonella typhimurium*, have effective pore diameters ranging from approximately 0.8 to 1.4 nm [8]. Assembled lipid nanoparticles (LNPs) measure roughly 50–200 nm in diameter [9]. Individual ionizable lipid molecules, like those used in the Moderna modified RNA COVID vaccine platform--SM-102 (approximately 710 Da) and ALC-0315 (approximately 766 Da)--exist primarily incorporated within the nanoparticle core. Some free-floating lipids may exist due to incomplete assembly or aggregation. These lipids are not freely diffusible under physiological conditions [10–13]. The substantial size disparity between bacterial porins and LNPs indicates that intact nanoparticles cannot traverse these channels. These structural conditions preclude direct exposure of bacterial genomic DNA to formulated LNPs, rendering such tests biologically irrelevant.

2.2. Free Lipid versus Formulated Behavior

Ionizable lipids in isolation, like SM-102 or ALC-0315, display physicochemical properties distinct from those when incorporated into LNPs, including altered charge states, aggregation behavior, and pH-dependent ionization in endosomal environments [14,15]. Regulatory genotoxicity assessments performed using free lipids do not recapitulate the complex LNP microenvironment, which includes ionic strength, impurity sequestration, and covalent adduct formations with encapsulated nucleic acids. These dynamics limit the interpretability of such assays for evaluating formulated product safety.

3. Cellular Uptake and PBMC Relevance

3.1. PBMC Cell Types and Uptake Capacity

PBMCs comprise a heterogeneous population of immune cells, including T lymphocytes, B lymphocytes, natural killer (NK) cells, and monocytes--each with distinct endocytic and metabolic capacities [17,18]. Resting lymphocytes, like naive T and B cells, exhibit minimal endocytosis and are

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largely refractory to uptake of extracellular macromolecules--whereas activated lymphocytes and monocytes/macrophages display higher endocytic activity. These characteristics allow the internalization of nanoparticles or lipid-nucleic acid complexes under appropriate conditions [19–21]. Experimental evidence using structurally diverse ionizable lipids demonstrates that lipid-RNA complexes can achieve measurable transfection in human PBMC cultures. In GFP reporter screens, certain lipids yielded detectable GFP expression primarily in monocytes and dendritic cell subsets, with transfection efficiencies ranging from a few percent to approximately 15-20 percent under optimized conditions, whereas resting T and B cells showed negligible uptake [22–24]. These observations highlight that cellular uptake is both cell type and activation dependent, driven by endocytic capacity, lipid ionization state, and nanoparticle surface characteristics.

The implications for genotoxicity testing are substantial. Standard in vitro micronucleus assays using non-activated PBMCs exposed to free ionizable lipids fail to replicate the physiological conditions required for intracellular delivery of LNP-associated nucleic acids, including endocytosis, endosomal escape, and potential nuclear access [25–27]. Consequently, negative results from such assays reflect a lack of cellular exposure rather than evidence of safety, as the compounds cannot efficiently reach intracellular compartments where covalent adduct formation or nucleic acid interactions may occur.

3.2. Implication for Micronucleus and Other PBMC In Vitro Assays

Gram-negative bacterial porins, including OmpF, OmpC, and their Salmonella homologs, possess effective pore diameters ranging approximately from 0.8 to 1.4 nanometers, forming selective aqueous channels that allow diffusion of small molecules, ions, and metabolites but exclude macromolecular complexes [28,29]. In contrast, fully assembled lipid nanoparticles formulated for mRNA delivery measure roughly 50 to 200 nanometers in diameter, with individual ionizable lipid molecules, like SM-102 (approximately 710 Da) or ALC-0315 (approximately 766 Da), sequestered within the nanoparticle structure rather than existing freely in solution [10]. The dimensional disparity between bacterial porins and intact LNPs exceeds two orders of magnitude, rendering LNPs physically incapable of translocating through these channels and preventing any direct interaction with bacterial genomic DNA. As a result, conventional bacterial genotoxicity assays like the Ames test are structurally incapable of evaluating mutagenic potential for these formulations, because the core substrate cannot access the intracellular target.

Furthermore, the behavior of ionizable lipids outside of LNP assemblies differs substantially from their properties within the nanoparticle. Free SM-102 or ALC-0315 in solution exists in distinct protonation states, with variable aggregation and pH-dependent ionization, whereas encapsulation within LNPs stabilizes the lipid in a defined microenvironment that favors complexation with nucleic acids and shields reactive groups from solvent exposure [14,30,31]. Regulatory tests performed using free lipid, rather than fully formulated LNPs, fail to replicate this microenvironment, including the effects of ionic strength, impurity sequestration, potential covalent adduct formation between lipids and mRNA [2]. Although not qualified in vivo, the potential for the impurities in the ionizable lipids to induce covalent bonds with human nucleic acids. Another concern is the presence of linearized DNA plasmid contamination inside the LNP and potentially free floating in solution [3]. These studies mechanistically cannot test for such contamination. As a result, the reported results may be false--not based on proper testing, but on improper testing included in FDA submissions. This disparity highlights a critical limitation: negative results obtained with free lipids or other free components like free modified RNA--which do not represent the drug product—do not reflect the biological exposure or mechanistic interactions occurring in vivo. In vivo, ionizable lipids and LNPs interact with nucleic acids, endosomal membranes, and cellular uptake pathways that determine intracellular localization. Such physicochemical constraints must be considered when interpreting standard genotoxicity assays, as they do not model the relevant size, charge, or compartmentalization that define the exposure and risk profile of LNP based therapeutics.

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4. What Companies Tested

4.1. Moderna

The EMA assessment report lists three genotoxicity studies performed in support of the platform: a bacterial reverse mutation assay using SM-102 in Salmonella typhimurium and Escherichia coli, an in vitro micronucleus assay in human peripheral blood lymphocytes using isolated SM-102, and an in vivo erythrocyte micronucleus test in rats using a Zika mRNA construct formulated in lipid nanoparticles [32]. These studies are described in the EMA dossier rather than in U.S. submissions [32–36]. Their relevance is limited because the assays were not conducted on the final mRNA LNP vaccine product but on the free lipid component or a different mRNA LNP formulation [33]. This distinction matters because lipid behavior, cellular uptake, and nucleic acid interactions are governed by the assembled nanoparticle context rather than by isolated lipid molecules.

The EMA's own wording confirms this separation between the lipid and the full formulation. For example, the dossier explicitly names the "SM-102 bacterial reverse mutation test in Salmonella typhimurium and Escherichia coli," confirming that only the standalone lipid was assessed [32]. That testing framework does not model endocytosis, compartmental delivery, linearized DNA plasmid contamination impacts, or potential nucleic acid adduct formation that could occur when SM-102 is part of the LNP architecture housing mRNA and trace plasmid DNA [2,3,32,37].

4.2. U.S. FDA and Other Regulators

FDA summary documents available for the authorized Moderna product do not describe the Zika mRNA in vivo erythrocyte micronucleus assay referenced in EMA materials [33–35]. The omission is significant because any in vivo genotoxicity observations generated outside the U.S. dossier were not incorporated into FDA's determination of non-mutagenicity. Instead, the FDA review relies on statements like "No genotoxicity studies were conducted for the finished product" and platform-based reasoning that ribonucleotides are not expected to be mutagenic [3,32,38]. In some cases, negative Ames and PBMC micronucleus assays performed on the novel lipid are cited, but again, these are not assays conducted on the full LNP [32–36]. This matters because a negative result using free ionizable lipid dispersed in solvent does not replicate intracellular conditions, nanoparticle charge shifting, or interactions with modified RNA or plasmid impurities [2]. The absence of formulated testing means the regulatory conclusion is built on legacy small-molecule assumptions rather than evidence of how LNPs behave in human cells.

4.3. Pfizer/BioNTech Approach

Public regulatory summaries for Pfizer/BioNTech indicate that genotoxicity assessments centered on the novel ionizable lipid ALC-0315, using either in vitro bacterial reverse-mutation assays or computational (QSAR) read-across [38,39]. The documentation does not show direct testing of the fully formulated LNP. Instead, the justification relies on prior platform data or chemical structure arguments applied to the lipid in isolation. In at least some regulatory filings, the company asserts that no genotoxicity testing on the mRNA lipid nanoparticle was warranted because the components were presumed non-mutagenic [32–36,38,39].

This gap is important because lipid RNA interactions, nanoparticle size, and intracellular routing are properties that emerge only in the formulated product. Without testing the intact LNP in a human cell system that supports uptake and nuclear or cytosolic delivery, negative findings in bacterial or PBMC assays lack exposure relevance and cannot exclude the potential for DNA interaction, adduct formation, proteomic concerns, genotoxic impact, or innate immune signaling.

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4.4. Older or Foreign Platform Constructs Not Included in U.S. Submissions

The Zika mRNA LNP micronucleus study included in the EMA packet does not appear in the U.S. FDA review materials for the authorized COVID-19 vaccine. Public FDA documents do not reference or analyze those data [32–39]. The fact that this study was generated for a related lipid–mRNA nanoparticle but excluded from FDA's genotoxicity evaluation is critical. It shows that the agency formed safety conclusions without integrating in vivo results that could have illuminated potential nuclear or cytosolic consequences of LNP delivery.

The regulatory disconnect underscores that negative conclusions are not based on direct evidence from the final product or even on all platform data available elsewhere. Without inclusion and critical evaluation of those findings, there is no continuity of evidence demonstrating that LNP associated risks were systematically ruled out across formulations or jurisdictions.

5. Evidence That Reactive Impurities in Ionizable Lipids Form Adducts

Packer et al. demonstrated that electrophilic impurities generated during synthesis, storage of ionizable lipids, and with increases in temperature can react covalently with mRNA molecules encapsulated within lipid nanoparticles [2]. Using reversed-phase ion-pair high-performance liquid chromatography (RP-IP HPLC), they quantified these modifications, finding that a measurable fraction of mRNA molecules formed lipid-derived adducts under the experimental conditions reported, with frequency of these adducts forming between the cationic ionizable lipids and the modified RNA with increase in temperature; the top temperature reported being close to human body temperature [2]. The tertiary amine moieties of ionizable lipids are prone to oxidation or hydrolysis, forming reactive electrophilic species that undergo nucleophilic attack on nucleobases or ribose residues of mRNA, resulting in stable covalent modifications [2]. Covalent modifications go beyond just electrostatic interactions. These findings establish that the ionizable lipid chemistry within the LNP microenvironment can generate reactive species capable of directly modifying nucleic acids.

The mechanistic relevance of these adducts extends beyond the modified RNA itself: if similar electrophilic species were to interact with linearized DNA plasmid contaminants, chromosomal DNA, or other endogenous nucleic acids within human cells, there is potential for genomic or epigenomic perturbation. Standard bacterial reverse-mutation assays (Ames tests) are incapable of modeling the in-LNP chemistry, as the nanoparticles cannot penetrate bacterial porins and thus cannot expose DNA to the reactive species [40–42]. Likewise, in vitro micronucleus assays performed on non-activated PBMCs with free lipid fail to replicate intracellular LNP chemistry, including endocytosis, endosomal release, and nucleic acid modification, and therefore would miss these covalent interactions entirely [43,44]. These observations underscore the need for assays that account for the formulated LNP environment and intracellular compartments to evaluate mutagenic or immunomodulatory risks accurately.

6. Linearized DNA Plasmid Contaminants and Potential Intracellular Interactions

Varying amounts of linearized biotech plasmid DNA are present as manufacturing impurities in mRNA vaccine preparations, which represents a mechanistically distinct potential genotoxic hazard [3]. Chemically modified mRNA associated with ionizable lipids may exhibit altered degradation kinetics, not just from the addition of N1-Methylpseudouridine, but also from the electrostatically attached ionizable lipids. These lipids may impede breakdown by ribonucleases [45–47]. Additionally, covalent association between ionizable lipids and modified RNA can reduce translation efficiency [2]. This effect is experimentally supported and may include the possibility of frameshifting, production of truncated proteins, and downstream effects on protein function.

Ionizable lipids may also interact electrostatically with residual linearized plasmid DNA contained in the mRNA COVID vaccines, as the positively charged ionizable lipids can bind to the

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negatively charged DNA plasmid (and other negatively charged nucleic acids or at other bonding sites) [3,47]. The existence of electrostatic interactions between positively charged lipids and negatively charged DNA is chemically plausible and well established in lipid-nucleic acid chemistry [48,49]. If plasmid DNA reaches the nucleus, such interactions could theoretically alter the electrostatic environment of the nuclear core, which is tightly organized with chromatin, nucleosomes, and histones [50,51]. This could influence chromatin compaction, nucleosome positioning, and accessibility to repair machinery. Other interactions may include effects on gene expression. Another concern, beyond the theoretical integration of linearized DNA plasmid into the human genome causing unknown effects, is adduct formation between the cationic ionizable lipids and linearized DNA plasmid fragments if they enter the nucleus. In this case, mutation could be a hypothetical concern via adduction or point mutation alone via the ionizable lipids, without any integration event. This potential interaction raises serious biological concerns if a tumor suppressor gene or an oncogene experiences covalent adduction from ionizable lipid interactions with chromatin [2,51–53]. However, the extent to which lipid-DNA interactions meaningfully perturb nuclear structure in vivo is not directly demonstrated; it remains a mechanistic hypothesis. Lipid-bound plasmid DNA could also resist nuclease degradation, which is again a plausible but untested outcome.

Plasmid DNA that remains in the cytosol after endosomal escape may engage DNA-sensing pathways like cGAS-STING, a well-characterized mechanism for cytosolic DNA [54]. Ionizable lipids could also interact with cytosolic proteins, including ion channels, motor proteins, and nucleic-acid binding proteins [55]. While these interactions are mechanistically plausible, direct evidence in the context of vaccine formulations or intracellular LNP delivery is lacking.

Importantly, standard genotoxicity assays, including bacterial reverse-mutation tests and in vitro PBMC micronucleus assays, cannot capture these mechanisms [32–39]. The LNP mediated delivery of nucleic acids, covalent adduct formation [2], potential genomic disturbances like integration from linearized DNA biotech plasmid contamination [56,57], DNA plasmid exosome formation, or electrostatic perturbation of nuclear and cytosolic proteins occurs in compartments and conditions not modeled by these legacy assays [58]. This highlights the critical gap in experimental evaluation and underscores the need for advanced human-cell systems that can reproduce intracellular LNP uptake, nuclear access, and molecular interaction analysis to accurately assess genotoxic and immunomodulatory risks.

7. Limitations of Conventional Ames and PBMC Assays Relative to ICH M7 Guidance

Current regulatory frameworks, including ICH M7, require that genotoxicity testing meaningfully assess DNA reactive impurities under conditions relevant to patient exposure, encompassing both the chemical form of the test article and the biologically relevant cellular environment [59]. The guideline explicitly states that test systems must allow adequate intracellular exposure to the compound in its intended formulation and that negative results cannot be interpreted as absence of risk if exposure is compromised [60]. In the context of mRNA vaccines formulated in lipid nanoparticles, conventional bacterial reverse-mutation tests, like the Ames assay, and in vitro micronucleus assays using isolated human peripheral blood mononuclear cells fundamentally violate this principle. The test articles in these studies using free ionizable lipids or isolated bacterial systems differ substantially from the formulated LNPs administered to patients. Bacterial cells lack endocytic pathways capable of internalizing LNPs, and the nanoparticle size vastly exceeds the 0.8–1.4 nm pore diameter of Gram-negative porins, preventing any intracellular entry and interaction with genomic DNA [61]. As a result, negative Ames results cannot be interpreted as evidence of absence of mutagenicity, because the test does not truly replicate biologically relevant exposure for the full drug product itself.

Similarly, in vitro PBMC micronucleus assays reported for Moderna and other mRNA vaccines utilized non activated peripheral blood lymphocytes, which exhibit very low endocytosis rates.

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Monocytes and activated antigen-presenting cells within PBMC populations are capable of LNP uptake and endosomal escape, but resting lymphocytes largely fail to internalize either free ionizable lipids or formulated nanoparticles [62]. Therefore, observed negative micronucleus results in non-activated PBMCs reflect a lack of intracellular exposure rather than confirmation of non-genotoxicity. Mechanistically, ionizable lipids can form covalent adducts with chemically modified RNA [2], and similar interactions with residual linearized plasmid DNA are mechanistically plausible. If plasmid DNA enters the nucleus, positively charged lipids entering the nucleus with the linearized biotech DNA plasmid could perturb the electrostatic environment of the nuclear core, altering chromatin compaction, nucleosome positioning, and DNA accessibility to repair machinery [63]. These positively charged lipids could, in theory, interact with chromatin in the nucleus following mechanisms like those tested in vitro by Moderna in the Packer et al. study. Such interactions may allow covalent bonding to occur within chromatin. This could potentially produce mutation events without the linearized biotech DNA plasmid integrating into the human genome, leading to unexpected and unknown negative effects. Lipid association may also hinder enzymatic degradation of nucleic acids. This could increase the persistence of DNA plasmid and modified RNA. Consequently, there is a higher likelihood of unintended interactions. These may include covalent adduct formation with nuclear proteins, covalent bonding with human RNA, and interactions in other tissue compartments. In the cytosol, plasmid DNA can activate cGAS-STING signaling, eliciting innate immune responses independent of nuclear entry [65]. Ionizable lipids may additionally interact electrostatically with cytosolic proteins, including ion channels, motor proteins, other proteins, DEAD box proteins, and nucleic acid-binding proteins, further contributing to potential cellular disruption. None of these mechanistic phenomena are captured in conventional Ames or PBMC assays, which do not deliver the test article into the cellular compartments where these interactions occur.

8. Recommended Approach

Accurate evaluation of the genotoxic and immunomodulatory potential of ionizable lipids and fully formulated LNP therapeutics requires a multi-tiered strategy integrating analytical, cellular, and in vivo methods. Analytical assessment should include RP-IP HPLC on final vaccine lots to quantify lipid: nucleic acid adducts [2], reporting the fraction of adducted mRNA and identifying reactive electrophiles, complemented by mass spectrometry and nuclear magnetic resonance for detailed structural characterization of modifications [66]. In vitro studies should expose clinically relevant human cell types, including resting and activated PBMCs, primary human hepatocytes, dendritic cells, and dividing cell lines, to fully formulated LNPs [43]. Animal studies including sex organs, fertility, and full organ studies in anima should be a part of testing and not replaced with computer models or organoid systems. Endpoints should encompass micronucleus formation, γH2AX and 53BP1 foci, comet assays for DNA strand breaks, DNA mutation studies, transcriptomic profiling including interferon-stimulated genes, cGAS STING activation, and proteomic analyses to detect perturbations of nucleic-acid binding protein interactions, other nucleic acid interactions, and other cellular machinery [67,68] Sensitive PCR and next-generation sequencing assays are recommended to detect rare plasmid integration events following repeated passages [69]. Additional functional assessments, including high-resolution imaging of intracellular trafficking, proteomic mapping of lipid interactions, and microRNA profiling, can provide insight into downstream regulatory consequences of LNP exposure. In vivo studies may be warranted using mammalian tissues, with endpoints including comet and micronucleus assays following exposure to the complete LNP formulation at clinically relevant doses and routes. Comprehensive reporting should reflect fully formulated LNPs rather than isolated components to satisfy ICH M7 requirements for biologically relevant exposure and mechanistic relevance, ensuring that all potential interactions affecting genomic integrity and cellular proteomics are adequately captured.

Discussion

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The current analysis underscores multiple mechanistic and exposure related gaps in the genotoxicity assessment of ionizable lipids and fully formulated LNP therapeutics. Physicochemical constraints, including nanoparticle size and the positive charge of ionizable lipids, prevent meaningful intracellular access in bacterial systems to genetic material and non-activated PBMCs, rendering conventional assays like the Ames test and standard micronucleus assays potentially mechanistically irrelevant. Beyond physical exclusion, reactive electrophilic impurities associated with ionizable lipids can covalently modify mRNA [2]. These alterations could potentially reduce translation efficiency, cause frameshifting, and generate truncated or nonfunctional proteins [2]. These interactions may also affect residual linearized plasmid DNA in COVID vaccines. The plasmid, bound to positively charged lipids, may degrade more slowly. Together, the plasmids and lipids can move to different areas of the cell, including the nucleus. This movement could potentially cause unknown and potentially catastrophic effects. The linearized DNA plasmid, if electrostatically or covalently bound to ionizable lipids, could form adducts with the human genome, cause point mutations, interact with oncogenes or tumor suppressor genes, and result in other genomic consequences. These positively charged lipids, if bound to the DNA plasmid, could also perturb chromatin organization, nucleosome positioning, and histone-DNA electrostatics, with potential consequences for DNA accessibility, repair, and degradation. Cytosolic plasmid retained after endosomal escape may activate innate immune pathways like cGAS-STING, while lipid interactions with micro RNAs, other RNAs, and proteins may influence broader cellular physiology [7].

Regulatory review reveals substantial variation between jurisdictions [32–36,38,39]. EMA documentation explicitly lists testing of isolated lipids (SM-102) using bacterial reverse mutation and PBMC micronucleus assays, and in vivo evaluation of unrelated mRNA constructs, but does not provide evidence that fully formulated LNPs were tested [4,5]. FDA summaries note negative Ames and PBMC micronucleus results and suggest that ribonucleotides are “not expected to be genotoxic”, with reliance on platform data and in silico QSAR modeling. These differences highlight a divergence in regulatory interpretation, with the potential for omission of mechanistically relevant exposure pathways being treated as acceptable, despite ICH M7 guidance emphasizing biologically relevant exposure and adequate assessment of DNA-reactive impurities.

From a policy perspective, the discrepancy between testing methodology and mechanistic risk raises concerns regarding material misrepresentation versus scientific omission. While there is no evidence of intentional misconduct, failure to test the relevant formulation under conditions that reproduce intracellular LNP delivery constitutes a regulatory and scientific gap with implications for public safety assessment. Current evidence is limited by lack of data on actual adduct incidence in marketed vaccine lots, variable efficiency of LNP uptake across cell types, and incomplete characterization of lipid-plasmid or lipid-protein interactions. Addressing these gaps requires a roadmap integrating analytical quantification of lipid-nucleic acid adducts, human-cell exposure studies, sensitive integration assays, and comprehensive transcriptomic and proteomic profiling. Such a framework would align testing with the original intent of ICH M7 and provide regulators and researchers with actionable evidence regarding potential genotoxic and immunomodulatory risks before public roll out of gene therapy products, and those containing modified RNA of any type, and lipid formulations.

Conclusions

Current genotoxicity testing applied to SM-102 and ALC-0315 as isolated components is inadequate to evaluate the safety of fully formulated lipid nanoparticles used in mRNA vaccines. Standard bacterial assays and isolated PBMC studies fail to reproduce the cellular uptake, endosomal escape, nuclear access, and intracellular microenvironment relevant to patient exposure. Fully formulated LNPs and other modified RNA therapeutics of any kind must be assessed in human-cell–relevant systems capable of detecting lipid: nucleic acid adducts, chromatin perturbations, rare plasmid integration, and downstream proteomic and transcriptomic effects, including protein

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impacts, microRNA interactions, and activation of DNA-sensing pathways like cGAS-STING. Regulatory reliance on component and organoid only testing leaves potential gaps in identifying genotoxic and immunomodulatory risks that could contribute to adverse events in patients. Public health safety depends on pharmaceutical sponsors providing complete formulation data and on regulators enforcing testing that aligns with mechanistic realities of LNP therapeutics. Implementing such comprehensive, mechanism informed testing is critical to anticipate and mitigate risks before large scale clinical use. Current models may leave gaps that, if unaddressed, could affect public health and confidence in regulatory oversight.

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