Article

Not peer-reviewed version

Synthetic mRNA Vaccines and Transcriptomic Dysregulation: Evidence from New-Onset Adverse Events and Cancers Post-Vaccination

Natalia von Ranke , Wei Zhang , Philipp Anokin , Nicolas Hulscher , Kevin J. McKernan , Peter A. McCullough , John A. Catanzaro *

Posted Date: 25 July 2025

doi: 10.20944/preprints202507.2155.v1

Keywords: mRNA vaccine; new-onset post-vaccination symptoms; transcriptomics; GSEA; mitochondrial dysfunction; immune dysregulation; spike protein; epigenetic instability; adverse events; post-vaccine cancer

Article

Synthetic mRNA Vaccines and Transcriptomic Dysregulation: Evidence from New-Onset Adverse Events and Cancers Post-Vaccination

Natalia von Ranke [1], Wei Zhang [1], Philipp Anokin [1], Nicolas Hulscher [3], Kevin J. McKernan [3], Peter A. McCullough [2] and John A. Catanzaro [1,*]

[1] Neo7Biosience

[2] McCullough Foundation

[3] Medicinal Genomics

* Correspondence: john.catanzaro@neo7bioscience.com

Abstract

Background/Objectives: Synthetic mRNA vaccines have raised concerns regarding prolonged spike protein expression, immune activation, and potential off-target effects. This study investigates transcriptomic alterations in individuals with new-onset adverse events or cancer following mRNA COVID-19 vaccination. **Methods:** Bulk RNA sequencing was performed on peripheral blood from two patient groups: individuals with new-onset nonmalignant adverse events and individuals newly diagnosed with cancer post-vaccination. A control group of healthy individuals was used for comparison. Differential gene expression was analyzed using DESeq2, and Gene Set Enrichment Analysis (GSEA) was conducted using the MSigDB database and custom gene sets. **Results:** Both vaccine patient groups displayed widespread transcriptional dysregulation. In the nonmalignant adverse event group, hallmark enrichments included mitochondrial dysfunction, proteasome-mediated stress, transcriptomic instability, and systemic inflammation. The cancer group exhibited additional hallmarks of genomic instability, and epigenetic reprogramming. Nonsense-mediated decay (NMD), ribosomal stress, and MYC activation were prominent in both groups, while immune signaling via TLRs and type I interferons was particularly elevated in cancer patients. **Conclusions**: The observed transcriptomic profiles indicate persistent cellular stress responses, mitochondrial dysfunction, and immune dysregulation following exposure to mRNA vaccines, potentially in susceptible individuals. Shared and distinct molecular signatures in both cohorts demonstrate underlying mechanisms contributing to post-vaccine symptomatology and complications including oncogenesis and or progression of malignant disease. These findings underscore the need for a deeper investigation into the long-term safety of mRNA vaccines and host response variability.

Keywords: mRNA vaccine; new-onset post-vaccination symptoms; transcriptomics; GSEA; mitochondrial dysfunction; immune dysregulation; spike protein; epigenetic instability; adverse events; post-vaccine cancer

1. Introduction

Synthetic mRNA vaccines have introduced a novel platform in human immunization by encoding viral antigens within lipid nanoparticle–encapsulated, chemically modified mRNA molecules. While initially promoted for their rapid production and strong immunogenicity, a growing body of evidence has identified a range of adverse events, including myocarditis, thrombosis, cerebrovascular accidents, amyloidogenesis, arthralgia, menstrual disorders, reproductive concerns, and new-onset malignancies [1–7]. These events have prompted renewed

 doi:10.20944/preprints202507.2155.v1

scrutiny of the molecular mechanisms triggered by synthetic mRNA constructs and their intracellular fate [8,9].

Unlike endogenous mRNA, vaccine-derived transcripts incorporate non-natural features such as N1-methylpseudouridine (m1Ψ) substitution, extended poly(A) tails, and optimized untranslated regions (UTRs) to enhance stability and translational efficiency. These same modifications may alter RNA metabolism and surveillance, contributing to ribosomal infidelity, +1 frameshifting, and defective protein folding [10,11]. Independent studies have reported reverse transcription of vaccine mRNA into DNA via endogenous LINE-1 elements, highlighting issues over genomic integration and persistent expression [10,12]. Recent analyses of BNT162b2 vaccine vials revealed residual plasmid DNA, including SV40 enhancer and antibiotic resistance genes, raising concerns over potential oncogenic risk from unintended genomic exposure [13–17]. Moreover, recent research has shown that spike protein translated from mRNA vaccines can persist in the circulation for extended periods, far beyond initial pharmacokinetic expectations, potentially sustaining inflammatory signaling and immune activation [18–20]. Finally, some studies suggest that SARS-CoV-2 bears molecular signatures consistent with synthetic genome assembly, raising further concerns about engineered RNA platforms and their unforeseen biological consequences [21]. These features collectively raise concerns regarding unintended cellular consequences of synthetic mRNA exposure.

To address this gap, we conducted a comparative transcriptomic analysis using bulk RNA-sequencing data from whole blood of patients with new-onset adverse events (Group 1) and patients with new-onset cancers (Group 2) following COVID-19 mRNA vaccination, alongside healthy controls. Differential expression analysis and Gene Set Enrichment Analysis (GSEA) were performed to identify hallmark-specific molecular pathways perturbed in each group. By comparing these two profiles, this study aims to highlight potential transcriptomic disturbances associated with vaccine-related pathology and explore whether distinct or shared molecular hallmarks may underlie different post-vaccination clinical trajectories.

2. Materials and Methods

2.1. Patient Population Characteristics and Sample Collection

We enrolled adult participants (ages 18–70) who developed either new-onset symptoms following administration of mRNA-based COVID-19 vaccines (BNT162b2 [Pfizer] or mRNA-1273 [Moderna]). All participants provided written informed consent prior to inclusion in the study. Among the symptomatic individuals with nonmalignant adverse events, reported manifestations included cardiovascular injury, thrombosis, chronic fatigue, and neurological dysfunction, emerging shortly after vaccination. Another group of participants was diagnosed with cancer as a new-onset condition within a short time frame following mRNA vaccine administration. The study population was divided into two groups: the first consisted of three individuals who developed new-onset post-vaccination symptoms (group 1), and the second included seven individuals with new-onset cancer diagnoses (group 2). A cohort of 803 healthy individuals was used as the reference control group.

Peripheral blood samples were collected in accordance with institutional biosafety guidelines by licensed nurses at clinical sites affiliated with Neo7Bioscience. Venipuncture was performed using standard sterile technique. Whole blood was drawn into pre-labelled Streck tubes, stored at 4 °C, and transported the same day in cooled containers to the Genomics Center at the University of North Texas for processing.

2.2. RNA Extraction

Total RNA was extracted using the Quick-DNA/RNA Viral Kit (Zymo Research), following the manufacturer's protocol for whole blood RNA extraction. All procedures were conducted in a biosafety level 2 (BSL-2) cabinet (Esco Labculture A2). Briefly, lysis buffer was added to blood samples to inactivate pathogens, followed by ethanol precipitation and column-based RNA purification. Eluted RNA was quantified and stored at −80°C until library preparation.

 doi:10.20944/preprints202507.2155.v1

2.3. Library Preparation and cDNA Synthesis and High-Throughput Sequencing

RNA libraries were prepared using the Illumina TruSeq Stranded Total RNA Kit, incorporating ribosomal RNA depletion and strand-specific cDNA synthesis. RNA was fragmented enzymatically, reverse-transcribed to first-strand cDNA, and converted to double-stranded cDNA. Following end repair and adenylation, Illumina-specific adapters were ligated, and libraries were amplified by PCR. Quality and fragment size distribution were assessed using the High Sensitivity DNA kits on the TapeStation 4200 (Agilent Technologies). Libraries were stored at −20°C prior to sequencing.

Normalized and pooled libraries were sequenced on the Illumina NextSeq 550 platform using the NextSeq 500/550 High Output Kit v2.5 (300 cycles), targeting a coverage depth of 200× per sample. PhiX was added at 1% as an internal control. Paired-end sequencing reads were collected, and sequencing quality metrics (e.g., Q30 scores, cluster density) were monitored in real-time using Illumina's Sequencing Analysis Viewer.

2.4. Data Preprocessing, Quality Control, and Alignment

The raw sequencing data, in the form of paired-end Fastq files, were first organized by sample and read type (R1 and R2). These Fastq files were stored in a dedicated directory, and the cat command was used to group them based on their sample identifiers. The R1 and R2 files for each sample were then merged into a single file for each read type, resulting in forward and reverse merged files that were ready for downstream analysis.

Quality control checks were performed before the alignment step to ensure the integrity of the data. The sequencing reads were aligned to the human reference genome (hg38) using STAR [22], a widely used aligner for RNA sequencing data. STAR efficiently handles read alignment in a multi-threaded environment, optimizing processing time. The aligned reads were then outputted as BAM files, sorted by coordinate, which is standard practice for subsequent analysis steps.

2.5. Differential Expression Analysis and Volcano Plot Visualization

To investigate transcriptomic alterations associated with mRNA vaccine–related outcomes, we performed two distinct differential expression analyses, each comparing one patient group to a common pool of healthy controls (n = 803). The first comparison included individuals from group 1, who developed nonmalignant new-onset symptoms following mRNA vaccination (n = 3), while the second comprised individuals from group 2, diagnosed with cancer as a new-onset condition shortly after vaccination (n = 7). For both analyses, raw gene counts generated from Salmon [23] were input into DESeq2 [24], which applied internal normalization and modeled dispersion using the negative binomial distribution. Shrinkage of $\log_2$ fold changes was performed using the "apeglm" method to improve effect size estimation, particularly for genes with low read counts.

To visualize the global patterns of gene expression, volcano plots were generated for each comparison. In these plots, the x-axis represents the $\log_2$ fold change, and the y-axis represents the negative $\log_{10}$ of the adjusted p-value. Genes with $\log_2$ fold change greater than +1 and an adjusted p-value (padj) below 0.05 were considered significantly upregulated and were colored red, while those with $\log_2$ fold change less than -1 and padj < 0.05 were considered significantly downregulated and were colored blue. All remaining genes that did not meet these criteria were displayed in gray.

2.6. Gene Set Enrichment Analysis

To investigate transcriptomic disruptions associated with mRNA vaccine exposure, we analyzed RNA-seq data from three individuals with new-onset vaccine-related adverse effects and 803 healthy controls [25]. Differential gene expression analysis was conducted using DESeq2 [24], and genes were ranked by $\log_2$ fold change to generate the input file for enrichment analysis.

We then performed Gene Set Enrichment Analysis (GSEA) using the Preranked mode of the Broad Institute's GSEA 4.4.0 tool [26]. This approach was selected due to the significant class imbalance (3 vs. 803 samples), as it improves the stability and interpretability of enrichment results.

 doi:10.20944/preprints202507.2155.v1

The "weighted" enrichment statistic was applied to incorporate both gene ranking and expression magnitude, enhancing sensitivity to biologically relevant perturbations. We used 1,000 gene set permutations to estimate statistical significance and selected the "No collapse" option to retain gene symbols without alias mapping.

GSEA was systematically conducted across multiple MSigDB [27] collections: H (Hallmark), C2 (KEGG and REACTOME), C5 (GOBP), C7 (Immunologic Signatures), and custom gene sets including Gavish and curated GSE-derived expression signatures. Enrichment results were interpreted based on the normalized enrichment score (NES) and false discovery rate (FDR q-value), selecting gene sets that were strongly enriched (positive NES) or suppressed (negative NES), which indicated upregulated or downregulated biological functions, respectively. For downstream analysis, only gene sets with FDR < 0.25 and absolute NES ≥ 1.5 were retained. The top enriched and suppressed gene sets were manually grouped into higher-order molecular pathway categories.

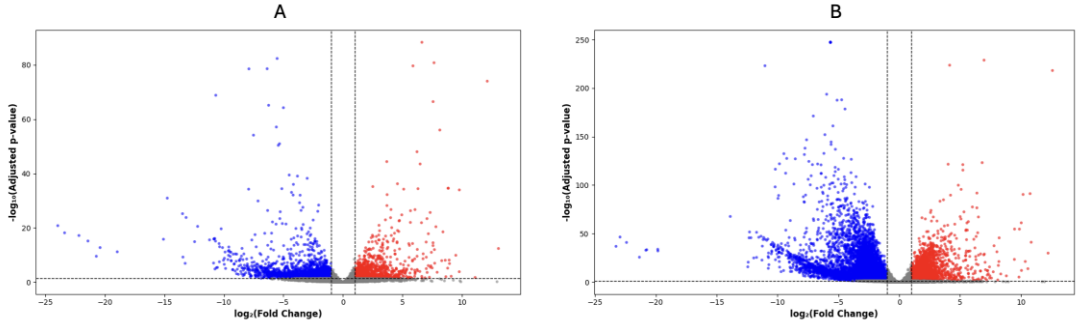
To improve biological relevance, we excluded gene sets associated with non-blood tissues (e.g., brain, retina, skin) and embryonic development, as these are unlikely to reflect transcriptional activity in peripheral blood. This filtering minimized noise and focused the analysis on pathways relevant to immune, inflammatory, and systemic responses.

2.7. Protein-Protein Interaction Map

For constructing the gene networks, interactions information of Differential expressed genes from the STRING database were loaded onto the Cytoscape software platform [28,29]. STRING is a database of known and predicted protein–protein interactions that includes both physical and functional protein associations. The STRING database currently covers 24,584,628 proteins from 5090 organisms [30]. STRING generates a network from an input list of proteins based on associations from a variety of data sources including genomic context predictions, high-throughput lab experiments, automated text mining, and previous knowledge in databases [31]. The network can be viewed within STRING or exported for visualization and analysis outside of STRING; for example, the network can be exported directly to Cytoscape. Cytoscape combines biomolecular interaction networks with high-throughput expression data and other molecular states into an integrated conceptual network model.

3. Results

To characterize the global transcriptional impact of mRNA vaccination in individuals with post-vaccination adverse outcomes, we conducted differential gene expression analyses comparing each patient group to the healthy control cohort. As shown in Figure 1, the volcano plots visualize the distribution of gene expression changes for both group 1, new-onset nonmalignant post-vaccination symptoms (A), and group 2, new-onset cancer diagnoses (B). In both groups, a clear transcriptomic shift is observed, with hundreds of genes showing significant dysregulation. Notably, the cancer group exhibits a broader distribution of downregulated genes, including several with extreme fold change magnitudes, suggesting a profound suppression of key regulatory pathways.



 doi:10.20944/preprints202507.2155.v1

Figure 1. Transcriptome-wide differential expression profiles in vaccine-affected individuals. (**A**) Volcano plot showing differential gene expression in individuals with new-onset adverse events following mRNA COVID-19 vaccination (n = 3) compared to healthy controls (n = 803). (**B**) Volcano plot showing differential gene expression in individuals diagnosed with new-onset cancer shortly after receiving mRNA COVID-19 vaccination (n = 7) compared to the same control cohort. Each point represents a single gene plotted by $\log_2$ fold change (x-axis) and $-\log_{10}$ adjusted p-value (y-axis). Genes with significant upregulation ($\log_2FC > 1$, padj < 0.05) are marked in red, while significantly downregulated genes ($\log_2FC < -1$, padj < 0.05) are shown in blue. Non-significant genes appear in gray. These plots reveal widespread transcriptional dysregulation in both patient groups, serving as the foundation for subsequent pathway enrichment analysis.

Gene Set Enrichment Analysis (GSEA) was performed separately for both study groups: Group 1, comprising individuals with new-onset adverse events following mRNA COVID-19 vaccination, and Group 2, comprising individuals diagnosed with new-onset cancer after vaccination, to characterize transcriptomic disruptions associated with vaccine exposure. GSEA was conducted using the predefined MSigDB gene set collections, and the enriched gene sets were systematically grouped according to their relevance to higher-order molecular hallmark pathways. This pathway-based grouping enabled integrative interpretation of transcriptomic alterations across systems biology domains.

In Group 1, the top enriched gene sets clustered into six key hallmark categories: (1) Mitochondrial Electron Transport Dysfunction & Reactive Oxygen Species (ROS), (2) Proteasome-Mediated Protein Degradation Stress, (3) Transcriptomic Instability and Translational Stress, (4) Systemic Inflammatory and Immune Response, (5) Endothelium Dysfunction, and (6) Proliferative Signaling and Suppressed Tumor Control. In contrast, Group 2 exhibited a distinct but overlapping enrichment pattern. The hallmarks identified in this group include: (1) Transcriptomic Instability and Translational Stress, (2) Systemic Inflammatory and Immune Response, (3) Endothelium Dysfunction, (4) Proliferative Signaling and Suppressed Tumor Control, and (5) Genomic Instability and Epigenetic Shift. As shown in Table 1, multiple gene sets within each hallmark category demonstrated strong enrichment scores (NES > 1.5 or < –1.5) and statistically significant FDR q-values (FDR < 0.05), supporting the presence of coordinated molecular dysregulation. Representative enrichment score (ES) curves are provided in Supplemental Figure S1 to illustrate the distribution of gene ranks contributing to each hallmark.

Table 1. Gene Set Enrichment Results Categorized by Molecular Pathway Disruptions in Patients with COVID-19 Vaccine–Associated Adverse Events.

Molecular Pathways	Systematic MSigDB ID	Brief description	NES	FDR
Mitochondrial Electron Transport Dysfunction & ROS	M47970	Mitochondrial complex - UCP1 in Thermogenesis.	1.86	0.11
	M47683	Electron transfer in Complex I.	1.70	0.10
	M47706	Mutation-inactivated PINK1 to electron transfer in Complex I.	1.73	0.09
	M47685	Mutation-caused aberrant Abeta to electron transfer in Complex I	1.69	0.09
	M47705	Mutation-caused aberrant SNCA to electron transfer in Complex I.	1.66	0.11
	M5936	Genes encoding proteins involved in oxidative phosphorylation.	1.43	>0.001
Proteasome-Mediated Protein Degradation Stress	M47713	Mutation-caused aberrant Abeta to 26S proteasome-mediated protein degradation.	1.78	0.12
	M47748	Mutation-inactivated VCP to 26S proteasome-mediated protein degradation.	1.74	0.13

 doi:10.20944/preprints202507.2155.v1

	M47747	Mutation-caused aberrant SOD1 to 26S proteasome-mediated protein degradation.	1.73	0.09
	M23267	The acquisition, loss, or modification of macromolecules within a complex, resulting in the alteration of an existing complex.	-2.11	>0.001
Transcriptomic Instability and Translational Stress	M27686	Eukaryotic Translation Initiation.	3.11	>0.001
	M567	SRP-dependent cotranslational protein targeting to membrane.	3.08	>0.001
	M27921	Nonsense-Mediated Decay (NMD)	2.9	>0.001
	M47786	Genes involved in ribosome assembly and start codon recognition during translation initiation.	3.0	>0.001
	M10085	The chemical reactions and pathways resulting in the formation of a protein in the cytoplasm. This is a ribosome-mediated process in which the information in messenger RNA (mRNA) is used to specify the sequence of amino acids in the protein.	2.60	>0.001
	M12963	A cellular process that results in the biosynthesis of constituent macromolecules, assembly, and arrangement of constituent parts of a small ribosomal subunit; includes transport to the sites of protein synthesis.	2.35	>0.001
	M16031	A cellular process that results in the biosynthesis of constituent macromolecules, assembly, and arrangement of constituent parts of a large ribosomal subunit; includes transport to the sites of protein synthesis.	2.34	>0.001
Systemic Inflammatory and Immune Response	M4475	Genes up-regulated in comparison of naive CD4 CD8 T cells versus unstimulated dendritic cells (DC).	2.43	>0.001
	M6217	Genes up-regulated in polymorphonuclear leukocytes (9h): control versus infection by A. phagocytophilum.	2.36	>0.001
	M5668	Genes up-regulated in comparison of untreated peripheral blood mononuclear cells (PBMC) versus PBMCs treated with CSF3	2.27	>0.001
	M5670	Genes down-regulated in comparison of untreated peripheral blood mononuclear cells (PBMC) versus PBMCs treated with CSF3	-1.93	>0.001
	M5662	Genes down-regulated in comparison of peripheral blood mononuclear cells (PBMC) from patients with acute E. coli infection versus PBMC from patients with acute S. pneumoniae infection.	-1.9	>0.001
	M10380	An immune humoral response against microbes mediated through a body fluid.	-1.95	>0.001
	M13774	An immune humoral response mediated through a body fluid.	-1.91	0.006
Endothelium Dysfunction	M5944	Genes up-regulated during formation of blood vessels (angiogenesis).	-1.94	>0.001
Proliferative Signaling and Suppressed Tumor Control	M5926	A subgroup of genes regulated by MYC	1.64	>0.001
	M46569	Genes upregulated in within various tumors	1.77	0.005
	M5956	Genes down-regulated by KRAS activation.	-1.68	>0.001
	M5939	Genes involved in p53 pathways and networks.	-1.50	-0.038
	M47835	Wnt signaling modulation, Wnt inhibitor.	-1.56	0.89

[1]The table displays the top gene sets within each category, along with their corresponding MSigDB identifiers, Normalized Enrichment Scores (NES), and false discovery rate (FDR) q-values. Positive NES values indicate

 doi:10.20944/preprints202507.2155.v1

upregulation, while negative NES values indicate downregulation of the gene sets in vaccinated samples (group 1) relative to controls. Gene sets were selected based on biological relevance and NES > |1.5| with FDR q < 0.25.

Table 2. Gene Set Enrichment Results Categorized by Molecular Pathway Disruptions in New-Onset Cancer Patients Following RNA-Based COVID-19 Vaccination.

Molecular Pathways	**Systematic MSigDB ID**	**Brief description**	**NES**	**FDR**
Transcriptomic Instability and Translational Stress	M27342	Genes involved in suppression of rRNA transcription regulated by SIRT1.	3.08	>0.001
	M27691	Genes active during the transition of RNA polymerase I from initiation to elongation.	2.61	>0.001
	M27458	Genes associated with transcriptional control mediated by small RNAs.	2.63	>0.001
	M29668	Genes linked to rRNA expression activated through the B-WICH chromatin remodeling complex.	2.60	>0.001
	M27426	Genes involved in epigenetic mechanisms that promote rRNA transcription.	2.54	>0.001
	M22151	Genes required for processing and maturation of large subunit (LSU) rRNA from a single transcript.	2.03	0.027
Systemic Inflammatory and Immune Response	M47908	Genes involved in type I interferon signaling through the JAK–STAT pathway.	2.64	>0.001
	M47564	Genes activated in response to HIV Tat via TLR2/4 and NF-κB signaling.	2.73	>0.001
	M47452	Genes mediating cellular responses to type I interferons.	2.64	>0.001
	M47616	Genes linking RIG-I activation to NF-κB–mediated transcription.	2.54	>0.001
	M47450	Genes involved in Toll-like receptor 3 signaling through IRF7 activation.	2.46	>0.001
	M47897	Genes triggered by TLR7, TLR8, or TLR9 signaling through IRF5.	2.29	>0.001
	M47617	Genes involved in TLR7 and TLR9–mediated signaling through IRF7.	2.27	>0.001
	M47782	Genes linked to MDA5 activation of IRF7 and IRF3 pathways.	2.0	0.006
	M47964	Genes involved in interferon signaling through RIPK1 and RIPK3.	2.0	0.006
	M47576	Genes activated downstream of RIG-I through IRF7 and IRF3.	2.0	0.007
	M46699	Genes associated with chromatin state changes in CD8+ T cells.	1.78	0.068
Endothelium Dysfunction	M15272	Genes related in process that stops, prevents, or reduces the rate or extent of endothelial cell proliferation	-1.98	0.0017
	M12042	Genes related in process that stops, prevents, or reduces the frequency, rate or extent of coagulation.	-1.98	0.0017
Proliferative Signaling and Suppressed Tumor Control	M27658	Genes involved in forming the origin recognition complex (ORC) during the initiation of DNA replication.	2.93	>0.001
Genomic Instability and Epigenetic Shift	M27429	Genes involved in the addition of methyl groups to DNA, a key epigenetic regulatory mechanism.	3.18	>0.001

 doi:10.20944/preprints202507.2155.v1

	M27166	Genes regulating chromatin compaction during early mitotic prophase.	2.93	>0.001
	M47539	Genes involved in cytosolic DNA sensing via cGAS–STING activation of type I interferon response.	2.43	>0.001
	M46570	Gene signature associated with chromatin-related transcriptional changes in malignant cells.	1.91	0.05
	M23298	Genes involved in the positioning and structuring of nucleosomes within chromatin.	2.33	0.01

[1]The table displays the top gene sets within each category, along with their corresponding MSigDB identifiers, Normalized Enrichment Scores (NES), and false discovery rate (FDR) q-values. Positive NES values indicate upregulation, while negative NES values indicate downregulation of the gene sets in vaccinated samples relative to controls. Gene sets were selected based on biological relevance and NES > |1.5| with FDR q < 0.25.

To further explore the molecular landscape associated with vaccine-induced transcriptomic disruptions, protein–protein interaction (PPI) network analysis was conducted for the most significantly dysregulated genes in each study group. As shown in Figure 2, the PPI map for Group 1 (individuals with new-onset adverse events post-vaccination) and Figure 3 shows the PPI map for Group 2 (individuals with new-onset cancer following vaccination)

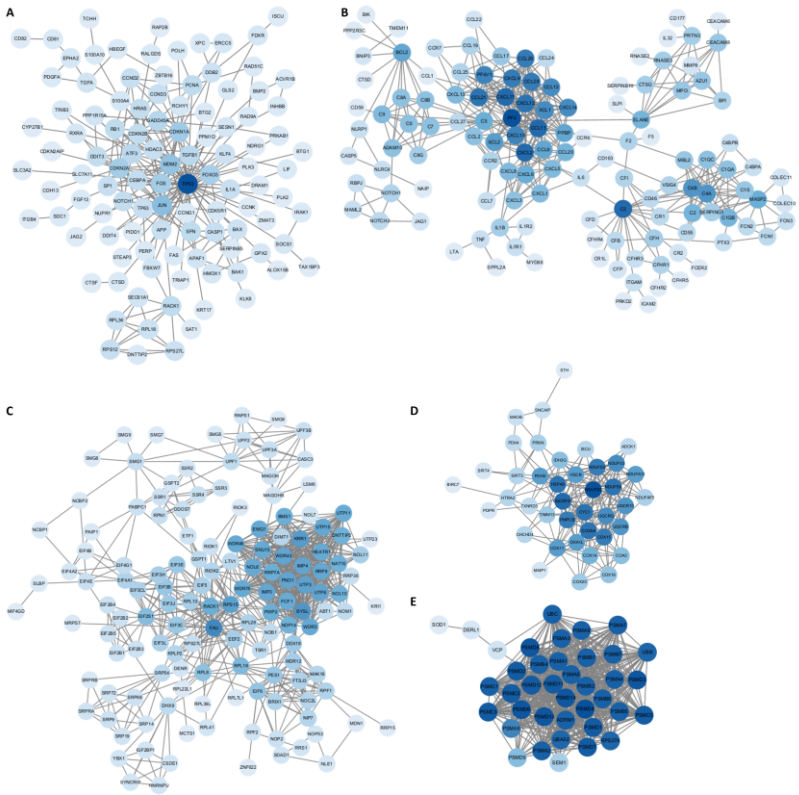


Figure 2. Protein–protein interaction (PPI) network of the most dysregulated genes in Group 1. Node color intensity reflects the degree of interaction (connectivity), with darker nodes indicating higher connectivity or a hub status within the network. (**A**) Differential expressed genes that are related to Proliferative Signaling and Tumor control. (**B**) Differential expressed genes that are related to Systemic Inflammatory immune. (**C**) Differential expressed genes related to Transcriptomic instability. (**D**) Differential expressed genes that are related to Mitochondrial. (**E**) Differential expressed genes that are related to Proteasome-degradation.

 doi:10.20944/preprints202507.2155.v1

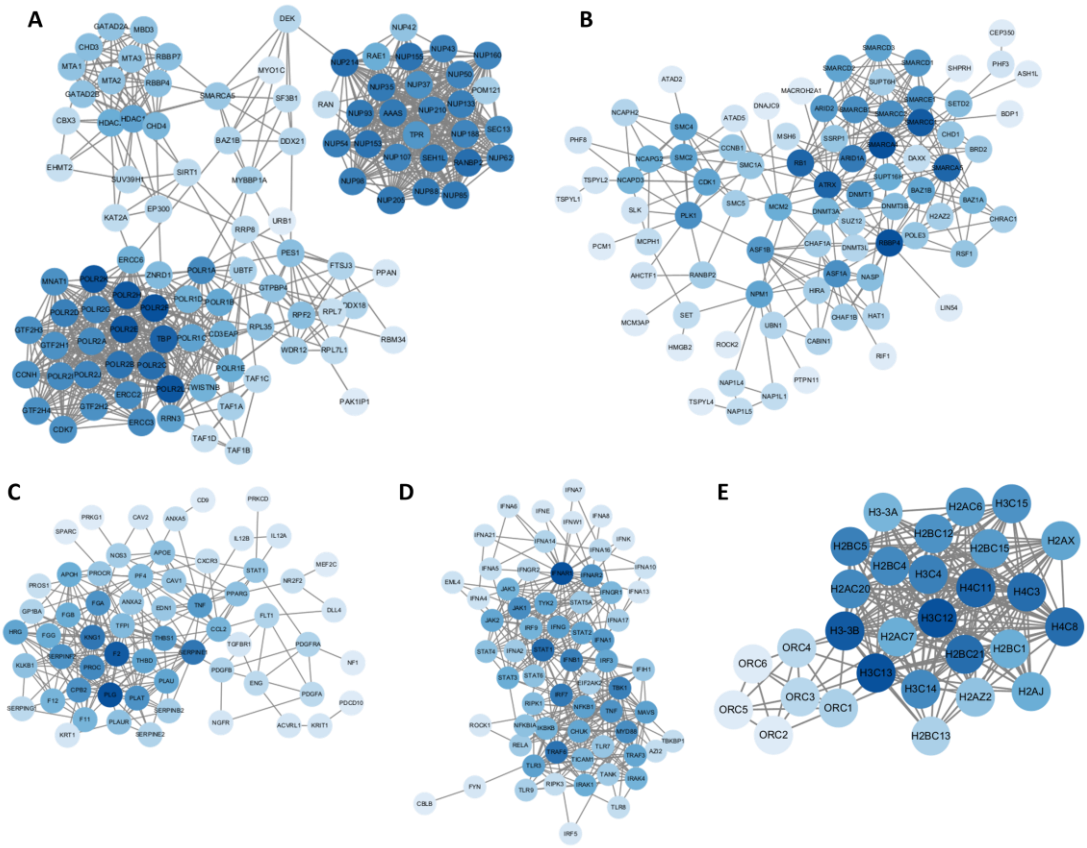


Figure 3. Protein–protein interaction (PPI) network of the most dysregulated genes in Group 2. Node color intensity reflects the degree of interaction (connectivity), with darker nodes indicating higher connectivity or a hub status within the network. (A) Protein-protein interaction network for upregulated genes that are related to Transcriptomic Instability, Translational Stress. (B) Protein-protein interaction network for upregulated genes that are related to Genomic Instability and Epigenetic Shift. (C) Protein-protein interaction network for downregulated genes associated with endothelial dysfunction. (D) Protein-protein interaction network for downregulated genes that are related to Systemic Inflammatory and Immune Response. (E) Protein-protein interaction network for upregulated genes that are related to Proliferative Signaling and Suppressed Tumor Control.

4. Discussion

The rapid global rollout of RNA-based COVID-19 vaccines introduced a novel therapeutic platform involving synthetic mRNA and lipid nanoparticle (LNP) delivery systems. While initially deployed to mitigate the spread of SARS-CoV-2, the long-term biological effects were unknown. Unlike conventional vaccines, these formulations induce host cells to express a viral spike glycoprotein from exogenous mRNA templates, raising concerns about unintended cellular responses [32]. Emerging clinical reports of persistent symptoms following vaccination—including neurological, cardiovascular, and immunological disturbances—have prompted scientific scrutiny into the molecular mechanisms potentially triggered by prolonged exposure to synthetic mRNA, its translation products, and associated immune activation [2,3,33,34]. To investigate these concerns, we performed gene set enrichment analyses (GSEA) on transcriptomic data from two distinct patient cohorts: individuals with new-onset post-vaccination adverse effects (group 1) and patients who developed cancer following mRNA vaccination (group 2). A summary of the transcriptomic alterations observed in each group is illustrated in Figure 4.

 doi:10.20944/preprints202507.2155.v1

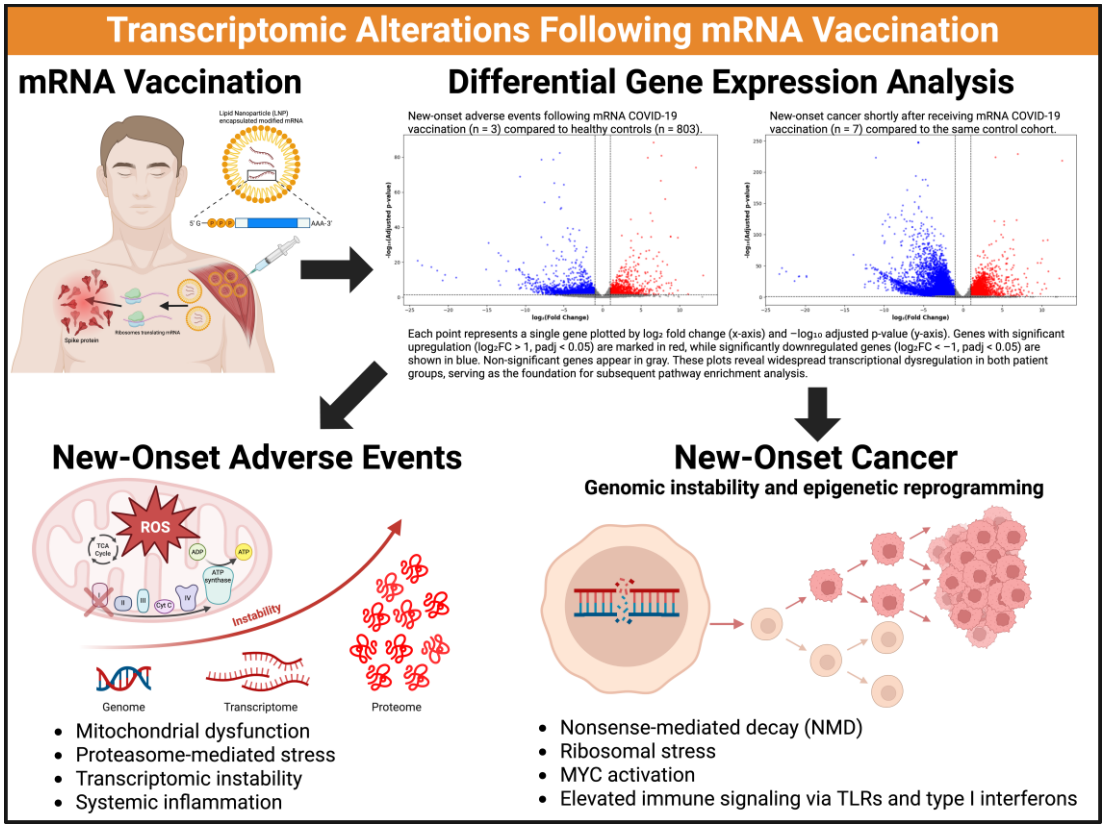


Figure 4. Transcriptomic Alterations Following mRNA Vaccination. This central illustration summarizes the experimental design, core findings, and proposed molecular mechanisms underlying transcriptomic dysregulation following synthetic mRNA COVID-19 vaccination. **Top Left:** Schematic of mRNA vaccination, showing lipid nanoparticle (LNP)–encapsulated, chemically modified mRNA encoding spike protein delivered into host cells, initiating persistent translation and immunologic engagement. **Top Right:** Volcano plots depict global differential gene expression in peripheral blood samples from two affected cohorts versus healthy controls (n = 803). Left: individuals with new-onset nonmalignant adverse events (n = 3). Right: individuals with new-onset cancer (n = 7). Upregulated genes ($log_2FC > 1$, padj < 0.05) are shown in red; downregulated genes ($log_2FC < –1$, padj < 0.05) in blue; non-significant genes in gray. **Bottom Left (New-Onset Adverse Events):** Transcriptomic analysis reveals enrichment of pathways linked to mitochondrial electron transport dysfunction and reactive oxygen species (ROS), proteasome-mediated protein degradation stress, mRNA surveillance activation, and systemic inflammatory signaling. **Bottom Right (New-Onset Cancer):** Cancer patients exhibit hallmarks of oncogenesis, including genomic instability, epigenetic reprogramming, nonsense-mediated decay (NMD), ribosomal stress, MYC-driven proliferative signaling, and persistent immune activation via Toll-like receptors (TLRs) and type I interferons. **Abbreviations:** mRNA, messenger ribonucleic acid; ROS, reactive oxygen species; TCA, tricarboxylic acid; NMD, nonsense-mediated decay; TLRs, Toll-like receptors; MYC, myelocytomatosis oncogene. ***Created with BioRender.com**

In the group 1 cohort, enrichment of gene sets related to mitochondrial electron transport indicates transcriptional disruption in core components of oxidative phosphorylation, particularly at complex I. The presence of variant-related enrichments involving PINK1, ABETA, and SNCA suggests convergence associated with impaired mitophagy, neurodegeneration, and defective electron flow. These findings suggest that spike-mediated inflammation disrupts mitochondrial homeostasis. Indeed, previous studies indicate that the spike protein alters mitochondrial fusion-fission dynamics, suppressing the biogenesis of its regulators (NRF1/2, PGC1α, TFAM), and increasing mitochondrial damage [35]. In addition, numerous studies have independently reported persistent mitochondrial dysfunction as a hallmark of Long COVID [36–38], further supporting a mechanistic link between spike protein exposure and bioenergetic imbalance.

 doi:10.20944/preprints202507.2155.v1

In the group 1 cohort, enrichment of proteasome-associated gene sets indicated activation of components of the ubiquitin–proteasome system. Upregulated proteins such as ubiquitin-40S ribosomal protein S27a (RPS27A), 26S proteasome subunit SEM1, polyubiquitin-B (UBB), and ubiquitin-60S ribosomal protein L40 (UBA52), along with several core subunits of the 20S and 19S proteasome complexes, including PSMA2–5, PSMA8, PSMC1, and PSMD7 reflect increased transcriptional demand for protein degradation machinery, likely in compensation for misfolded or aggregated proteins. The enrichment of variant-associated gene sets linked to VCP and SOD1 dysfunction further supports convergence with proteostasis disruption, a phenomenon commonly observed in neurodegenerative and proteinopathy-associated conditions. One potential driver of this sustained proteasomal activation is the prolonged presence of synthetic mRNA in circulation, which has been shown to persist beyond initial translation and may continuously stimulate the production of spike protein or aberrant translation products [18–20]. Additionally, emerging evidence suggests that the SARS-CoV-2 spike protein contains prion-like domains that are prone to misfolding and aggregation. These properties may be amplified in vaccine-induced expression, leading to persistent proteotoxic stress and neurodegenerative-like transcriptional profiles [10].

Transcriptomic Instability and Translational Stress was a dominant hallmark in both patient groups. Enrichment of gene sets related to translation initiation, ribosome biogenesis, and mRNA surveillance suggests sustained activation of the protein synthesis machinery. In group 1, upregulated pathways such as translation initiation, cytoplasmic translation, and ribosomal subunit biogenesis indicate persistent engagement of ribosomes and translational apparatus, likely driven by prolonged spike protein expression from stabilized synthetic mRNA [10,18,20]. Enrichment of nonsense-mediated decay (NMD) related genes in patients with new-onset vaccine-associated adverse events contrasts with previous findings that SARS-CoV-2 virus suppresses NMD to protect its RNA genome [39,40]. This suggests that, unlike the viral genome, synthetic mRNA used in vaccination may instead provoke a compensatory activation of RNA surveillance mechanisms, potentially due to persistent translation or accumulation of aberrant transcripts. The opposing patterns of NMD regulation in these two contexts point to distinct cellular responses and warrant further investigation. In group 2, as in the first group, we observed significant enrichment in gene sets related to ribosome biogenesis, cytoplasmic translation, and mRNA surveillance. However, the cancer group displayed more pronounced activation of ribosomal stress pathways, particularly those linked to RNA Polymerase I promoter escape, rRNA maturation, and positive epigenetic regulation of rRNA expression, indicating hyperactivation of nucleolar functions and elevated translational throughput. While these transcriptional programs are classically associated with tumor cells to support uncontrolled proliferation [41–43], their detection in peripheral blood samples likely reflects systemic consequences of underlying malignancy, such as systemic immune alterations or stress responses induced by tumor-related inflammation and signaling [44–46]. While the vaccine-adverse event group showed activation of nonsense-mediated decay pathways, the cancer group displayed additional signals related to small RNA–mediated transcriptional regulation and epigenetic repression of ribosomal gene activity. The coexistence of transcriptional silencing and increased rRNA production may reflect underlying transcriptional stress affecting cellular balance.

Enrichments related to the hallmark of Systemic Inflammatory and Immune Response were identified in both groups analyzed in this work. In group 1, the most highly connected upregulated genes in the protein–protein interaction (PPI) network included LOX, CD28, CCR7, and SELL. Recent findings demonstrate that the use of N1-methylpseudouridine in mRNA constructs can induce +1 ribosomal frameshifting, resulting in the production of off-target proteins that may elicit unintended cellular immune responses [11]. This aberrant antigen production may represent a novel mechanism contributing to systemic inflammation and immune dysregulation. In addition, emerging evidence suggests that cytoplasmic fragmentation of vaccine-derived mRNA may generate short RNA sequences with miRNA-like properties capable of hybridizing host immune transcripts, such as interferons and anti-inflammatory regulators. This unintended post-transcriptional interference could contribute to systemic immune dysregulation and inflammatory responses, particularly in

 doi:10.20944/preprints202507.2155.v1

individuals with predisposing comorbidities or impaired RNA degradation pathways [47]. In addition, numerous studies have previously demonstrated the inflammatory activity of mRNA lipid nanoparticles [48–50]. Compared to group 1, where immune imbalance was more skewed toward aberrant cellular activation and humoral suppression, group 2 displayed a distinct pattern of innate immune activation. Gene set enrichment analysis revealed the upregulation of pathways involving type I interferons, Toll-like receptors (TLRs), and NF–κB–driven inflammatory signaling. Enrichment of the RIG-I/MDA5–IRF7 axis, TLR3/7/8/9–IRF5/7, and the JAK-STAT interferon cascade suggests persistent engagement of RNA-sensing mechanisms and their downstream proinflammatory transcriptional programs. While these responses are central to antiviral immunity, their chronic activation in the peripheral blood of cancer patients might be related to inflammation, immune exhaustion, and tumor immune editing [51–53].

For group 1, a negative NES for angiogenesis hallmark suggests transcriptional downregulation or post-transcriptional inhibition of key angiogenic mediators. This may be linked to the RNA fragmentation hypothesis supported by [47], wherein miRNA-like fragments derived from cleaved vaccine mRNA hybridize with endothelial transcripts, suppressing their translation and contributing to systemic endothelial dysfunction and impaired vascular repair. In the cancer group, transcriptomic analysis revealed significant downregulation of gene sets involved in negative regulation of endothelial cell proliferation and negative regulation of coagulation. The suppression of these regulatory pathways may indicate uncontrolled endothelial activation or a vascular pro-thrombotic shift, conditions that can be linked to spike protein adverse events and tumor progression [54–57].

For both groups 1 and 2, we could note an enrichment in the Proliferative Signaling and Suppressed Tumor Control Hallmark genes signatures. For group 1, positive enrichment in gene sets regulated by MYC suggests an active oncogenic transcriptional program that favors tumor growth and adaptation. This proliferative bias is compounded by the downregulation of tumor-suppressive pathways, including the KRAS-inhibited signature, p53 regulatory networks, and inhibitors of the Wnt pathway. These findings suggest a microenvironment conducive to unchecked cellular expansion, loss of apoptotic surveillance, and diminished responsiveness to anti-proliferative signals. Notably, many previous studies have reported that the spike protein can trigger the mitogen pathway, through the downregulation of ACE2 expression, which promotes an angiotensin II type I receptor (AT1R)-mediated signaling cascade, inducing the transcriptional regulatory molecules nuclear factor-κB (NF-κB) and activator protein 1 (AP-1)/c-Fos via mitogen-activated protein kinase (MAPK) activation [58–60]. In accordance with this molecular pathway, our investigated patients from group 1 and group 2 also presented a very downregulated ACE2 expression compared to normal control (logfold change: -4.3 and -4.8, respectively). In relation to this hallmark, group 2 showed an enrichment of pathways involved in the DNA replication process, which may reflect increased proliferation or replicative stress in circulating immune cells, potentially driven by tumor-related systemic effects or prolonged immune activation [51–53].

Only group 2 presented enrichments related to the Genomic Instability and Epigenetic Shift hallmark. Key pathways enriched show aberrant regulation of histone modification, DNA packaging, and epigenetic silencing, which are commonly observed in oncogenic events. In parallel, enrichment of the cGAS–STING signaling pathway points to innate immune recognition of cytoplasmic DNA fragments, a well-established marker of DNA damage and chromosomal instability [61]. This pathway can be related to tumor-promoting inflammation and immune editing [51–53].

5. Conclusions

This study provides transcriptomic evidence of molecular disruptions in two patient populations—those with new-onset nonmalignant adverse events and those with newly diagnosed cancers—months to years following COVID-19 mRNA vaccination. Using differential expression analysis and GSEA, we identified hallmark signatures of mitochondrial dysfunction, translational stress, immune dysregulation, endothelial disturbance, and proliferative signaling across both cohorts. Notably, while both groups shared transcriptional perturbations in immune and

 doi:10.20944/preprints202507.2155.v1

translational pathways, the cancer group exhibited additional signatures of genomic instability and epigenetic remodeling.

Persistent spike protein expression, prolonged synthetic mRNA activity, and RNA modifications such as N1-methylpseudouridine appear to contribute to sustained aberrant ribosomal activity, proteostasis stress, and immune activation. Our findings also highlight transcriptional signals indicative of tumor-promoting conditions, including suppressed p53 networks, activated MYC targets, and altered interferon signaling, particularly in the context of epigenetic dysregulation in the cancer cohort. These observations suggest that vaccine-induced transcriptomic reprogramming may differentially affect individuals, genetically or immunologically over a long period of time after vaccination.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

Author Contributions: N.v.R. and W.Z. led the discussion and interpretation of results. P.A. prepared the datasets for analysis. N.H. K.M., and P.M. provided scientific support and project oversight. J.C. served as the principal investigator and supervised the research. All authors reviewed and approved the final manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards (or Ethics Committee) of the following clinics:

Rejenesis Plus	Dr James Joseph	IRB00014446
Forum Health Center	NP Susan Harris	IRB00013617
Medical Health Institute	Dr Rudolph Eberwein	IRB00014296
Dr Petra MD	Dr Petra Davelaar	IRB00014502
Humanizing Medicine	Dr Mark Hancock	IRB00014854

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study

Data Availability Statement: We encourage all authors of articles published in MDPI journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required. Suggested Data Availability Statements are available in section “MDPI Research Data Policies” at https://www.mdpi.com/ethics.

Acknowledgments: We thank the University of North Texas Genomics Center for sequencing support, Neo7Bioscience for data interpretation assistance, and the McCullough Foundation for scientific collaboration. We also acknowledge the participants who made this study possible.

Conflicts of Interest: This research received no governmental funding. Neo7Bioscience is a private biotechnology company focused on the development of personalized therapeutic peptides. The McCullough Foundation provided independent support for scientific investigation. The University of North Texas participated in sample processing and transcriptomic analysis. The authors declare no affiliations or involvements that could be interpreted as influencing the integrity of this work.

Abbreviations

The following abbreviations are used in this manuscript:

mRNA – Messenger Ribonucleic Acid
UTR – Untranslated Region
NMD – Nonsense-Mediated Decay
SV40 – Simian Virus 40
ORC – Origin Recognition Complex
rRNA – Ribosomal RNA
cGAS – Cyclic GMP-AMP Synthase
STING – Stimulator of Interferon Genes

 doi:10.20944/preprints202507.2155.v1

IFN – Interferon
TLR – Toll-Like Receptor
ISG – Interferon-Stimulated Gene
PPI – Protein–Protein Interaction
DESeq2 – Differential Expression Sequencing (v2)
GSEA – Gene Set Enrichment Analysis
PBMC – Peripheral Blood Mononuclear Cell
PCR – Polymerase Chain Reaction
LNP – Lipid Nanoparticle
LINE-1 – Long Interspersed Nuclear Element-1

References

1. Acevedo-Whitehouse, K.; Bruno, R. Potential health risks of mRNA-based vaccine therapy: A hypothesis.
2. Li, Y.E.; Wang, S.; Reiter, R.J.; Ren, J. Clinical cardiovascular emergencies and the cellular basis of COVID-19 vaccination: from dream to reality? *International Journal of Infectious Diseases* **2022**, *124*, 1-10, doi:https://doi.org/10.1016/j.ijid.2022.08.026.
3. Mohseni Afshar, Z.; Tavakoli Pirzaman, A.; Liang, J.J.; Sharma, A.; Pirzadeh, M.; Babazadeh, A.; Hashemi, E.; Deravi, N.; Abdi, S.; Allahgholipour, A.; et al. Do we miss rare adverse events induced by COVID-19 vaccination? *Frontiers in Medicine* **2022**, *Volume 9 - 2022*.
4. Solomon, A.L.; Ratchford, E.V.; Armitage, K.B.; Kovacic, J.C. Vascular Disease Patient Information Page: Vascular considerations with COVID-19 vaccines. *Vascular Medicine* **2021**, *27*, 102-106, doi:10.1177/1358863X211066128.
5. Yoon, D.A.-O.; Jeon, H.A.-O.; Noh, Y.A.-O.; Choe, Y.A.-O.; Choe, S.A.-O.; Jung, J.A.-O.; Shin, J.A.-O. A Nationwide Survey of mRNA COVID-19 Vaccinee's Experiences on Adverse Events and Its Associated Factors.
6. Ferreira-da-Silva, R.; Lobo, M.F.; Pereira, A.M.; Morato, M.; Polónia, J.J.; Ribeiro-Vaz, I. Network analysis of adverse event patterns following immunization with mRNA COVID-19 vaccines: real-world data from the European pharmacovigilance database EudraVigilance. *Frontiers in Medicine* **2025**, *Volume 12 - 2025*.
7. Yasmin, F.; Najeeb, H.; Naeem, U.; Moeed, A.; Atif, A.R.; Asghar, M.A.-O.; Nimri, N.; Saleem, M.; Bandyopadhyay, D.; Krittanawong, C.; et al. Adverse events following COVID-19 mRNA vaccines: A systematic review of cardiovascular complication, thrombosis, and thrombocytopenia.
8. Nyström, S.; Hammarström, P. Amyloidogenesis of SARS-CoV-2 Spike Protein. *Journal of the American Chemical Society* **2022**, *144*, 8945-8950, doi:10.1021/jacs.2c03925.
9. Manniche, V.F., T.; Schmeling, M.; Gilthorpe, J.D.; Hansen, P.R. Rates of Successful Conceptions According to COVID-19 Vaccination Status: Data from the Czech Republic. *International Journal of Risk & Safety in Medicine* **2025**.
10. Seneff, S.; Kyriakopoulos, A.M.; Nigh, G.; McCullough, P.A. A Potential Role of the Spike Protein in Neurodegenerative Diseases: A Narrative Review. *Cureus* **2023**, *15*, e34872, doi:10.7759/cureus.34872.
11. Stiving, A.Q.; Roose, B.W.; Tubbs, C.; Haverick, M.; Gruber, A.; Rustandi, R.R.; Kuiper, J.; Schombs, M.; Schuessler, H.; Li, X. Functionality and translation fidelity characterization of mRNA vaccines using platform based mass spectrometry detection. *npj Vaccines* **2025**, *10*, 38, doi:10.1038/s41541-025-01082-4.
12. Lim, S.; Yocum, R.R.; Silver, P.A.; Way, J.C. High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection.
13. Šenigl, F.; Soikkeli, A.I.; Prost, S.; Schatz, D.G.; Slavková, M.; Hejnar, J.; Alinikula, J. The SV40 virus enhancer functions as a somatic hypermutation-targeting element with potential tumorigenic activity.
14. Dean, D.A.; Dean Bs Fau - Muller, S.; Muller S Fau - Smith, L.C.; Smith, L.C. Sequence requirements for plasmid nuclear import.
15. Kämmerer, U.; Schulz, V.; Steger, K. BioNTech RNA-Based COVID-19 Injections Contain Large Amounts Of Residual DNA Including An SV40 Promoter/Enhancer Sequence. **2024**.

16. Drayman, N.; Ben-Nun-Shaul, O.; Butin-Israeli, V.; Srivastava, R.; Rubinstein, A.M.; Mock, C.S.; Elyada, E.; Ben-Neriah, Y.; Lahav, G.; Oppenheim, A. p53 elevation in human cells halt SV40 infection by inhibiting T-ag expression.
17. Strayer, D.; Branco F Fau - Zern, M.A.; Zern Ma Fau - Yam, P.; Yam P Fau - Calarota, S.A.; Calarota Sa Fau - Nichols, C.N.; Nichols Cn Fau - Zaia, J.A.; Zaia Ja Fau - Rossi, J.; Rossi J Fau - Li, H.; Li H Fau - Parashar, B.; Parashar B Fau - Ghosh, S.; et al. Durability of transgene expression and vector integration: recombinant SV40-derived gene therapy vectors.
18. Patterson, B.K.; Yogendra, R.; Francisco, E.B.; Guevara-Coto, J.; Long, E.; Pise, A.; Osgood, E.; Bream, J.; Kreimer, M.; Jeffers, D.; et al. Detection of S1 spike protein in CD16+ monocytes up to 245 days in SARS-CoV-2-negative post-COVID-19 vaccine syndrome (PCVS) individuals. *Human Vaccines & Immunotherapeutics* **2025**, *21*, 2494934, doi:10.1080/21645515.2025.2494934.
19. Boros, L.G.; Kyriakopoulos, A.M.; Brogna, C.; Piscopo, M.; McCullough, P.A.; Seneff, S. Long-lasting, biochemically modified mRNA, and its frameshifted recombinant spike proteins in human tissues and circulation after COVID-19 vaccination. *Pharmacology Research & Perspectives* **2024**, *12*, e1218, doi:https://doi.org/10.1002/prp2.1218.
20. Yonker, L.M.; Swank, Z.; Bartsch, Y.C.; Burns, M.D.; Kane, A.; Boribong, B.P.; Davis, J.P.; Loiselle, M.; Novak, T.; Senussi, Y.; et al. Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis. *Circulation* **2023**, *147*, 867-876, doi:10.1161/CIRCULATIONAHA.122.061025.
21. Bruttel, V.; Washburne, A.; VanDongen, A. Endonuclease fingerprint indicates a synthetic origin of SARS-CoV-2. *bioRxiv* **2023**, 2022.2010.2018.512756, doi:10.1101/2022.10.18.512756.
22. Dobin, A.; Davis Ca Fau - Schlesinger, F.; Schlesinger F Fau - Drenkow, J.; Drenkow J Fau - Zaleski, C.; Zaleski C Fau - Jha, S.; Jha S Fau - Batut, P.; Batut P Fau - Chaisson, M.; Chaisson M Fau - Gingeras, T.R.; Gingeras, T.R. STAR: ultrafast universal RNA-seq aligner.
23. Patro, R.; Duggal, G.; Love, M.I.; Irizarry, R.A.; Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression.
24. Love, M.I.; Huber, W.; Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **2014**, *15*, 550, doi:10.1186/s13059-014-0550-8.
25. Anders, S.; Huber, W. Differential expression analysis for sequence count data. *Genome Biology* **2010**, *11*, R106, doi:10.1186/gb-2010-11-10-r106.
26. Subramanian, A.; Tamayo, P.; Mootha, V.K.; Mukherjee, S.; Ebert, B.L.; Gillette, M.A.; Paulovich, A.; Pomeroy, S.L.; Golub, T.R.; Lander, E.S.; et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences* **2005**, *102*, 15545-15550, doi:10.1073/pnas.0506580102.
27. Liberzon, A.; Birger, C.; Thorvaldsdóttir, H.; Ghandi, M.; Mesirov, J.P.; Tamayo, P. The Molecular Signatures Database (MSigDB) hallmark gene set collection.
28. Szklarczyk, D.; Franceschini, A.; Wyder, S.; Forslund, K.; Heller, D.; Huerta-Cepas, J.; Simonovic, M.; Roth, A.; Santos, A.; Tsafou, K.P.; et al. STRING v10: protein-protein interaction networks, integrated over the tree of life.
29. Rao, V.S.; Srinivas, K.; Sujini, G.N.; Kumar, G.N. Protein-protein interaction detection: methods and analysis.
30. Bebek, G. Identifying Gene Interaction Networks. In *Statistical Human Genetics: Methods and Protocols*, Elston, R.C., Satagopan, J.M., Sun, S., Eds.; Humana Press: Totowa, NJ, 2012; pp. 483-494.
31. Szklarczyk, D.; Kirsch, R.; Koutrouli, M.A.-O.; Nastou, K.A.-O.; Mehryary, F.A.-O.; Hachilif, R.; Gable, A.L.; Fang, T.A.-O.; Doncheva, N.A.-O.; Pyysalo, S.; et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest.
32. Speicher, D.J., Jessica Rose, L. M. Gutschi, David M. Wiseman, PhD, and Kevin McKernan. DNA Fragments Detected in Monovalent and Bivalent Pfizer/biontech and Moderna Modrna COVID-19 Vaccines from Ontario, Canada: Exploratory Dose Response Relationship with Serious Adverse Events. *OSF Preprints* **2023**.
33. Trougakos, I.P.; Terpos, E.; Alexopoulos, H.; Politou, M.; Paraskevis, D.; Scorilas, A.; Kastritis, E.; Andreakos, E.; Dimopoulos, M.A. Adverse effects of COVID-19 mRNA vaccines: the spike hypothesis.

 doi:10.20944/preprints202507.2155.v1

34. Fang, L.; Tang, T.; Hu, M.A.-O. Identification of Differentially Expressed Genes in COVID-19 and Integrated Bioinformatics Analysis of Signaling Pathways.
35. Chang, X.; Ismail, N.I.; Rahman, A.; Xu, D.; Chan, R.W.Y.; Ong, S.-G.; Ong, S.-B. Long COVID-19 and the Heart: Is Cardiac Mitochondria the Missing Link? *Antioxidants & Redox Signaling* **2022**, *38*, 599-618, doi:10.1089/ars.2022.0126.
36. Molnar, T.; Lehoczki, A.; Fekete, M.; Varnai, R.; Zavori, L.; Erdo-Bonyar, S.; Simon, D.; Berki, T.; Csecsei, P.; Ezer, E. Mitochondrial dysfunction in long COVID: mechanisms, consequences, and potential therapeutic approaches. *GeroScience* **2024**, *46*, 5267-5286, doi:10.1007/s11357-024-01165-5.
37. Rurek, M. Mitochondria in COVID-19: from cellular and molecular perspective. *Frontiers in Physiology* **2024**, *Volume 15 - 2024*.
38. Cao, X.; Nguyen, V.; Tsai, J.; Gao, C.; Tian, Y.; Zhang, Y.; Carver, W.; Kiaris, H.; Cui, T.; Tan, W. The SARS-CoV-2 spike protein induces long-term transcriptional perturbations of mitochondrial metabolic genes, causes cardiac fibrosis, and reduces myocardial contractile in obese mice. *Molecular Metabolism* **2023**, *74*, 101756, doi:https://doi.org/10.1016/j.molmet.2023.101756.
39. Mallick, M.; Boehm, V.; Xue, G.; Blackstone, M.; Gehring, Niels H.; Chakrabarti, S. Modulation of UPF1 catalytic activity upon interaction of SARS-CoV-2 Nucleocapsid protein with factors involved in nonsense mediated-mRNA decay. *Nucleic Acids Research* **2024**, *52*, 13325-13339, doi:10.1093/nar/gkae829.
40. Nuccetelli, V.; Mghezzi-Habellah, M.; Deymier, S.; Roisin, A.; Gérard-Baraggia, F.; Rocchi, C.; Coureux, P.D.; Gouet, P.; Cimarelli, A.; Mocquet, V.A.-O.; et al. The SARS-CoV-2 nucleocapsid protein interferes with the full enzymatic activation of UPF1 and its interaction with UPF2. LID - 10.1093/nar/gkaf010 [doi] LID - gkaf010.
41. Pitts, S.; Laiho, M. Regulation of RNA Polymerase I Stability and Function. LID - 10.3390/cancers14235776 [doi] LID - 5776.
42. Ferreira, R.; Schneekloth, J.S., Jr.; Panov, K.I.; Hannan, K.M.; Hannan, R.D. Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age. LID - 10.3390/cells9020266 [doi] LID - 266.
43. López, J.; Blanco, S. Exploring the role of ribosomal RNA modifications in cancer.
44. Abusanad, A. Utilizing peripheral blood inflammatory biomarker (PBIB) to predict response to systemic therapy in patients with breast cancer.
45. Huai, Q.; Luo, C.; Song, P.; Bie, F.; Bai, G.; Li, Y.; Liu, Y.; Chen, X.; Zhou, B.; Sun, X.; et al. Peripheral blood inflammatory biomarkers dynamics reflect treatment response and predict prognosis in non-small cell lung cancer patients with neoadjuvant immunotherapy. *Cancer Science* **2023**, *114*, 4484-4498, doi:https://doi.org/10.1111/cas.15964.
46. Xie, Y.; Yu, Q.; Zhu, Y.; Wu, W.; Xiao, R.; Wang, N.; Zhu, L.; Li, P.; Chen, T. The value of peripheral blood inflammation markers in risk assessment and prediction of lung cancer. *Future Science OA* **2025**, *11*, 2476870, doi:10.1080/20565623.2025.2476870.
47. Demongeot, J.A.-O.; Fougère, C. mRNA COVID-19 Vaccines-Facts and Hypotheses on Fragmentation and Encapsulation. LID - 10.3390/vaccines11010040 [doi] LID - 40.
48. Kiaie, S.H.; Majidi Zolbanin, N.; Ahmadi, A.; Bagherifar, R.; Valizadeh, H.; Kashanchi, F.; Jafari, R. Recent advances in mRNA-LNP therapeutics: immunological and pharmacological aspects. *Journal of Nanobiotechnology* **2022**, *20*, 276, doi:10.1186/s12951-022-01478-7.
49. Parry, P.A.-O.; Lefringhausen, A.; Turni, C.A.-O.; Neil, C.A.-O.; Cosford, R.A.-O.; Hudson, N.A.-O.; Gillespie, J. 'Spikeopathy': COVID-19 Spike Protein Is Pathogenic, from Both Virus and Vaccine mRNA. LID - 10.3390/biomedicines11082287 [doi] LID - 2287.
50. Sharma, P.; Hoorn, D.; Aitha, A.; Breier, D.; Peer, D. The immunostimulatory nature of mRNA lipid nanoparticles.
51. Hu, A.; Sun, L.; Lin, H.; Liao, Y.; Yang, H.; Mao, Y. Harnessing innate immune pathways for therapeutic advancement in cancer. *Signal Transduction and Targeted Therapy* **2024**, *9*, 68, doi:10.1038/s41392-024-01765-9.
52. Maiorino, L.; Daßler-Plenker, J.; Sun, L.; Egeblad, M. Innate Immunity and Cancer Pathophysiology.
53. Yi, M.; Li, T.; Niu, M.; Mei, Q.; Zhao, B.; Chu, Q.; Dai, Z.; Wu, K. Exploiting innate immunity for cancer immunotherapy. *Molecular Cancer* **2023**, *22*, 187, doi:10.1186/s12943-023-01885-w.

54. Blann, A.D. Endothelial cell activation markers in cancer. *Thrombosis Research* **2012**, *129*, S122-S126, doi:https://doi.org/10.1016/S0049-3848(12)70031-2.
55. Smeda, M.; Przyborowski, K.; Stojak, M.; Chlopicki, S. The endothelial barrier and cancer metastasis: Does the protective facet of platelet function matter? *Biochemical Pharmacology* **2020**, *176*, 113886, doi:https://doi.org/10.1016/j.bcp.2020.113886.
56. Zheng, Y.; Zhao, J.; Li, J.; Guo, Z.; Sheng, J.; Ye, X.; Jin, G.; Wang, C.; Chai, W.; Yan, J.; et al. SARS-CoV-2 spike protein causes blood coagulation and thrombosis by competitive binding to heparan sulfate.
57. Becker, R.C.; Tantry, U.S.; Khan, M.; Gurbel, P.A. The COVID-19 thrombus: distinguishing pathological, mechanistic, and phenotypic features and management. *Journal of Thrombosis and Thrombolysis* **2025**, *58*, 15-49, doi:10.1007/s11239-024-03028-4.
58. Valdes Angues, R.; Perea Bustos, Y. SARS-CoV-2 Vaccination and the Multi-Hit Hypothesis of Oncogenesis. *Cureus* **2023**, *15*, e50703, doi:10.7759/cureus.50703.
59. Patra, T.; Meyer, K.; Geerling, L.; Isbell, T.S.; Hoft, D.F.; Brien, J.; Pinto, A.K.; Ray, R.B.; Ray, R. SARS-CoV-2 spike protein promotes IL-6 trans-signaling by activation of angiotensin II receptor signaling in epithelial cells. *PLOS Pathogens* **2020**, *16*, e1009128, doi:10.1371/journal.ppat.1009128.
60. Suzuki, Y.J.; Gychka, S.G. SARS-CoV-2 Spike Protein Elicits Cell Signaling in Human Host Cells: Implications for Possible Consequences of COVID-19 Vaccines. *Vaccines* **2021**, *9*, doi:10.3390/vaccines9010036.
61. Kwon, J.; Bakhoum, S.F. The Cytosolic DNA-Sensing cGAS-STING Pathway in Cancer.