%RNA integrity issues in the mRNA COVID-19 injectable products - is this the reason why some people suffer adverse events and others do not?

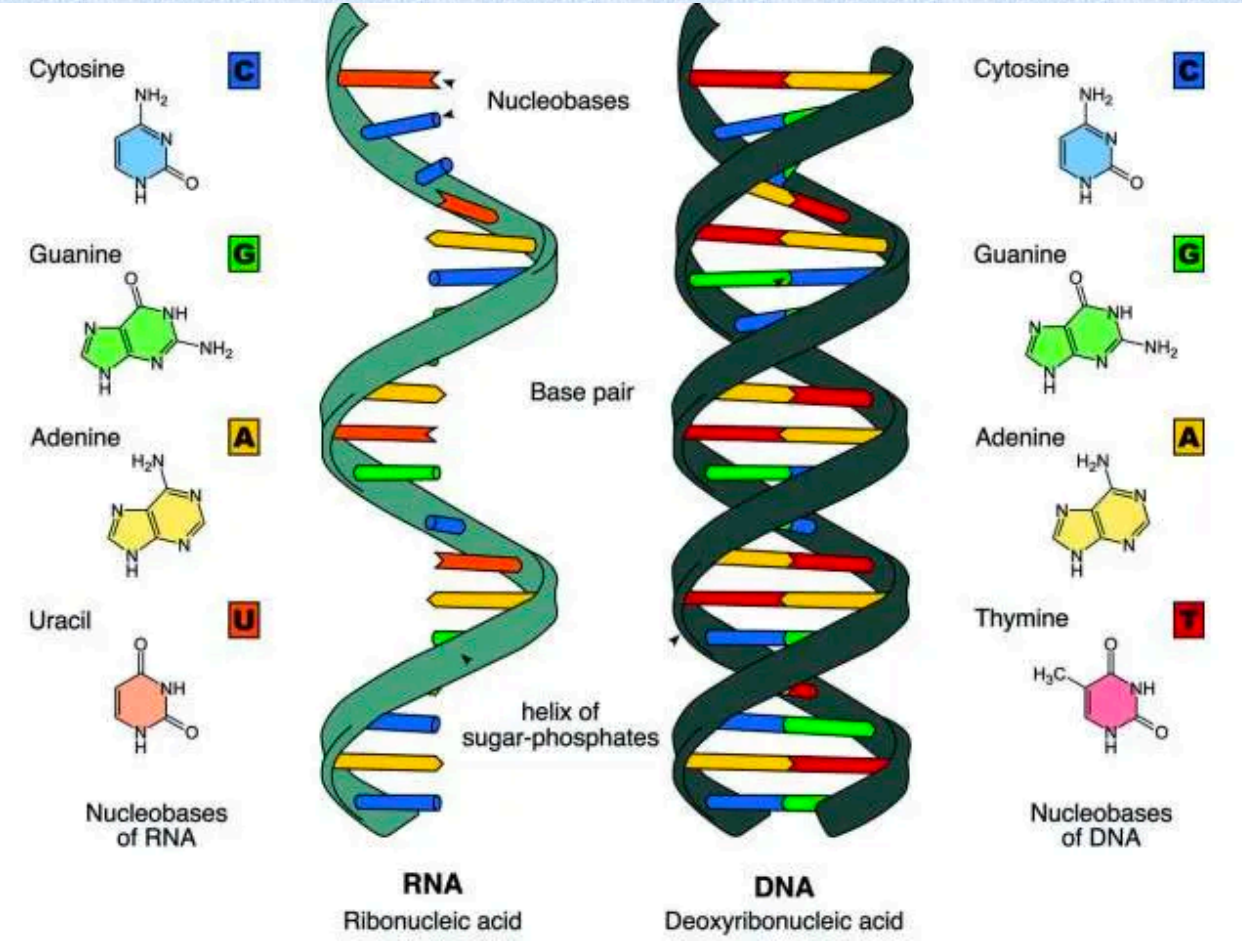
Rounding the Earth Podcast with Mathew Crawford

December 5, 2022

Dr. Jessica Rose

What is RNA?

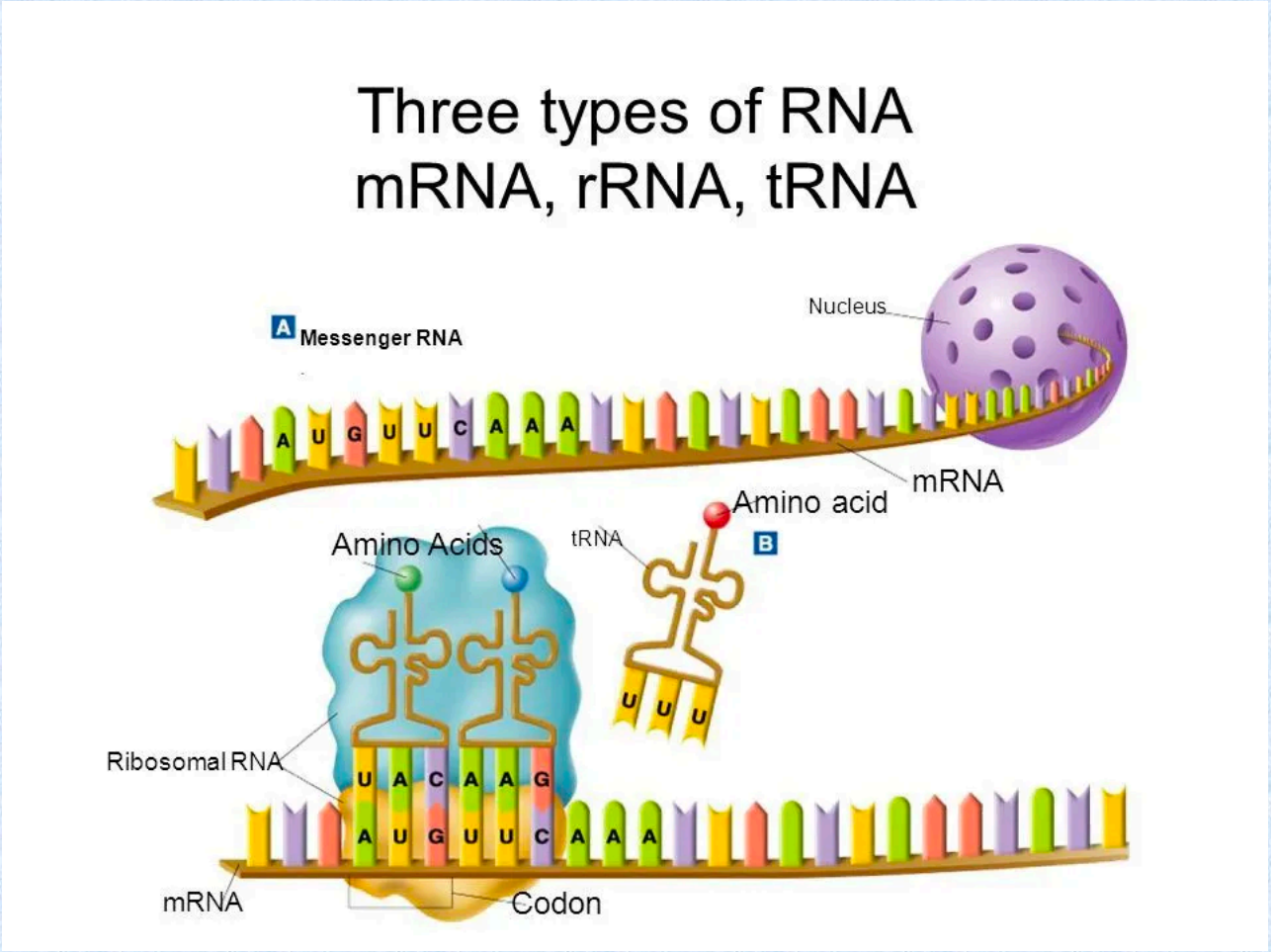
- RNA is functional genetic coding material made up of 4 nucleotide (nt) bases – Uracil, Guanine, Cytosine and Adenine
- It functions to catalyze biological reactions, control gene expression, sense and communicate and enable protein synthesis
- There are many types of RNA including messenger, transfer and ribosomal



Credit: Wikimedia Commons; https://phys.org/news/2018-09-ancestors-rna.html

What is messenger RNA (mRNA)?

- mRNA is the intermediate between DNA and protein and originates from a DNA template in the nucleus
- mRNA is trafficked from the nucleus to the cytoplasm where it is ‘read’ by cellular protein-making machinery
- Each ‘codon’ (set of 3 nts) is translated into a single amino acid to form a protein chain



Credit: https://www.pinterest.com.au/pin/74802043796409746/

What is a codon?

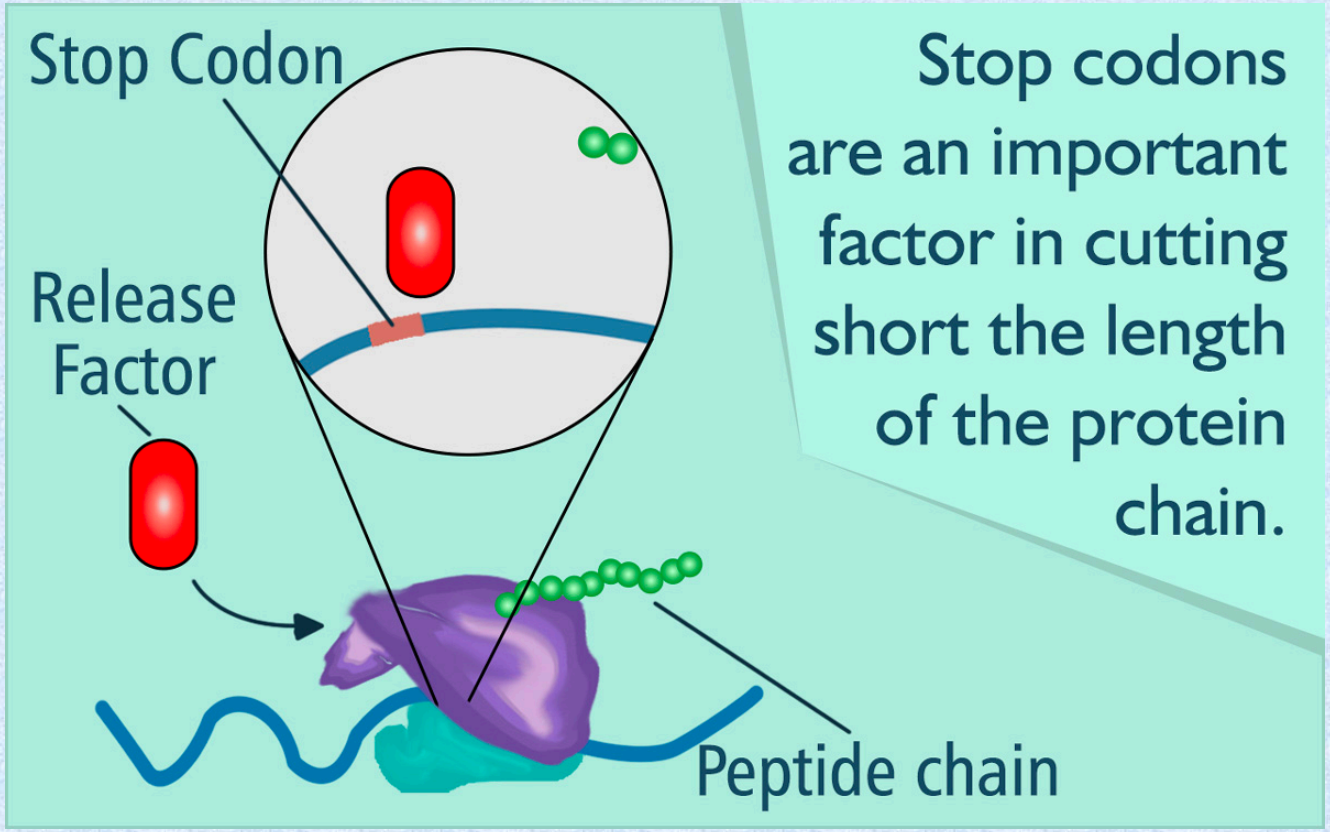
- A codon is a set of 3 nucleotide bases
- There are 64 possible codons that correspond to 20 amino acids + 1 stop codon - redundancy
- Codons are like nature's way of ensuring proper production of proteins during translation
- Specific or preferential codon usage (or codon bias) determines protein expression levels and protein function itself

First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; **UAA: Stop**; **UAG: Stop**	UGU, UGC: Cys; **UGA: Stop**; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; **AUG: Met**	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Credit: modification of work by NIH by https://openoregon.pressbooks.pub/mhccbiology112/chapter/the-genetic-code/

What is a stop codon?

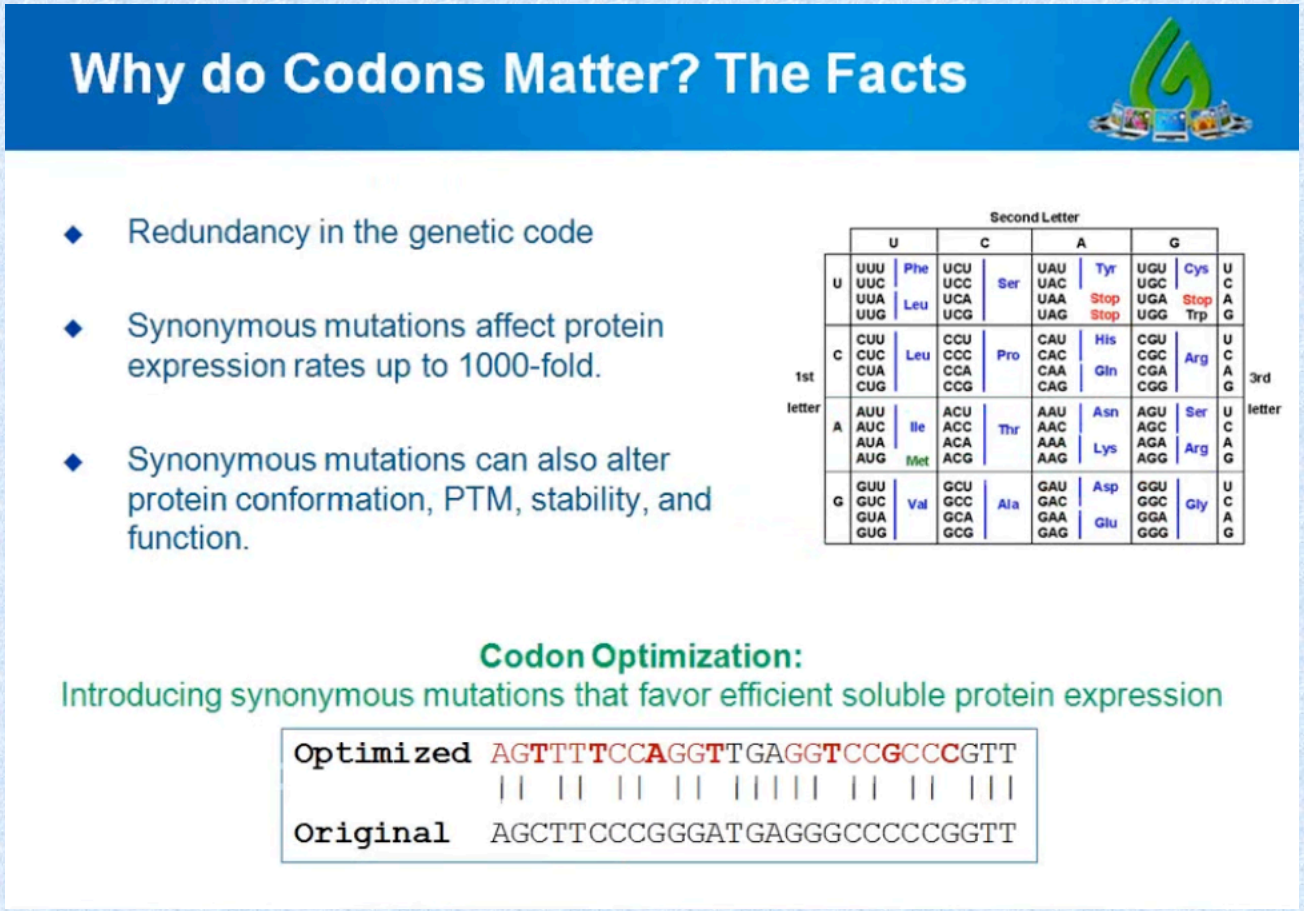
- **Stop** codons are nature's way to signal termination of the translation process to ensure the correct lengths of proteins
- RNA stop codons look like this: UAA; UAG; UGA



Credit: https://biologywise.com/stop-codon

What is a codon optimization?

- Codon usage is organism-specific and codon optimization is a process to maximize efficiency of translation and protein expression by accommodating the codon bias of the organism*
- Codons themselves can be altered artificially by swapping out one of the nts (called synonymous mutation) to optimize protein expression ➡️ the amino acid sequence will be the same, but protein expression could be more efficient

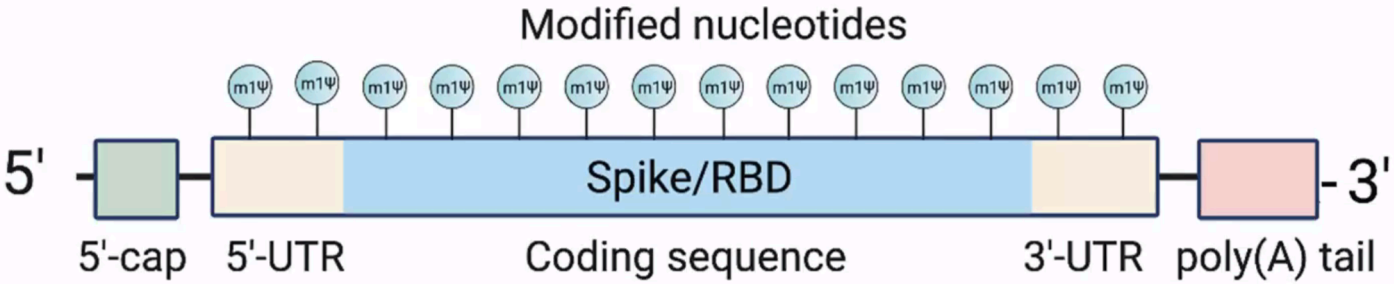


Credit: https://www.genscript.com/webinars/codon-optimization.html

*https://www.genewiz.com/en-GB/Public/Services/Gene-Synthesis/Codon-Optimization

How is codon optimization used in the spike protein development?

- The mRNA that codes for the SARS-CoV-2 spike protein was codon-optimized to ensure high fidelity translation and expression levels of spike protein
- The intention was to ensure that a lot of spike protein was made



- mRNA vaccines contain the genetic code to make spike protein
- The RNA is carefully engineered to resist breakdown
  - All of the uridines are replaced with 1-methyl-pseudouridine (m1Ψ)
- The mRNA is incorporated into a lipid particle along with polyethylene glycol (PEG)
- A synthetic cationic (positively charged) lipid is added as an adjuvant – very toxic to the cells
- The "humanized" mRNA is a stealth entry system for massive production of spike protein

*S Seneff et al. Food and Chemical Toxicology 2022; 164: 113008.

Seneff S, Nigh G, Kyriakopoulos AM, McCullough PA. Innate immune suppression by SARS-CoV-2 mRNA vaccinations: The role of G-quadruplexes, exosomes, and MicroRNAs. Food Chem Toxicol. 2022 Jun;164:113008.

Karikó K, Buckstein M, Ni H, Weissman D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. Immunity. 2005 Aug;23(2):165-75. doi: 10.1016/j.immuni.2005.06.008. PMID: 16111635.

Theoharides TC, Conti P. Be aware of SARS-CoV-2 spike protein: There is more than meets the eye. J Biol Regul Homeost Agents. 2021 May-Jun;35(3):833-838. doi: 10.23812/THEO_EDIT_3_21. PMID: 34100279.

What is RNA integrity?

- RNA integrity is the degree to which your RNA is intact
- mRNA can degrade quite readily due to temperature and pH changes, etc…
- There are many techniques for determining RNA integrity such as using RNA quality assessment and quantitation methods* or more algorithmic determinators like the RNA Integrity Number (RIN) method.

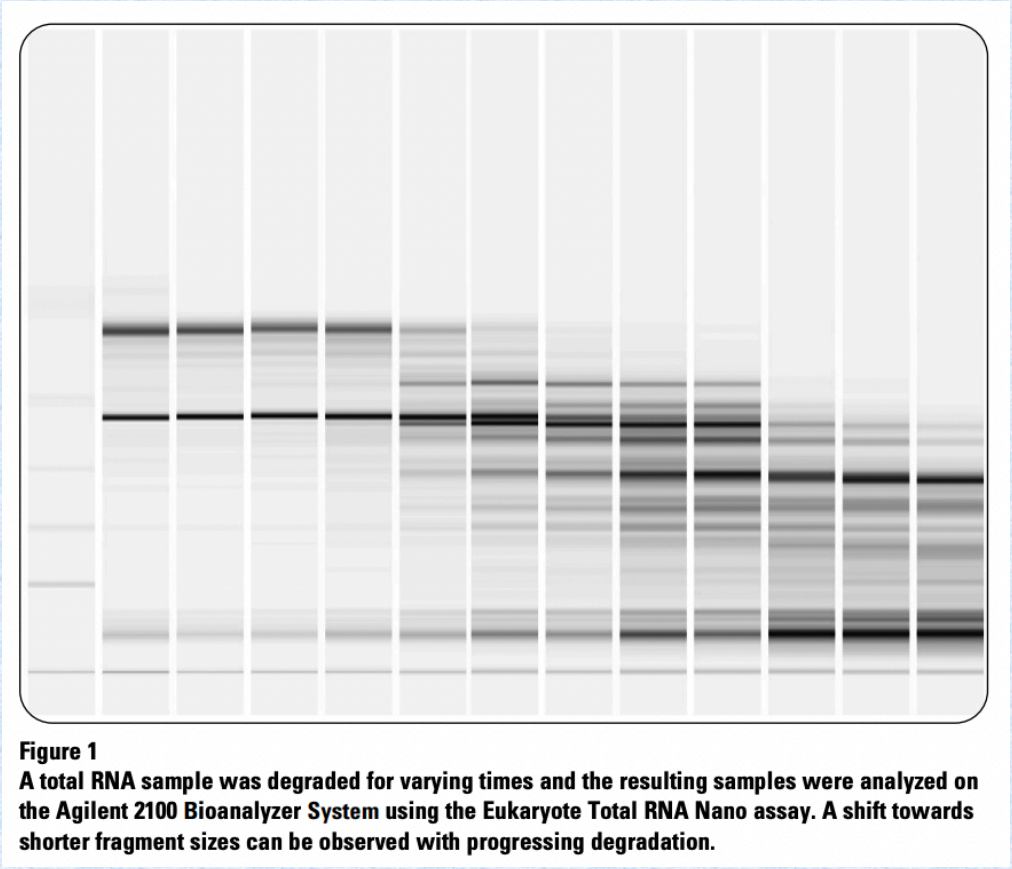


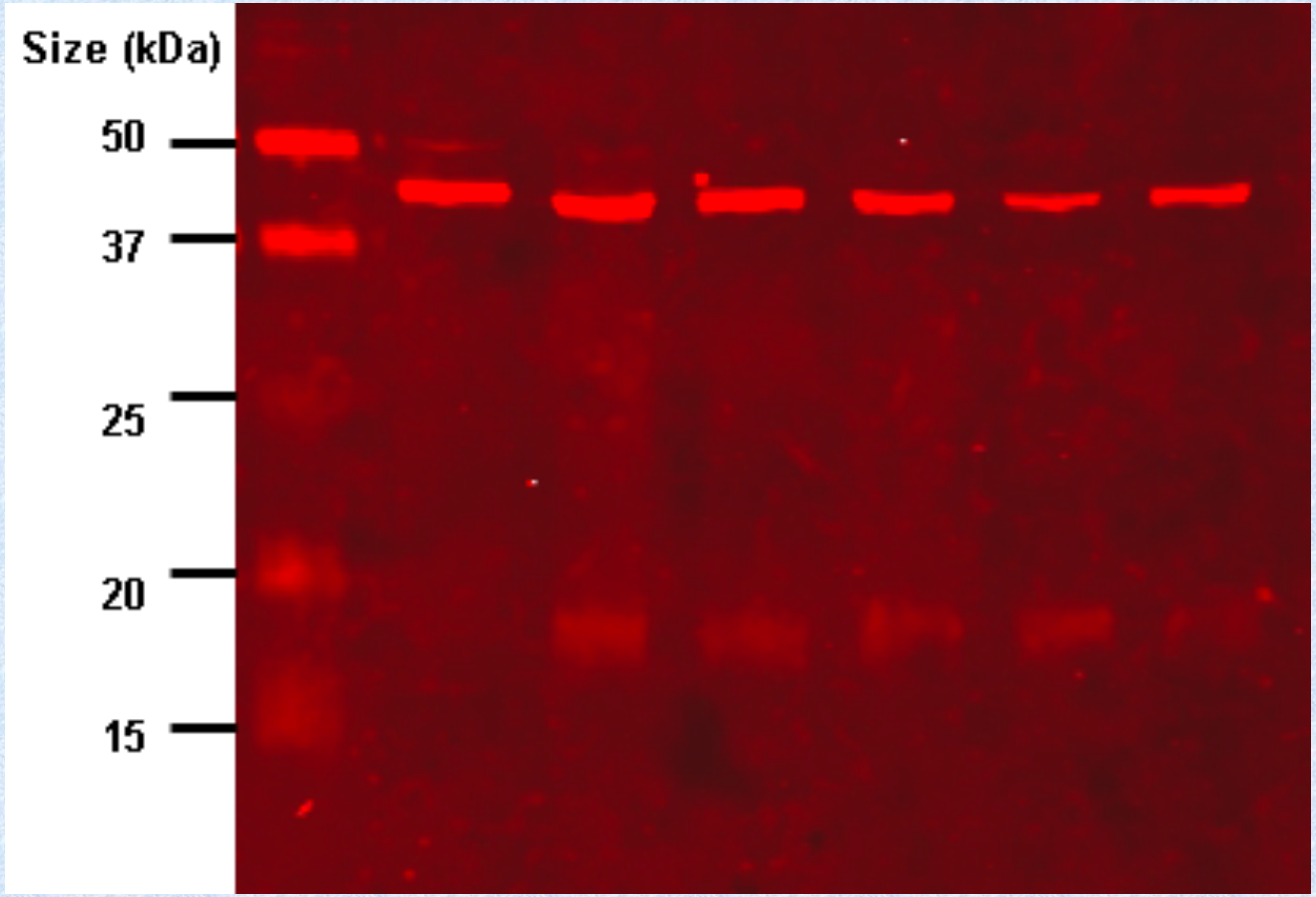
Figure 1
A total RNA sample was degraded for varying times and the resulting samples were analyzed on the Agilent 2100 Bioanalyzer System using the Eukaryote Total RNA Nano assay. A shift towards shorter fragment sizes can be observed with progressing degradation.

Credit: https://www.agilent.com/cs/library/applications/5989-1165EN.pdf

"Quantitation comparison of total RNA using the Agilent 2100 Bioanalyzer, ribogreen analysis, and UV spectrometry", Agilent Application Note, Publication Number 5988- 7650EN, 2002.
"A microfluidic system for highspeed reproducible DNA sizing and quantitation", Electrophoresis, 21(1), 128-34, 2000.

What is a Western Blot?

- A Western Blot is a biochemical lab technique used to characterize proteins in a sample, according to size.
- It is used for the qualitative detection of single proteins and protein-modifications
- “Additional data for the active substance are to be provided to confirm the identities of the observed Western Blot (WB) bands obtained by the in vitro expression assay.”*



Credit: Tim Vickers. https://en.wikipedia.org/wiki/Western_blot#/media/File:Anti-lipoic_acid_immunoblot.png

Relevance of RNA integrity?

- "It is important to recognize that the complete, intact mRNA molecule is essential to its potency as a vaccine. Even a minor degradation reaction, anywhere along a mRNA strand, can severely slow or stop proper translation performance of that strand and thus result in the incomplete expression of the target antigen."*

Table 3
Analytical Methods to Determine and Monitor Quality Attributes and Stability of mRNA Vaccine Bulk Drug Substance and Final Drug Product[a].

Assay	Purpose
Characterizing DNA templates and RNA transcripts	
DNA template sequencing/mRNA sequencing	Identification of mRNA
UV spectroscopy (A260 nm, A260/A280, A260/A230)	Quantification – purity dependent
Fluorescence-based assays (e.g., residual DNA)	Quantification – purity assessment
Agarose/acrylamide electrophoresis	Molecular mass, RNA integrity and quantification
Reverse transcriptase qPCR	Identification and quantification of mRNA
Blot for dsRNA	Quality assessment
mRNA capping analysis	Quality assessment
mRNA polyadenylated tail analysis	Quality assessment
Chromatographic assays[b]	Quantity and quality assessment
Characterizing mRNA-encoded translation products	
In vitro translation – cell free medium	Translation into target protein
mRNA evaluation using various cell-based systems	Translation product analysis and potential toxicity assay
Characterizing mRNA-lipid/protein complexes	
Light scattering[c]	Particle size (distribution)
(Gel) electrophoresis	Assessing bound/unbound mRNA and surface charge
Laser Doppler electrophoresis	Zeta potential
Chromatographic assays[b]/mass spectrometry	Quantification and integrity of carrier lipids/protein
Fluorescent dyes	Encapsulation efficiency
General pharmaceutical tests	
	Appearance, pH, osmolality, endotoxin concentration, sterility

[a] Adapted from Poveda et al., 2019[32] and Muralidhara et al., 2016.[19]
[b] Size-exclusion chromatography, anion-exchange chromatography, affinity chromatography, reversed-phase chromatography.
[c] Dynamic light scattering, static light scattering, nanoparticle tracking analysis.

Credit: Crommelin *at el.*,Table 3. Addressing the Cold Reality of mRNA Vaccine Stability

*Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the Cold Reality of mRNA Vaccine Stability. J Pharm Sci. 2021 Mar;110(3):997-1001. doi: 10.1016/j.xphs.2020.12.006. Epub 2020 Dec 13. PMID: 33321139; PMCID: PMC7834447.

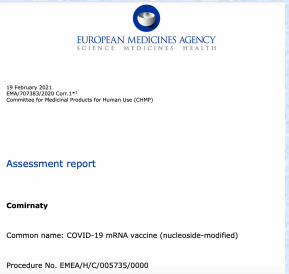
The risk of translating/translated proteins/peptides other than the intended spike protein is unknown

- RNA integrity was found to be 55% in commercial batches tested
- %RNA integrity in the mRNA of the COVID-19 injectable products was assessed by the EMA (European Medicines Agency) and it

Issue: A significant difference in %RNA integrity / truncated species has been observed between the clinical batches (~ 78% mRNA integrity) based on which the Interim analysis was performed and the proposed commercial batches (~ 55%).

The company claims that the efficacy of the drug product is dependent on the expression of the delivered RNA, which requires a **sufficiently intact RNA molecule.** The root cause for for the lower %RNA integrity at commercial batches has not yet been identified

Impact: The potential implications of this RNA integrity loss in commercial batches compared to clinical ones in terms of both safety and efficacy are yet to be defined. Whether or not the observed comparability issues could be a blocking point will depend on the relevance of these observations to safety and efficacy and the company will be requested to fully justify the lower %RNA integrity (and other differences noted).


EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

19 February 2021
EMA/707383/2020 Corr.1*
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Comirnaty

Common name: COVID-19 mRNA vaccine (nucleoside-modified)

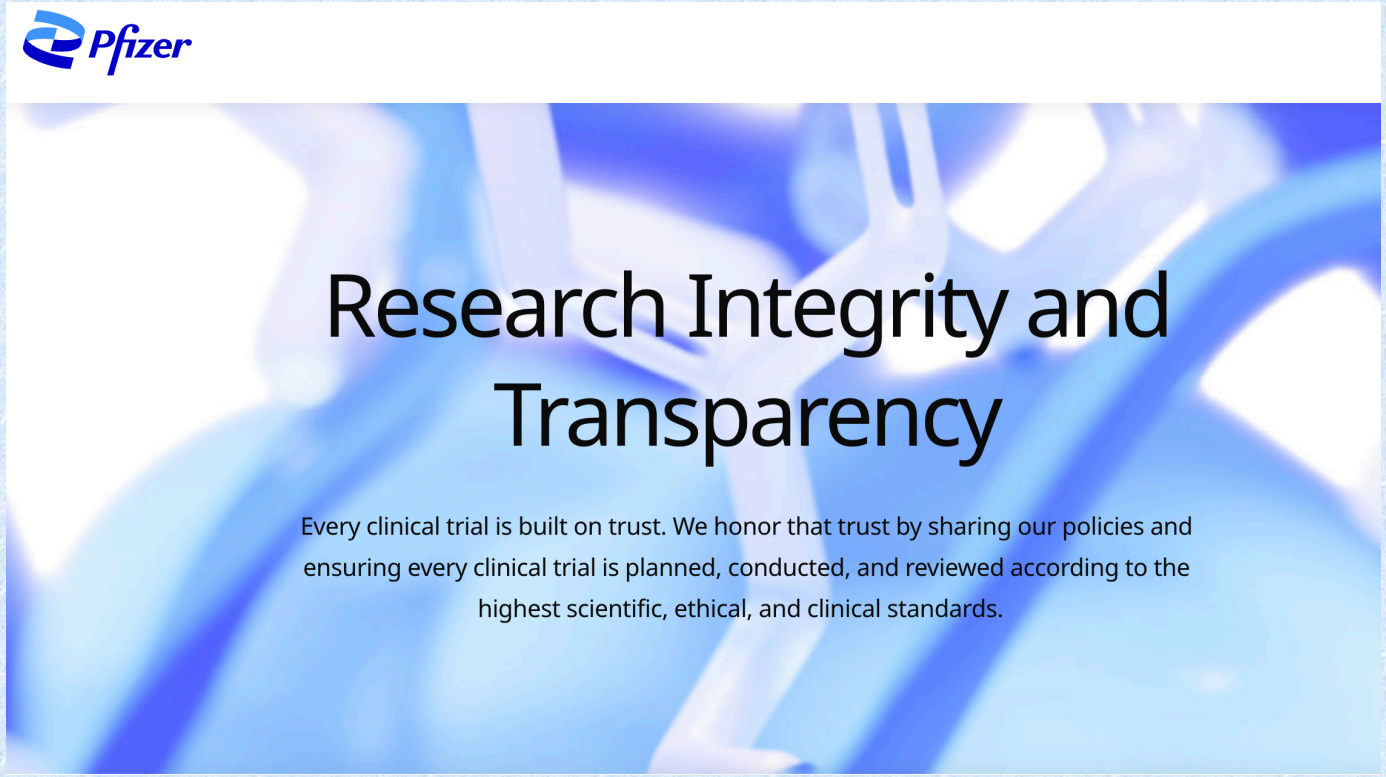
Procedure No. EMEA/H/C/005735/0000

Tinari Serena. The EMA covid-19 data leak, and what it tells us about mRNA instability **BMJ** 2021; 372 :n627 doi:10.1136/bmj.n627
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf
https://jessicar.substack.com/p/evidence-of-connection-between-severe

Pfizer thinks it's important to have integrity!

- What's concerning is that the manufacturer (Pfizer/BioNTech) claimed, "The efficacy of the drug product is dependent on the expression of the delivered RNA, **which requires a sufficiently intact RNA molecule**."

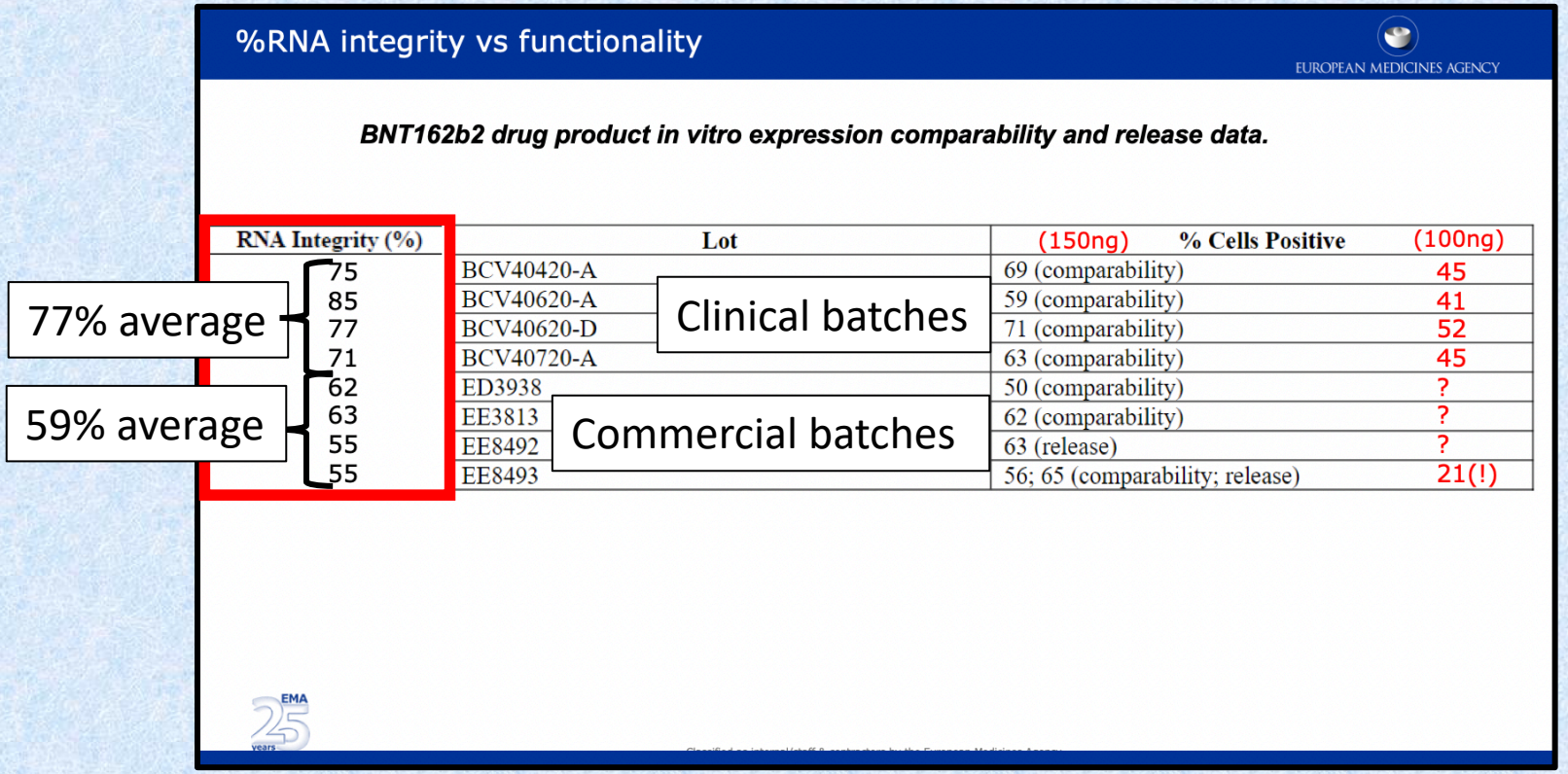
- **Sufficiently?**



https://www.pfizer.com/science/clinical-trials/integrity-and-transparency

EMA Quality Office CMC observations of BioNTech COVID-19 mRNA injectable products

- RNA integrity assays revealed low %RNA integrity in 'real vax lots' versus lab lots
- Is 18% lower integrity in commercial batches 'sufficient'?

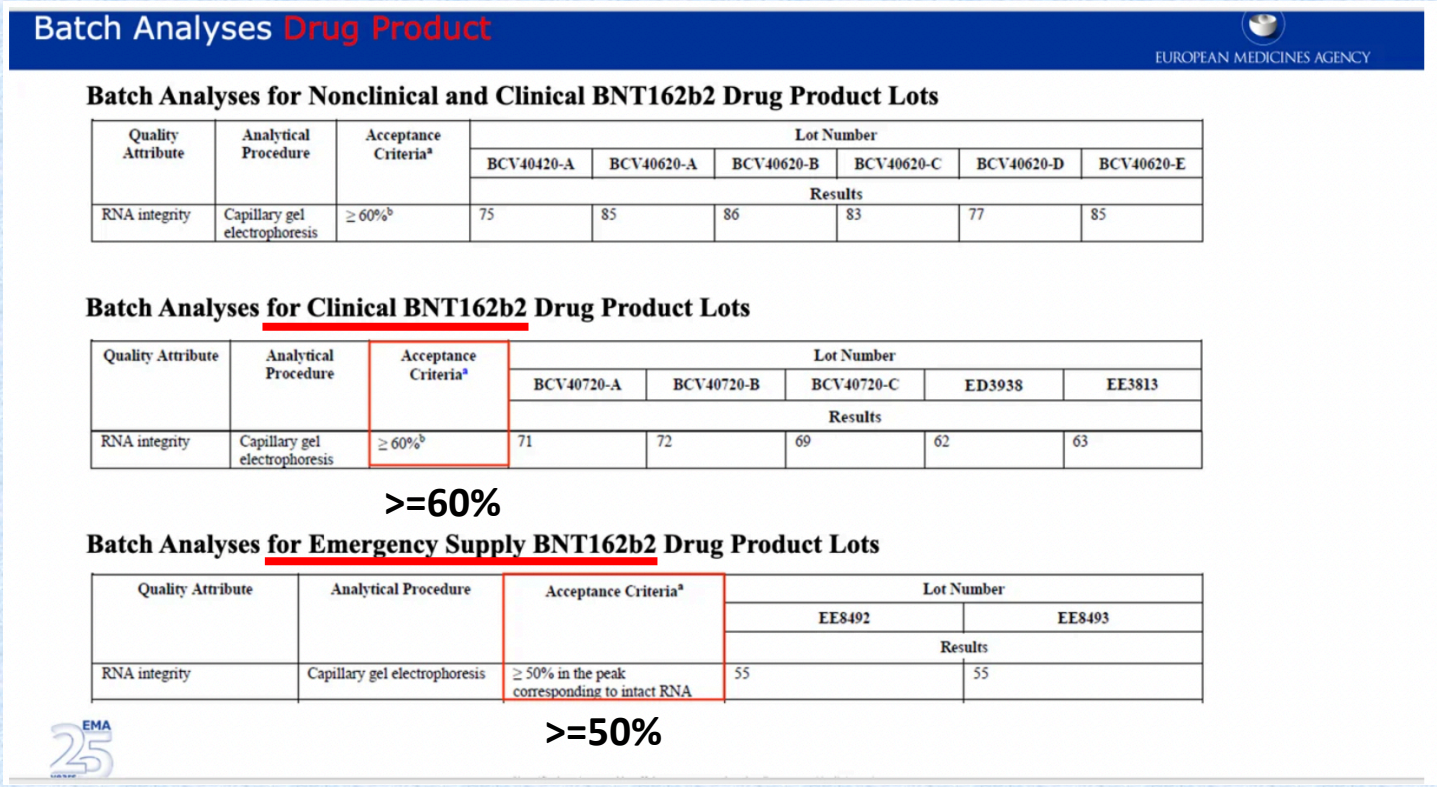


Credit: BNT CMC Peer Reviewers Ton der Stappen and Brian Dooley
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

*BioNTech COVID19 mRNA vaccine (nucleoside modified) EMA Quality Office CMC observations. BWP 24th November. Ton van der Stappen and Brian Dooley

They lowered the threshold for acceptable %RNA integrity for EU commercial products to get around the low %RNA integrity issue

- The stuff being injected into people likely has ~50% RNA integrity
- "…which requires a sufficiently intact RNA molecule" *Pfizer*
- **"However, when present in the cell there is a possibility that aberrant proteins will be expressed with possibilities for unwanted immunological events."***



Batch Analyses Drug Product

EUROPEAN MEDICINES AGENCY

Batch Analyses for Nonclinical and Clinical BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria[a]	Lot Number					
			BCV40420-A	BCV40620-A	BCV40620-B	BCV40620-C	BCV40620-D	BCV40620-E
			Results					
RNA integrity	Capillary gel electrophoresis	≥ 60%[b]	75	85	86	83	77	85

Batch Analyses for Clinical BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria[a]	Lot Number				
			BCV40720-A	BCV40720-B	BCV40720-C	ED3938	EE3813
			Results				
RNA integrity	Capillary gel electrophoresis	≥ 60%[b]	71	72	69	62	63

>=60%

Batch Analyses for Emergency Supply BNT162b2 Drug Product Lots

Quality Attribute	Analytical Procedure	Acceptance Criteria[a]	Lot Number	
			EE8492	EE8493
			Results	
RNA integrity	Capillary gel electrophoresis	≥ 50% in the peak corresponding to intact RNA	55	55

>=50%

Credit: BNT CMC Peer Reviewers Ton der Stappen and Brian Dooley*

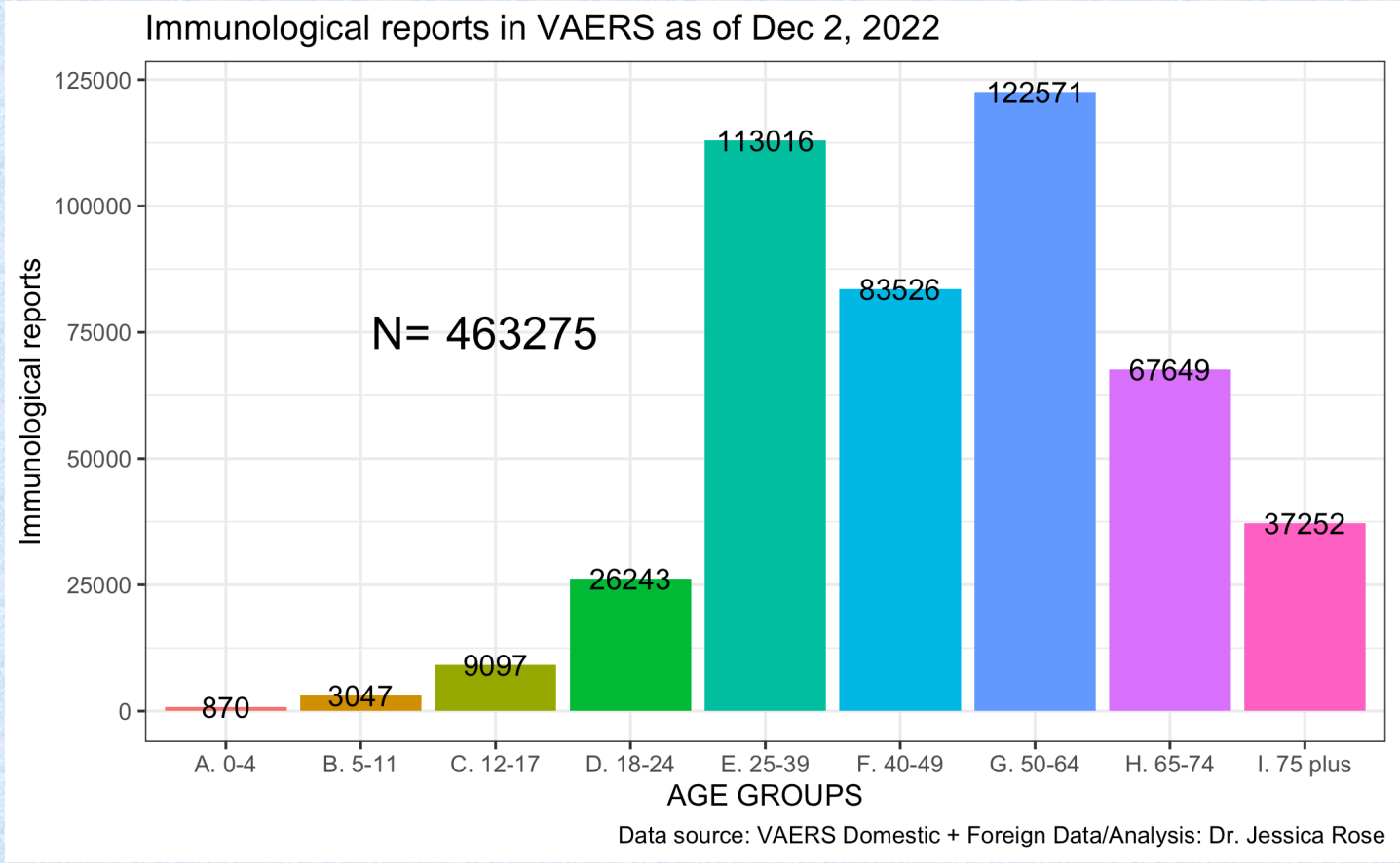
*BioNTech COVID19 mRNA vaccine (nucleoside modified) EMA Quality Office CMC observations. BWP 24th November. Ton van der Stappen and Brian Dooley

https://childrenshealthdefense.eu/eu-issues/a-further-investigation-into-the-leaked-ema-emails-confidential-pfizer-biontech-covid-19-vaccine-related-docs/

https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

Unwanted immunological effects, eh?

- Like what, you ask?
- Like any of the 1291 listed ‘adverse events of special interest’ documented in the FOIA-requested court-ordered Pfizer 5.3.6 Cumulative Analysis of Post-authorization Adverse Event Reports document?
- Like any of the 463,275 immunologically-related adverse event reports in VAERS as of Dec 2, 2022?



mRNA injectable products not stable after 6 months – degradation of product will ensue

Table 1
Current Stability Profile, Dose and Dosing Schedule of mRNA COVID-19 Vaccine Candidates in Development (Status December 05, 2020).

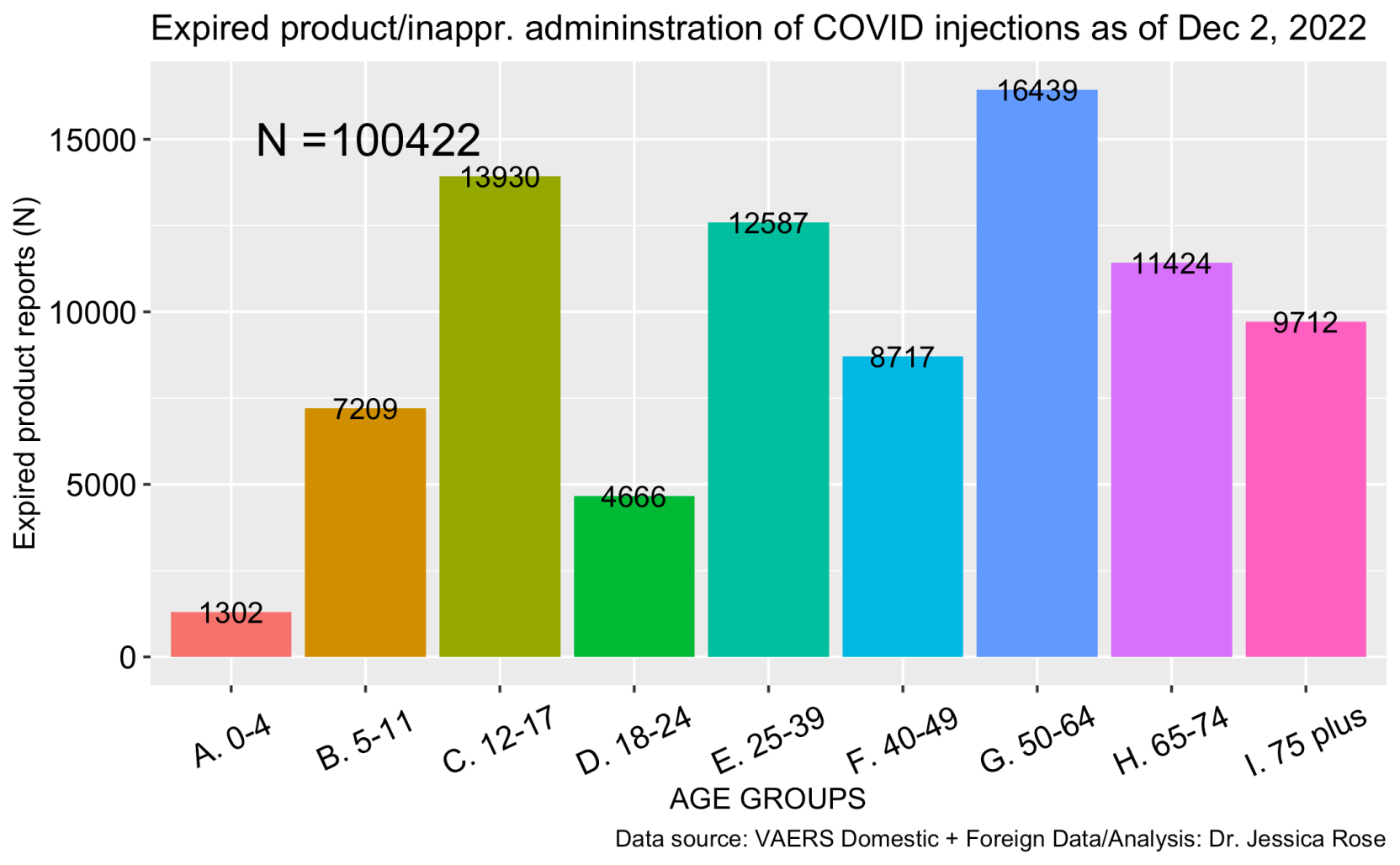
Manufacturer	Stability in Frozen State	Stability at 2–8 °C	Stability at Room Temperature	Dose (Injection Volume); Dosing Schedule	References
Moderna	−20 °C, up to 6 months	30 days	Up to 12 h	100 μg (0.5 mL); day 1, day 29	36,37
Pfizer-BioNTech	−80 °C to −60 °C, up to 6 months	up to 5 days	Up to 2 h (up to 6 h after dilution[a])	30 μg (0.3 mL); day 1, day 21	5,38,39
CureVac	≤ −60 °C, at least 3 months	At least 3 months	Up to 24 h	12 μg (no information); day 1, day 29	40–42

[a] The thawed vaccine must be diluted in its original multidose vial with unpreserved 1.8 mL sodium chloride 9 mg/mL (0.9%) solution for injection (not provided with the vaccine).[39]

Are expired batches being injected into people?
And is this resulting in more or fewer reported SAEs?

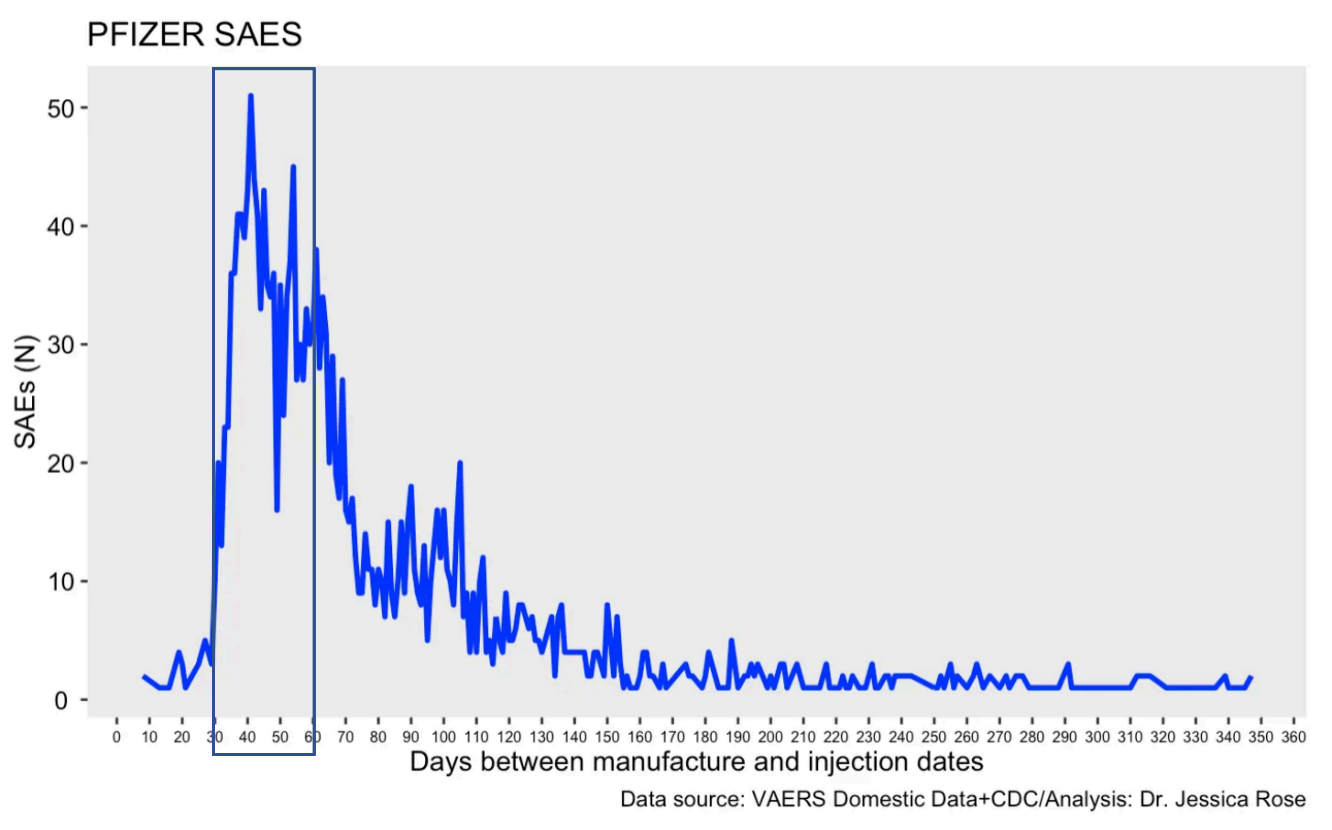
https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf

That would be a definitive: ‘YES’ to question 1



https://vaers.hhs.gov/

Maximum SAE count appears to fall inside the 1-2 month delta window

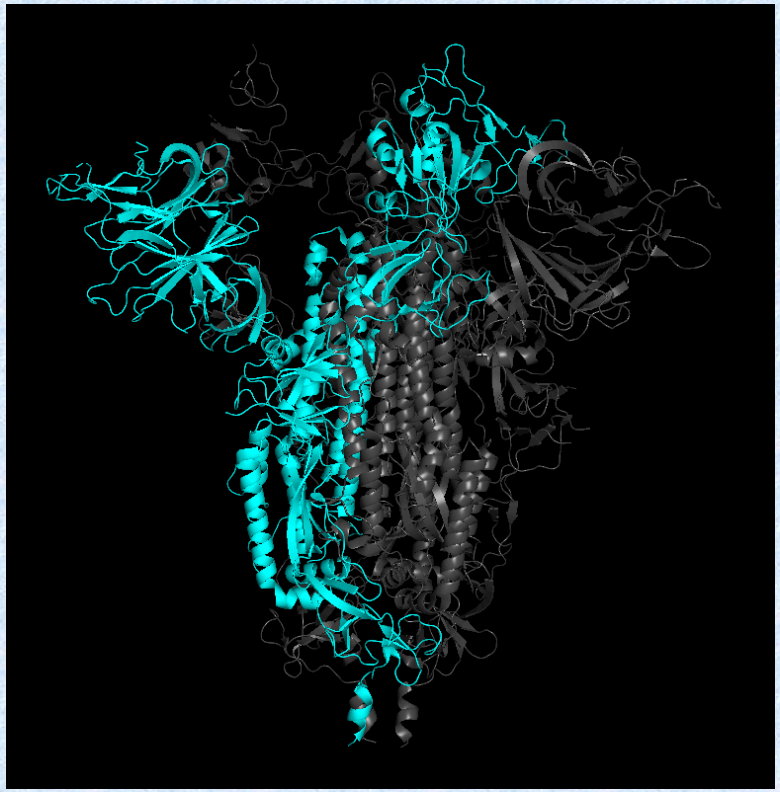


Plot showing the number of Severe Adverse Events versus the time between the manufacturing date and the injection date for the Pfizer mRNA COVID-19 injectable products.

https://jessicar.substack.com/p/evidence-of-connection-between-severe

Hypothetical scenarios

High (100) %RNA integrity
Full length spike

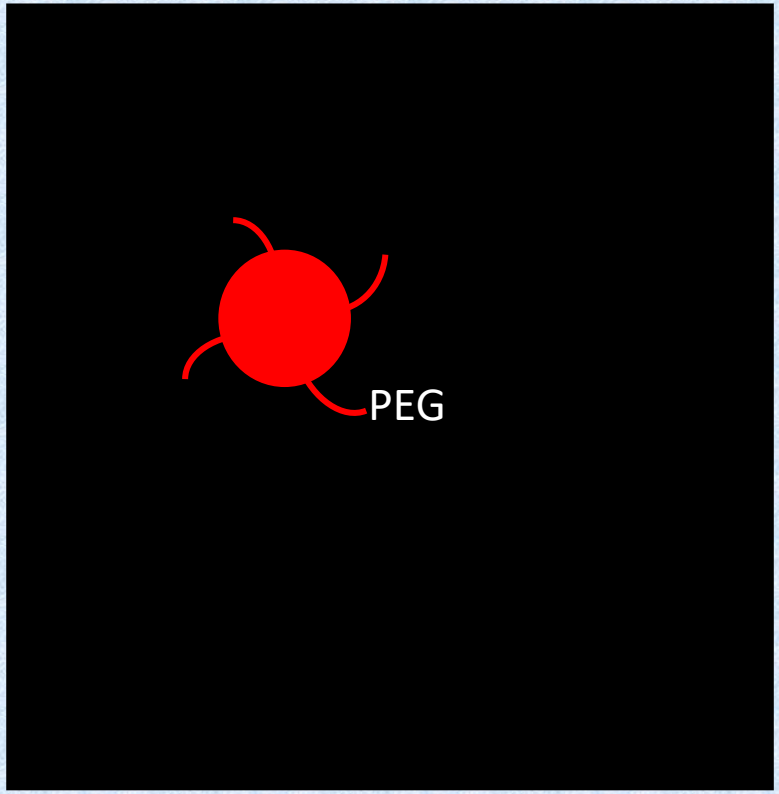


Large-scale production of full-length immunogenic spike proteins

Low (50) %RNA integrity
Truncated peptides

Large-scale production of full-length immunogenic spike proteins and smaller scale production of spike peptides

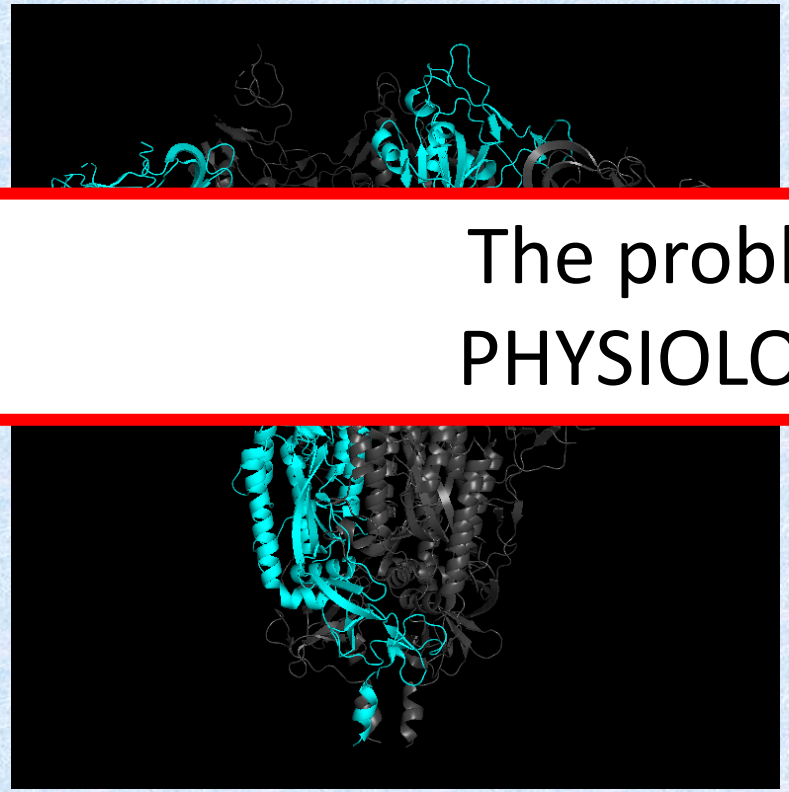
No (0) %RNA integrity
No spike protein



No production of spike protein or peptides

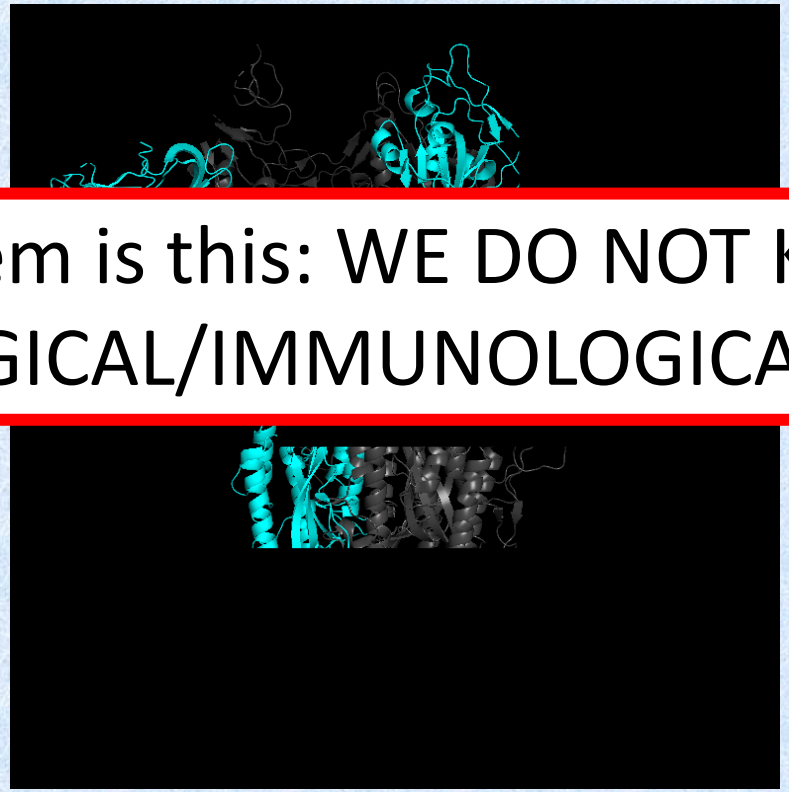
Hypothetical scenarios

High (100) %RNA integrity
Full length spike



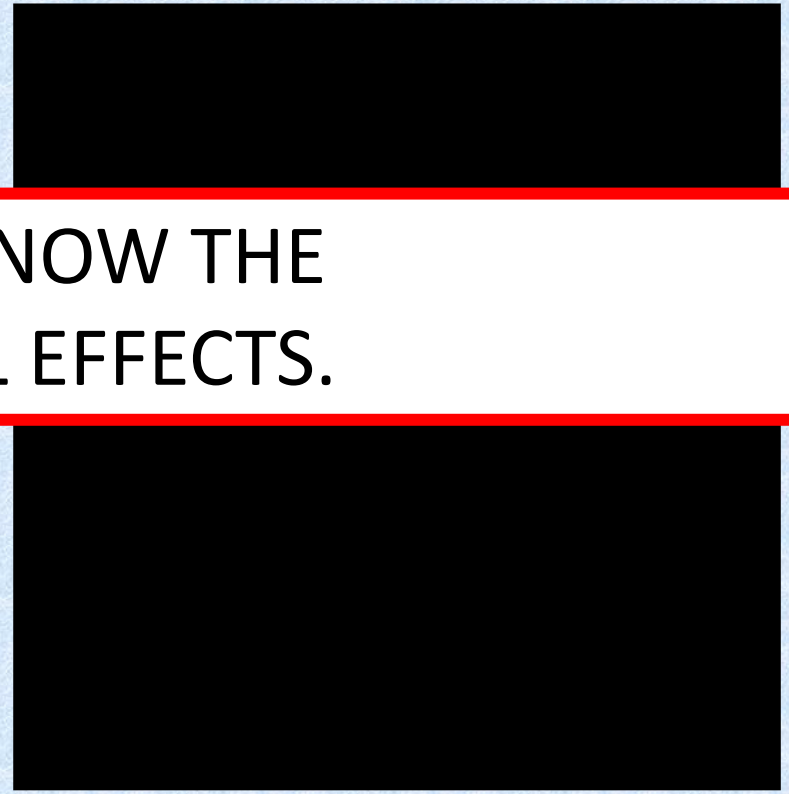
Spike-related cytotoxicity?

Low (50) %RNA integrity
Truncated peptides



Autoimmunity? Molecular mimicry?

No (0) %RNA integrity
No spike protein



'LNP problem?'

The problem is this: WE DO NOT KNOW THE PHYSIOLOGICAL/IMMUNOLOGICAL EFFECTS.

‘The speed of science’

The original *Trial Site News* report discussed what those CMC (Chemistry Manufacturing and Controls) issues were with Pfizer/BioNTech, particularly the loss of RNA integrity in the commercial batches and the unknown visible particles observed. Wathion tellingly states, ‘there are **still** issues’ speaks to the notion these ‘issues’ were not solved but had been ongoing. Wathion’s concern of ‘*compromising the robustness of the review*’ because of the need to authorise ‘on time’ is emphasized. This worry of speed over safety is reflected in other leaked emails, particularly by Wathion.

https://www.news.com.au/technology/science/human-body/pfizer-did-not-know-whether-covid-vaccine-stopped-transmission-before-rollout-executive-admits/news-story/f307f28f794e173ac017a62784fec414