DNA contamination in the mRNA vaccines

Decentralizing Peer Review

South Carolina, November 2025

Kevin McKernan, CSO Medicinal Genomics

Background

- 30 years in the Genomics Field
- One of 5 Genome Centers funded by Francis Collins ($27M)
  - Agencourt Biosciences, Whitehead/MIT, Baylor, Venter, WashU
  - Specialized in Plasmid Purification and Sequencing

- 60K Citations. Publications decorated the covers of multiple Journals
- Dozens of Patents
- Invented and Engineered the SOLiD sequencer ($6M NIH grant)
- Founded 4 genomics companies and have exited 3 of them

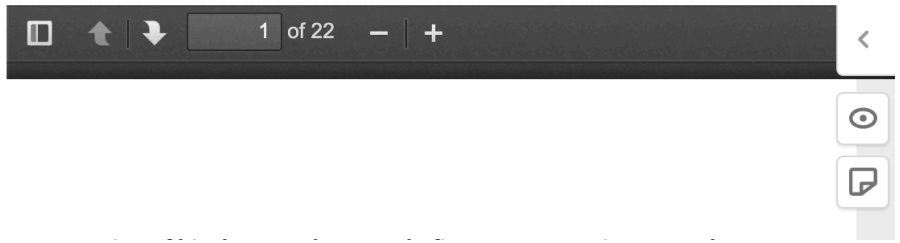
Illumina sequencing and RT-qPCR and qPCR

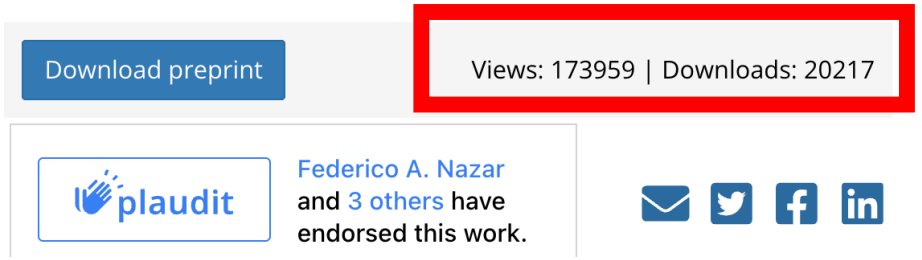
Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

AUTHORS
Kevin McKernan, Yvonne Helbert, Liam T. Kane, and Stephen McLaughlin

AUTHOR ASSERTIONS

CONFLICT OF INTEREST: Yes ▾ | PUBLIC DATA: Available ▾ | PREREGISTRATION: Not applicable ▾





All sequence data is public and qPCR assays and sequence publicly available.

Peer Reviewed- Speicher et al

AUTOIMMUNITY
2025, VOL. 58, NO. 1, 2551517
https://doi.org/10.1080/08916934.2025.2551517

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Quantification of residual plasmid DNA and SV40 promoter-enhancer sequences in Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines from Ontario, Canada

David J. Speicher[a], Jessica Rose[b] and Kevin McKernan[c]

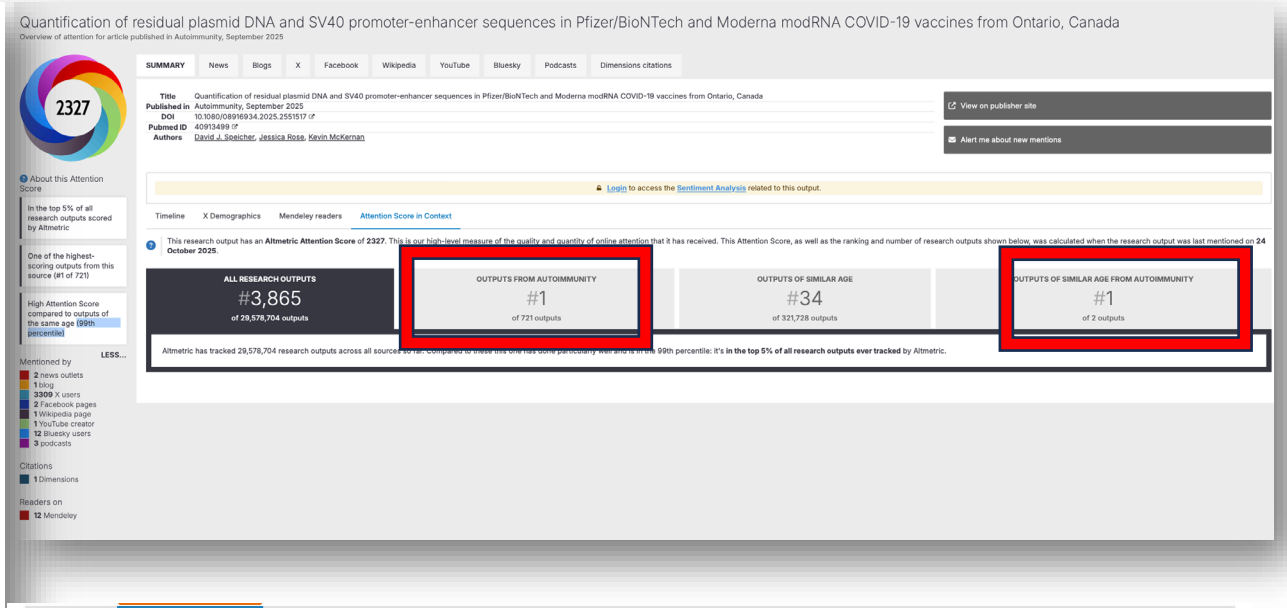
[a]Department of Pathobiology, University of Guelph, Guelph, ON, Canada; [b]Independent Researcher, Ontario, Canada; [c]Medicinal Genomics, Beverly, MA, USA

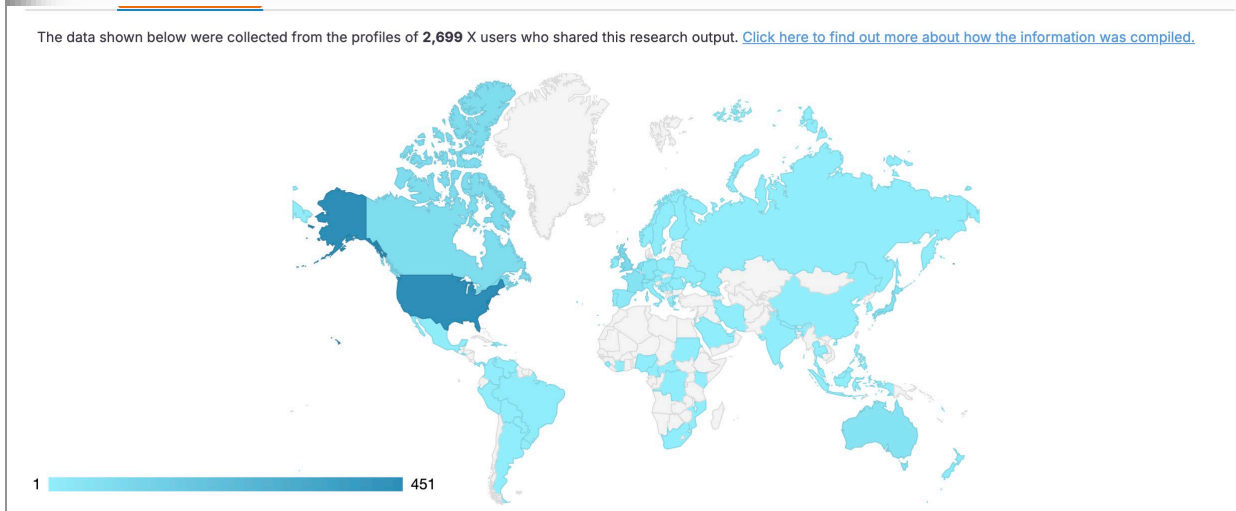
ABSTRACT

For some of the COVID-19 vaccines, the drug substances released to market were manufactured differently than those used in clinical trials. Manufacturing nucleoside-modified mRNA (modRNA) for commercial COVID-19 vaccines relies on RNA polymerase transcription of a plasmid DNA template. Previous studies identified high levels of plasmid DNA in vials of modRNA vaccines, suggesting that the removal of residual DNA template is problematic. Therefore, we quantified the DNA load in a limited number of Pfizer-BioNTech and Moderna COVID-19 modRNA vaccine vials using two independent methods. Total DNA and specific DNA targets were quantified by Qubit fluorometry and quantitative polymerase chain reaction (qPCR), respectively on 32 vials representing 16 unique vaccine lots. RNase A treatment was used to assess the impact of RNA crosstalk in DNA fluorometry. A preliminary assessment of DNA fragment length and DNase I sensitivity were also performed. Total DNA ranged 371–1,548 ng/dose and 1,130–6,280 ng/dose in Pfizer and Moderna products, respectively. Specific DNA of multiple plasmid DNA targets ranged 0.22–7.28 ng/dose for Pfizer, and 0.01–0.78 ng/dose for Moderna. The SV40 promoter-enhancer-*ori* (0.25–23.72 ng/dose) was only detected in Pfizer vials. Oxford Nanopore sequencing of one vial found mean and maximum DNA fragment lengths of 214 bp and 3.5 kb, respectively. These data demonstrate the presence of 1.23×10^{8} to 1.60×10^{11} plasmid DNA fragments per dose encapsulated in lipid nanoparticles. Using fluorometry, total DNA in all vials tested exceeded the regulatory limit for residual DNA set by the US Food & Drug Administration (FDA) and the World Health Authorization (WHO) by 36–153-fold for Pfizer and 112–627-fold for Moderna after accounting for nonspecific binding to modRNA. When tested by qPCR, all Moderna vials were within the regulatory limit, but 2/6 Pfizer lots (3 vials) exceeded the regulatory limit for the SV40 promoter-enhancer-*ori* by 2-fold. The presence of the SV40 promoter-enhancer element in Pfizer vials raises significant safety concerns. This study emphasizes the importance of methodological considerations when quantifying residual plasmid DNA in modRNA products, considering increased LNP transfection efficiency, and cumulative dosing presents significant and unquantified risks to human health.

ARTICLE HISTORY
Received 16 April 2025
Revised 16 July 2025
Accepted 10 August 2025

KEYWORDS
COVID-19; vaccine; modRNA; mRNA; residual DNA; DNA contamination





Geographical breakdown

Country	Count	As %
United States	451	17%
United Kingdom	93	3%
Canada	80	3%
Japan	53	2%
Netherlands	48	2%

Demographic breakdown

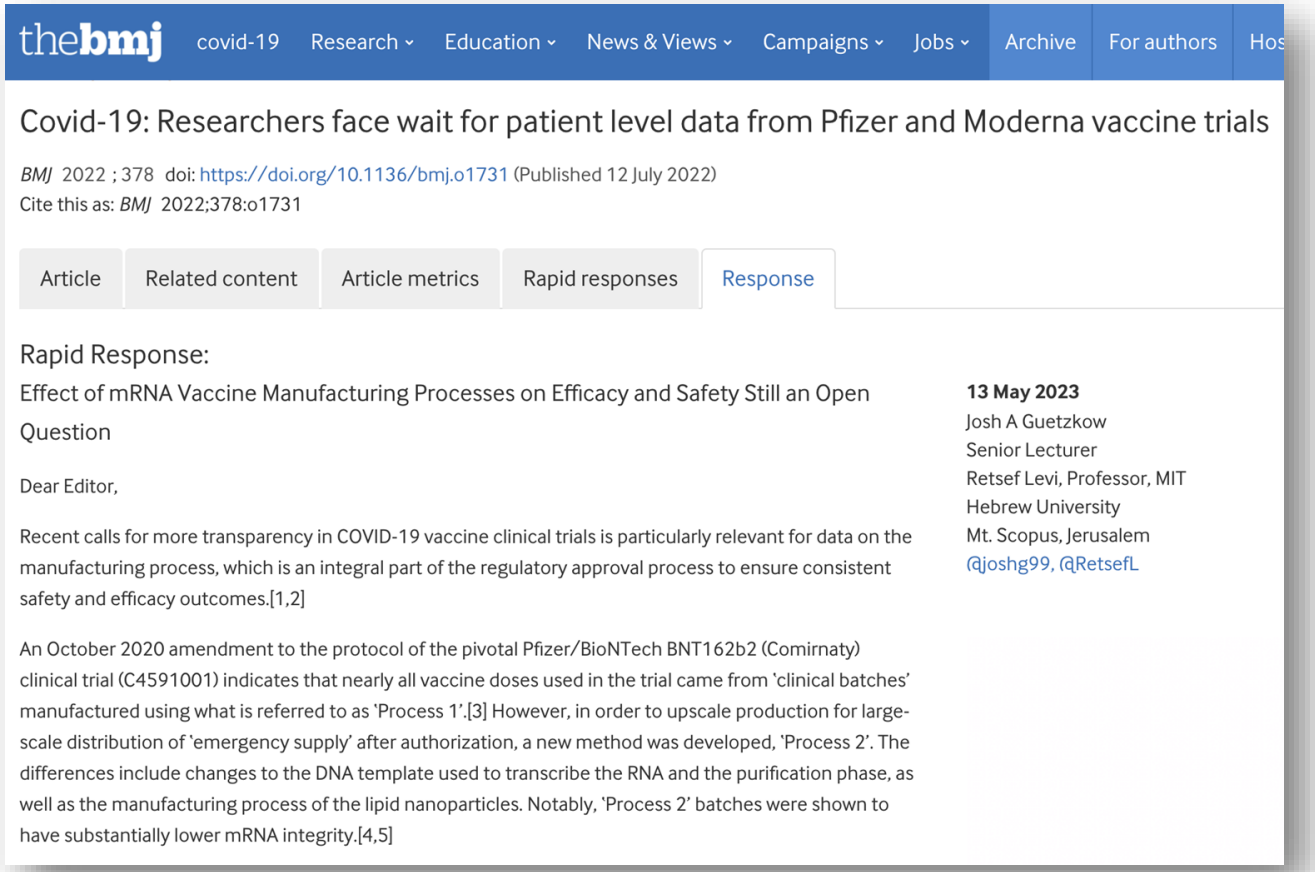
Type	Count	As %
Members of the public	2539	94%
Scientists	71	3%
Practitioners (doctors, other healthcare professionals)	67	2%
Science communicators (journalists, bloggers, editors)	21	<1%
Unknown	1	<1%

The 10ng limit is based on naked DNA

- It never considered LNP protected DNA
- Naked DNA has a 10 minute half life in the blood.
- LNP half life not known but assumed to be days to weeks.

Background- How did this happen?

Process 1 (IVT) vs Process 2 (E.coli)



thebmj covid-19 Research Education News & Views Campaigns Jobs Archive For authors Hos

Covid-19: Researchers face wait for patient level data from Pfizer and Moderna vaccine trials

BMJ 2022 ; 378 doi: https://doi.org/10.1136/bmj.o1731 (Published 12 July 2022)
Cite this as: *BMJ* 2022;378:o1731

Article | Related content | Article metrics | Rapid responses | Response

Rapid Response:

Effect of mRNA Vaccine Manufacturing Processes on Efficacy and Safety Still an Open Question

Dear Editor,

Recent calls for more transparency in COVID-19 vaccine clinical trials is particularly relevant for data on the manufacturing process, which is an integral part of the regulatory approval process to ensure consistent safety and efficacy outcomes.[1,2]

An October 2020 amendment to the protocol of the pivotal Pfizer/BioNTech BNT162b2 (Comirnaty) clinical trial (C4591001) indicates that nearly all vaccine doses used in the trial came from 'clinical batches' manufactured using what is referred to as 'Process 1'.[3] However, in order to upscale production for large-scale distribution of 'emergency supply' after authorization, a new method was developed, 'Process 2'. The differences include changes to the DNA template used to transcribe the RNA and the purification phase, as well as the manufacturing process of the lipid nanoparticles. Notably, 'Process 2' batches were shown to have substantially lower mRNA integrity.[4,5]

13 May 2023
Josh A Guetzkow
Senior Lecturer
Retsef Levi, Professor, MIT
Hebrew University
Mt. Scopus, Jerusalem
@joshg99, @RetsefL

The trial was run on Process 1 lots
250 people received Process 2 lots (plasmids)
The world received Process 2 lots

An October 2020 amendment to the protocol of the pivotal Pfizer/BioNTech BNT162b2 (Comirnaty) clinical trial (C4591001) indicates that nearly all vaccine doses used in the trial came from 'clinical batches' manufactured using what is referred to as 'Process 1'.[3] However, in order to upscale production for large-scale distribution of 'emergency supply' after authorization, a new method was developed, 'Process 2'. The differences include changes to the DNA template used to transcribe the RNA and the purification phase, as well as the manufacturing process of the lipid nanoparticles. Notably, 'Process 2' batches were shown to have substantially lower mRNA integrity.[4,5]

The protocol amendment states that "each lot of 'Process 2'-manufactured BNT162b2 would be administered to approximately 250 participants 16 to 55 years of age" with comparative immunogenicity and safety analyses conducted with 250 randomly selected 'Process 1' batch recipients. To the best of our knowledge, there is no publicly available report on this comparison of 'Process 1' versus 'Process 2' doses.

Two documents obtained through a Freedom of Information Act (FOIA) request[6] describe the vaccine batches and lots supplied to each of the trial sites through November 19, 2020[7] and March 17, 2021,[8] respectively. According to these documents, doses from 'Process 2' batch EE8493Z are listed at four trial sites prior to November 19, and four other sites are listed with 'Process 2' batch EJ0553Z in the updated document. Both batches were also part of the emergency supply for public distribution. The CDC's Vaccine Adverse Event Reporting System, known to be underreported,[9] lists 658 reports (169 serious, 2 deaths) for lot EE8493[10] and 491 reports (138 serious, 21 deaths) for lot EJ0553.[11]

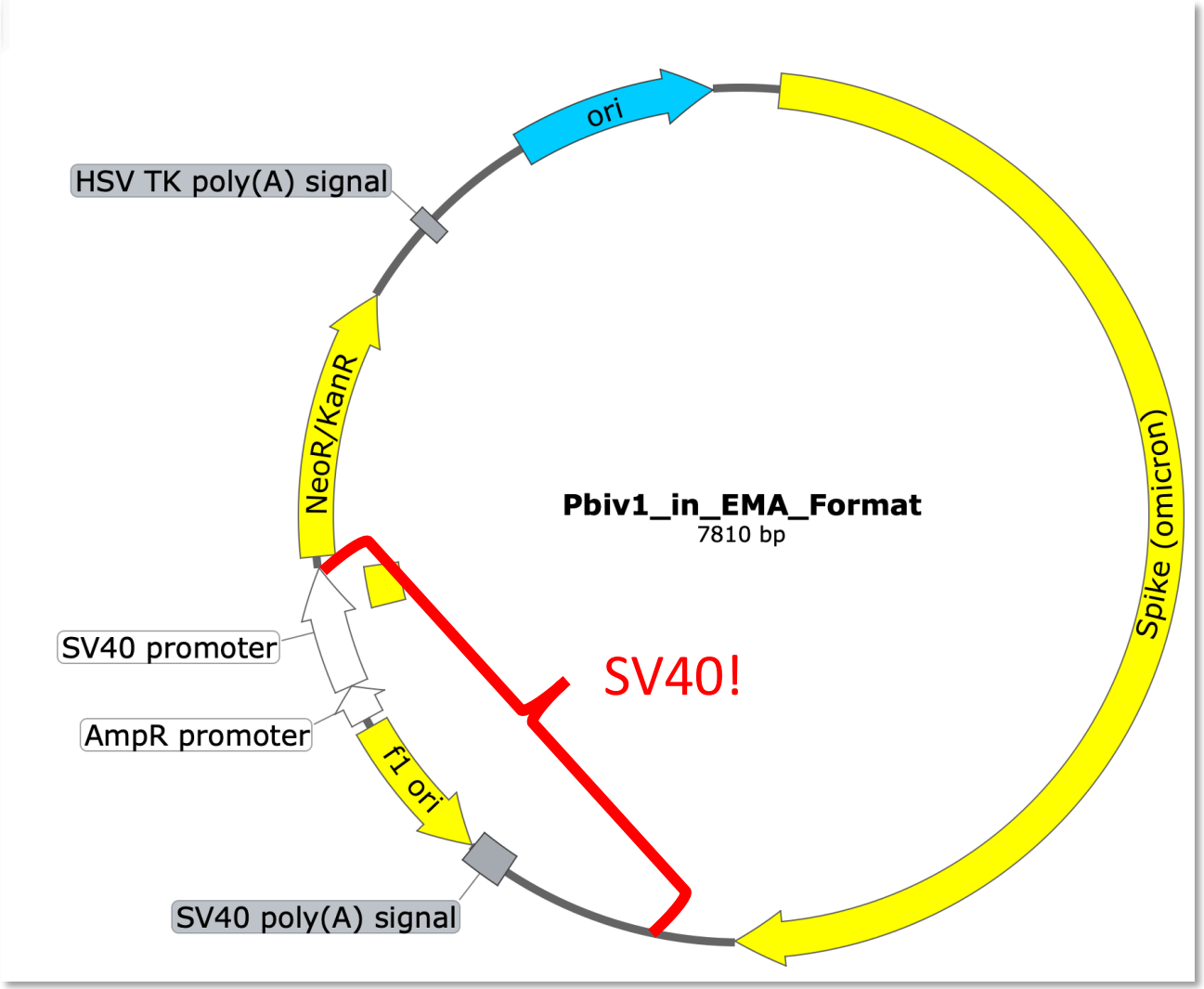
Furthermore, additional 'Process 1' batch EE3813 doses with distinct Pfizer lot numbers were added to the later batch document[7] at over 70% of trial sites, potentially supplied at a later stage to enable vaccination of placebo patients with BNT162b2. The 6-month interim clinical study report[12] from the Comirnaty trial notes that "the IR for any AE and at least 1 related AE and severe AE for participants who originally received placebo and then received BNT162b2 are greater (205.4 per 100 PY, 189.5 per 100 PY, 6.0 per 100 PY) than the IRs (83.2 per 100 PY, 62.9 per 100 PY, 4.3 per 100 PY) for participants who originally were randomized to BNT162b2" (p222). It is unclear whether there is a connection between the lots administered to the crossover placebo subjects and the elevated rate of AE's.

Finally, a recent study found significant variability in the rate of serious adverse events (SAEs) across 52 different lots of Comirnaty marketed in Denmark.[13] This finding underscores the importance of understanding better the potential impact of variability in the production process of COVID-19 mRNA vaccines on efficacy and safety.

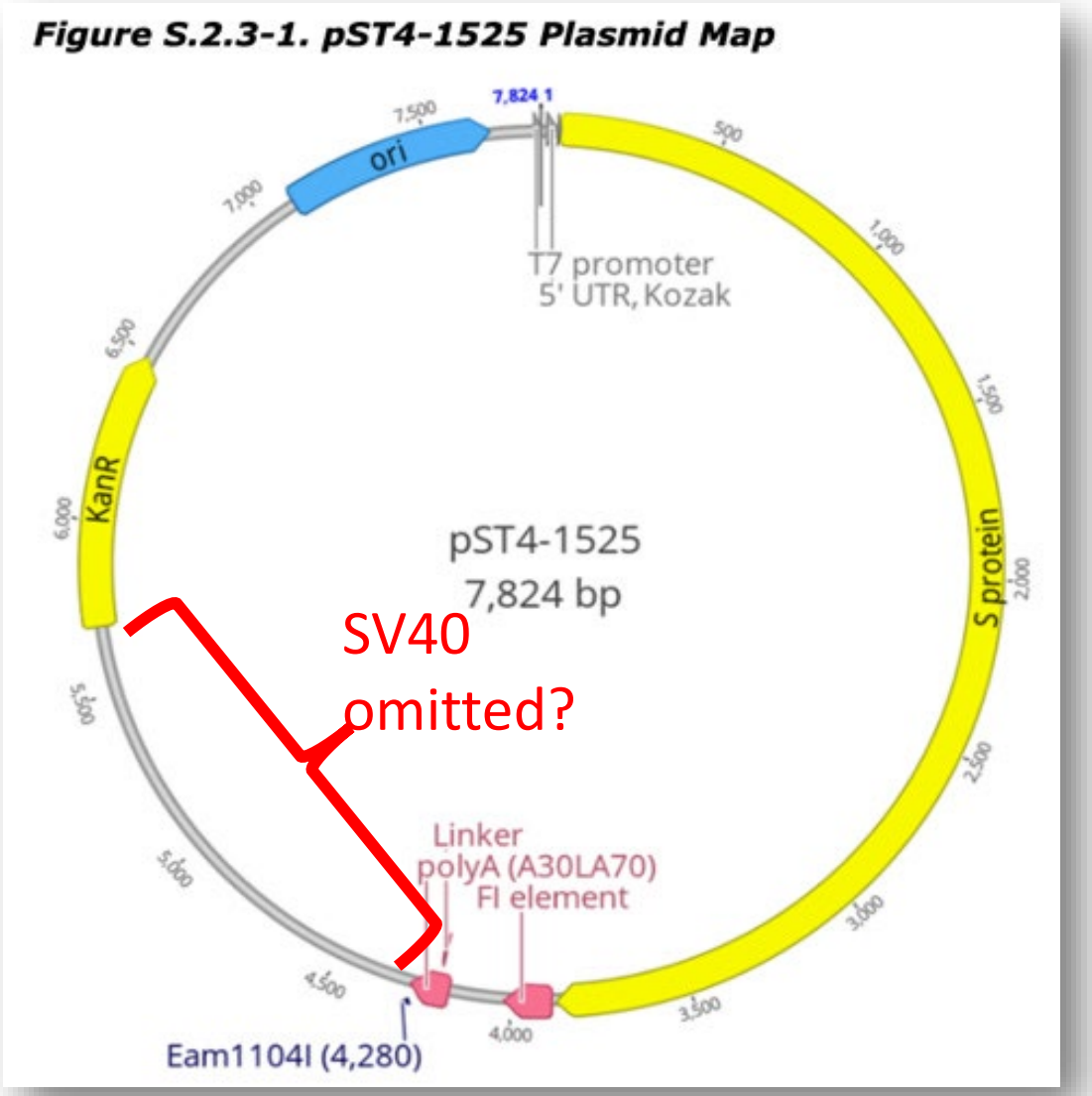
Evidence from existing research and trial documents highlights the importance of publicly disclosing the analysis comparing reactogenicity and safety of process 1 and 2 batches as specified in the trial protocol, and more generally patient-level batch and lot data from the trial.

Josh Guetzkow
Retsef Levi

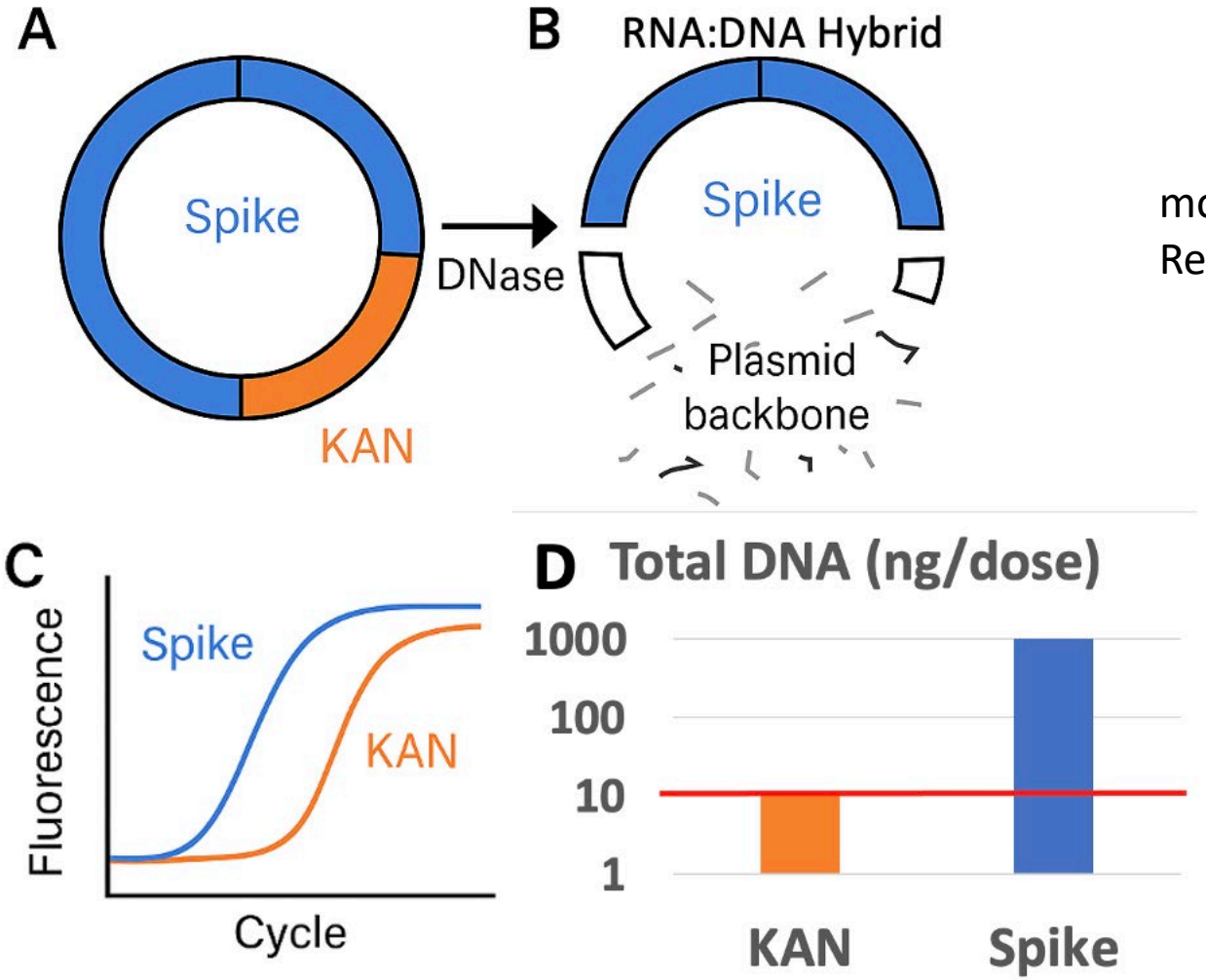
Independent Illumina sequencing



What was disclosed to the EMA



The regulators are looking in the wrong place



modRNA protects the spike DNA region from decay.
Regulators are only looking in the KAN region.

Initial Public Regulatory Response


1)Yes, SV40 is there


2)Yes, Pfizer did NOT spell this out


3)DNA is too small in length to matter


4)DNA is too small in quantity to matter


5)DNA is non-functional

 Regulators are correct

 Regulators are wrong

SV40 Enhancers are used in Gene Therapy: Nuclear Targeting Sequences (NTS)
Fact Checkers will not address this slide!

Dean Lab

Home

Projects

Publications

Lab Members

News

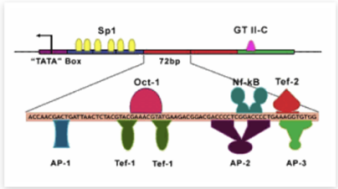
URMC / Labs / Dean Lab / Projects / Nuclear Targeting of Plasmids and Protein-DNA Complexes

Make a Gift

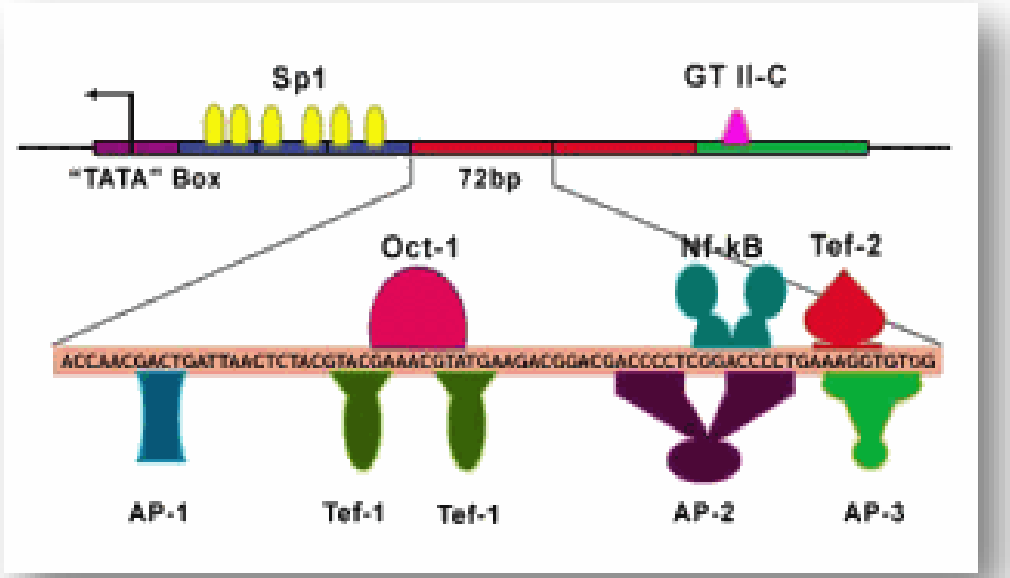
Nuclear Targeting of Plasmids and Protein-DNA Complexes

My laboratory studies the mechanisms and applications of plasmid and DNA-binding protein nuclear localization. Our long term goals are to develop gene therapy approaches to the treatment of a variety of human diseases by focusing on the development of novel non-viral intra- and extracellular delivery methods. Our main emphasis is in the area of pulmonary gene delivery and function. Perhaps the major problem hindering gene therapy is the inefficiency of gene transfer to slowly and non-dividing cells. While many aspects of non-viral vector design are being addressed, one critical area that has not received adequate attention is the nuclear import of vector DNA. Clearly, without the translocation of plasmid DNA into the nucleus, no gene expression, or "gene therapy" can take place. My laboratory continues to identify and characterize novel DNA sequences to promote nuclear import of non-viral vectors, both in cultured cells and in vivo, as well as sequences that promote cytoplasmic and intranuclear trafficking.

Over the past 15 years, work from our laboratory has addressed the nuclear targeting and entry of plasmid DNA. Using cultured cells, we have shown that plasmids are able to enter the nuclei of cells in the absence of cell division and its accompanying nuclear envelope breakdown. Assays used to follow the movement of DNA include in situ hybridization, reporter gene expression, GFP-, YFP-, and RFP- tagged proteins, and live cell imaging of fluorescently-labeled plasmids and RNAs. As for all other macromolecular exchange between the cytoplasm and nucleus, DNA nuclear entry is mediated by the nuclear pore complex.



Organization of transcription factor binding sites on the SV40 enhancer.

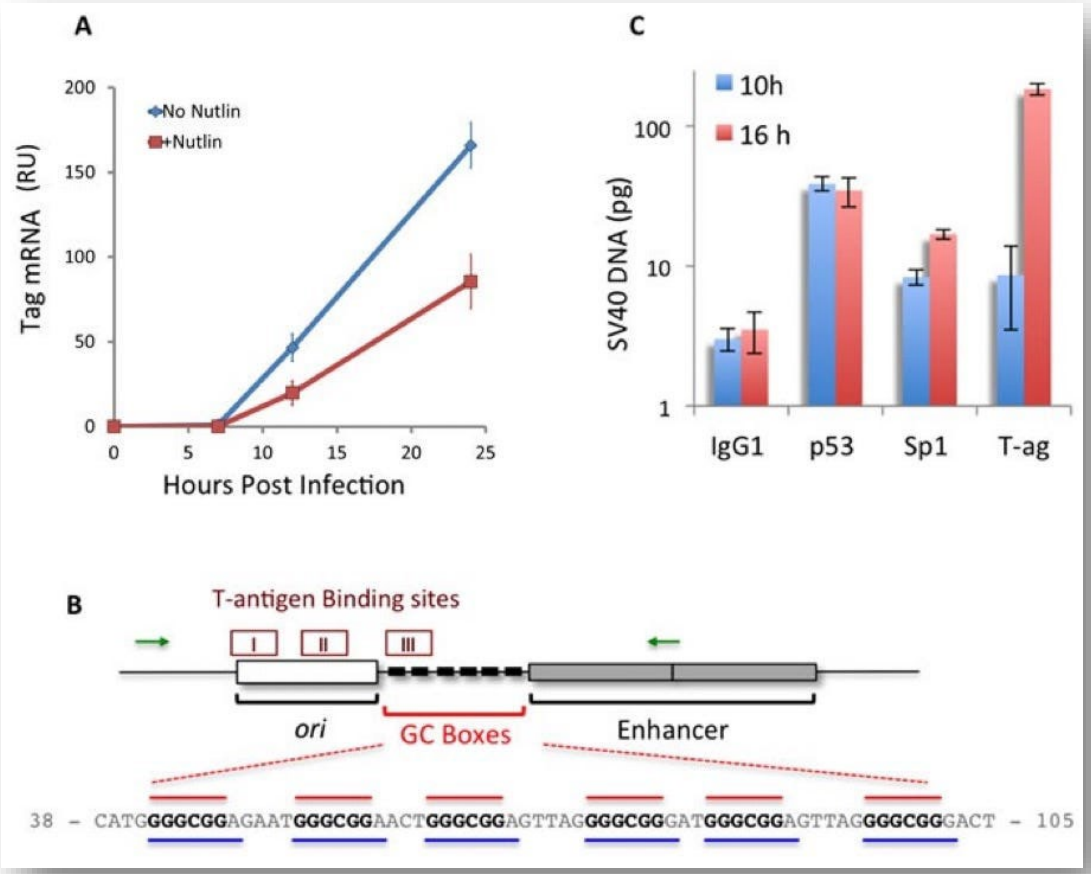


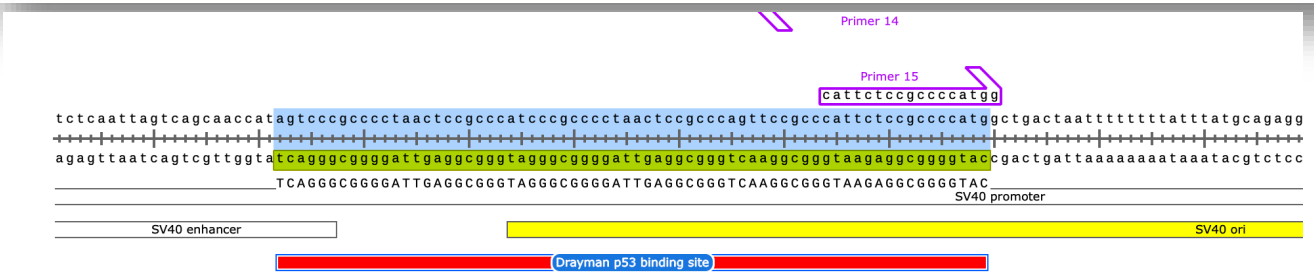
SV40 Promoter Binds to p53 Tumor Suppressor gene

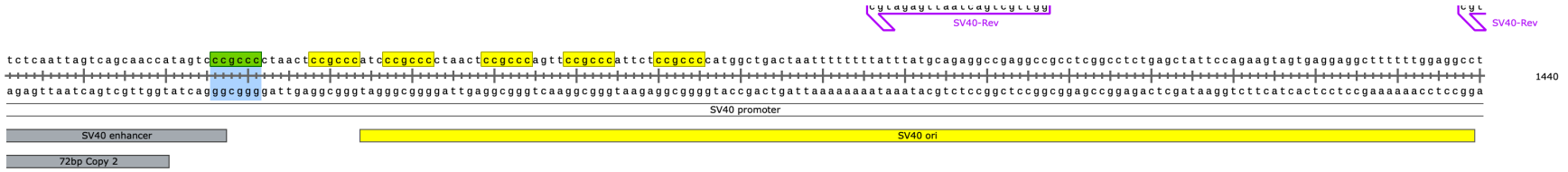
Figure 7

p53 binds to the SV40 early promoter, correlating with a decrease in T-ag mRNA

A. CV-1 cells, with or without 16 hours Nutlin3 pre-treatment, were infected with SV40 and the level of T-ag mRNA, represented as relative units, was measured by quantitative RT-PCR at the indicated time-points, with HPRT RNA as an internal standard. Note that the T-ag protein is seen at 9 hours post infection (Figure S4). The results shown are mean ± S.E. of 5 independent experiments. For the statistical analysis, we compared the area under the curves and found that it was significantly lower in Nutlin3 treated cells compared to untreated cells (680±50 AU vs. 1400±142 AU, respectively. *p*-value = 0.004). **B.** Diagram of the regulatory region of the SV40 genome presenting the *ori* - origin of replication, the GC-boxes and the Enhancer, composed of duplicated 72 bp. The 3 T-ag binding sites are shown on top, and DNA sequence of the GC-boxes with the overlapping Sp1 (red) and p53 (Blue) binding sites below (http://alggen.lsi.upc.es/cgi-bin/promo_v3/promo/promoinit.cgi?dirDB=TF_8.3). The green arrows designate the location of the PCR primers used in the ChIP experiments. **C.** Binding of Sp1, p53 and T-ag to SV40 DNA *in vivo* was determined by ChIP at the indicated time points. DNA recovered from the immune precipitate was quantified by PCR with SV40 DNA as an internal standard. Results are mean ± S.E. of 3 independent experiments.







50-500 Billion SV40 Enhancers in every dose

› Tumour Virus Res. 2024 Dec:18:200293. doi: 10.1016/j.tvr.2024.200293. Epub 2024 Oct 28.

The SV40 virus enhancer functions as a somatic hypermutation-targeting element with potential tumorigenic activity

Filip Šenigl [1], Anni I Soikkeli [2], Salomé Prost [3], David G Schatz [4], Martina Slavková [3], Jiří Hejnar [3], Jukka Alinikula [5]

Affiliations + expand

PMID: 39490533 PMCID: PMC11564006 DOI: 10.1016/j.tvr.2024.200293

Abstract

Simian virus 40 (SV40) is a monkey virus with tumorigenic potential in rodents and is associated with several types of human cancers, including lymphomas. A related Merkel cell polyomavirus causes carcinoma in humans by expressing truncated large tumor antigen (LT), with truncations caused by APOBEC family of cytidine deaminase-induced mutations. AID (activation-induced cytidine deaminase), a member of the APOBEC family, is the initiator of the antibody diversification process known as somatic hypermutation and its aberrant expression and targeting is a frequent source of lymphomagenesis. In this study, we investigated whether AID could cause mutations in SV40 LT. We demonstrate that the SV40 enhancer has strong somatic hypermutation targeting activity in several cell types and that AID-induced mutations accumulate in SV40 LT in B cells and kidney cells and cause truncated LT expression in B cells. Our results argue that the ability of the SV40 enhancer to target somatic hypermutation to LT is a potential source of LT truncation events that could contribute to tumorigenesis in various cell types, thereby linking SV40 infection with malignant development through a novel mutagenic pathway.

Keywords: AID; Enhancer; Large tumor antigen; SV40; Somatic hypermutation; Tumorigenesis.

- This paper demonstrates these sequences recruit mutagenic enzymes and you don't need Large Tumor Antigen for this to occur.

FDA guidelines were derived from Cell Substrate gDNA

10pg of gDNA was the limit pre-NCVIA

10ng of gDNA = 1,000 copies of the human genome

10ng of 200bp DNA = ~50 Billion copies

Many more active DNA ends
(3'Hydroxyls and 5' Phosphates)

Sheng-Fowler- FDA

equals 6×10^5 pg, or 600 ng. Thus, if the amount of residual cell-substrate DNA in a product is 10 ng, then the safety factor with respect to an infectious event for cellular DNA containing an infectious viral genome is 600 ng ÷ 10 ng, or 60. If the cell contains more than a single viral genome, then this safety factor would be reduced accordingly. As stated above, safety factors of $\geq 10^7$ have been considered appropriate with respect to cell-substrate DNA, and thus, a safety factor of 60 or lower would be insufficient. To obtain a safety factor in the $\geq 10^7$ range, either the level of cell-substrate DNA would need to be lowered below 10 ng, or the biological activity of the DNA would need to be reduced by nuclease digestion or chemical inactivation. Assuming that only one copy of the retroviral DNA was present, then the amount of residual cell-substrate DNA would need to be 10 fg or lower. However, if there were 100 copies of the infectious viral genome, the amount of DNA would need to be reduced to 100 ag. Reducing residual cell-substrate DNA to these levels, even with the hardiest of viral vaccines, would likely be impractical and difficult to document. Therefore, with certain cell substrates, additional treatments of the DNA might be recommended.

nature › scientific reports › articles › article

Article | Open Access | Published: 26 April 2023

High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection

Samuel Lim, R. Rogers Yocum, Pamela A. Silver & Jeffrey C. Way

Scientific Reports **13**, Article number: 6835 (2023) | Cite this article

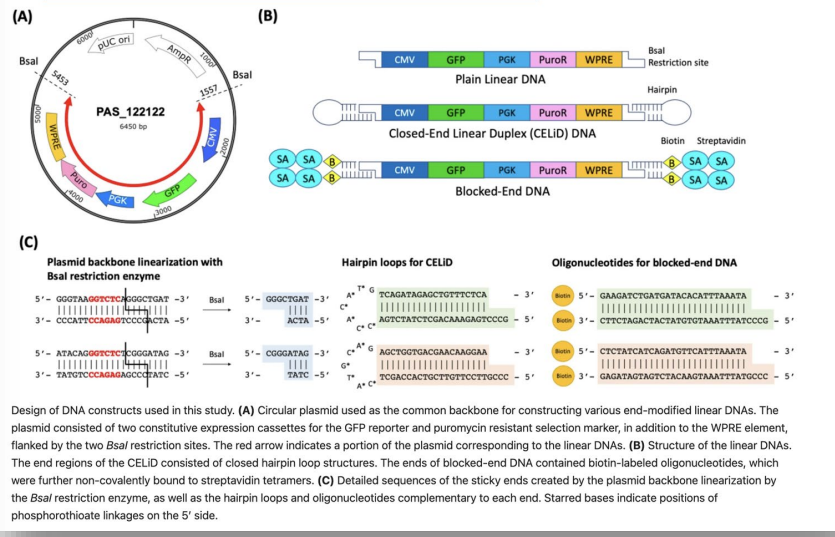
5153 Accesses | **199** Altmetric | Metrics

Abstract

In gene therapy, potential integration of therapeutic transgene into host cell genomes is a serious risk that can lead to insertional mutagenesis and tumorigenesis. Viral vectors are often used as the gene delivery vehicle, but they are prone to undergoing integration events. More recently, non-viral delivery of linear DNAs having modified geometry such as closed-end linear duplex DNA (CELiD) have shown promise as an alternative, due to prolonged transgene expression and less cytotoxicity. However, whether modified-end linear DNAs can also provide a safe, non-integrating gene transfer remains unanswered. Herein, we compare the genomic integration frequency upon transfection of cells with expression vectors in the forms of circular plasmid, unmodified linear DNA, CELiDs with thioester loops, and Streptavidin-conjugated blocked-end linear DNA. All of the forms of linear DNA resulted in a high fraction of the cells being stably transfected—between 10 and 20% of the initially transfected cells. These results indicate that blocking the ends of linear DNA is insufficient to prevent integration.

Figure 1

From: High spontaneous integration rates of end-modified linear DNAs upon mammalian cell transfection



Design of DNA constructs used in this study. **(A)** Circular plasmid used as the common backbone for constructing various end-modified linear DNAs. The plasmid consisted of two constitutive expression cassettes for the GFP reporter and puromycin resistant selection marker, in addition to the WPRE element, flanked by the two *BsaI* restriction sites. The red arrow indicates a portion of the plasmid corresponding to the linear DNAs. **(B)** Structure of the linear DNAs. The end regions of the CELiD consisted of closed hairpin loop structures. The ends of blocked-end DNA contained biotin-labeled oligonucleotides, which were further non-covalently bound to streptavidin tetramers. **(C)** Detailed sequences of the sticky ends created by the plasmid backbone linearization by the *BsaI* restriction enzyme, as well as the hairpin loops and oligonucleotides complementary to each end. Starred bases indicate positions of phosphorothioate linkages on the 5′ side.

Moderna Patent speaks to the risk of insertional mutagenesis from DNA contamination

(12) **United States Patent**
de Fougerolles et al.

(10) **Patent No.: US 10,898,574 B2**
(45) **Date of Patent: *Jan. 26, 2021**

(54) **DELIVERY AND FORMULATION OF ENGINEERED NUCLEIC ACIDS**

(71) Applicant: **ModernaTX, Inc.**, Cambridge, MA (US)

(72) Inventors: **Antonin de Fougerolles**, Waterloo (BE); **Sayda M. Elbashir**, Cambridge, MA (US)

(73) Assignee: **ModernaTX, Inc.**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/927,730**

(22) Filed: **Mar. 21, 2018**

(65) **Prior Publication Data**

US 2019/0060458 A1 Feb. 28, 2019

Related U.S. Application Data

(60) Continuation of application No. 15/379,284, filed on Dec. 14, 2016, now Pat. No. 9,950,068, which is a division of application No. 14/337,513, filed on Jul. 22, 2014, now Pat. No. 9,533,047, which is a continuation of application No. 13/897,362, filed on May 18, 2013, now abandoned, which is a continuation of application No. 13/437,034, filed on Apr. 2, 2012, now Pat. No. 8,710,200.

(58) **Field of Classification Search**
None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,034,506	A	7/1991	Summerton et al.
5,426,180	A	6/1995	Kool
5,489,677	A	2/1996	Sanghvi et al.
5,512,439	A	4/1996	Hornes et al.
5,591,722	A	1/1997	Montgomery et al.
5,637,459	A	6/1997	Burke et al.
5,639,873	A	6/1997	Barascut et al.
5,641,400	A	6/1997	Kaltenbach et al.
5,789,578	A	8/1998	Burton et al.
5,808,039	A	9/1998	Reddy et al.
5,989,911	A	11/1999	Fournier et al.
6,022,715	A	2/2000	Merenkova et al.
6,022,737	A	2/2000	Niven et al.
6,248,268	B1	6/2001	Cook
6,303,378	B1	10/2001	Bridenbaugh et al.
6,423,492	B1	7/2002	Harbron
6,511,832	B1	1/2003	Guarino et al.
6,521,411	B2	2/2003	Hecker et al.
7,691,569	B2	4/2010	Wohlgemuth et al.
8,075,780	B2	12/2011	Pearce
8,093,367	B2	1/2012	Kore et al.
8,664,194	B2	3/2014	de Fougerolles et al.
8,680,069	B2	3/2014	de Fougerolles et al.
8,691,750	B2	4/2014	Constien et al.
8,710,200	B2	4/2014	Schrum et al.
8,716,465	B2	5/2014	Rossi et al.
8,802,438	B2	8/2014	Rossi et al.
8,822,663	B2	9/2014	Schrum et al.

(Continued)

FOREIGN PATENT DOCUMENTS

CA	2028849	A1	9/1991
CA	2473135	A1	6/2003

(Continued)

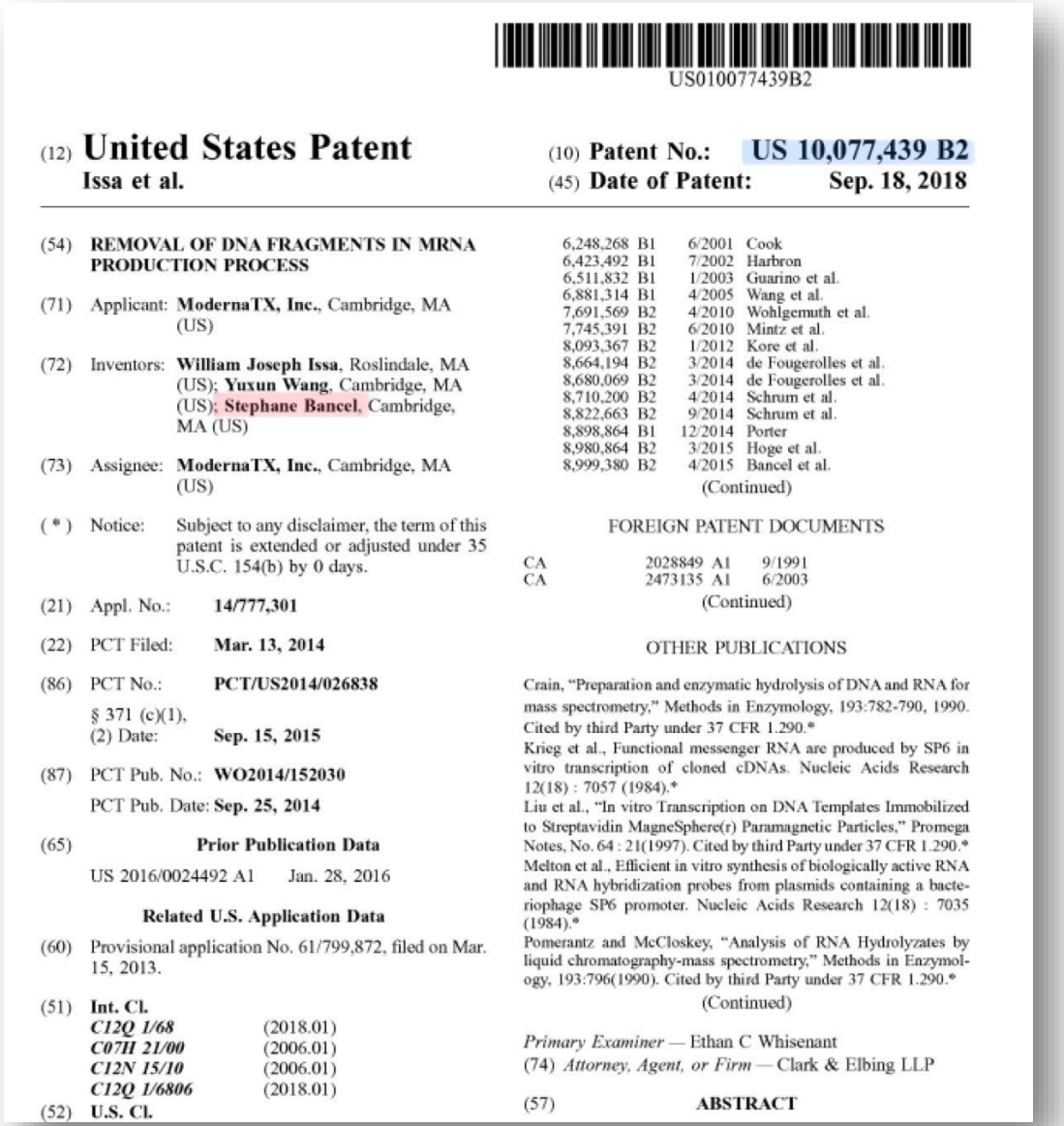
35

BACKGROUND OF THE INVENTION

There are multiple problems with prior methodologies of delivering pharmaceutical compositions in order to achieve effective protein expression both for therapeutics and bio- 40
processing applications. For example, introduced DNA can integrate into host cell genomic DNA at some frequency, resulting in alterations and/or damage to the host cell genomic DNA. Alternatively, the heterologous deoxyribonucleic acid (DNA) introduced into a cell can be inherited by 45
daughter cells (whether or not the heterologous DNA has integrated into the chromosome) or by offspring.

In addition, there are multiple steps which must occur after delivery but before the encoded protein is made which can effect protein expression. Once inside the cell, DNA 50

Moderna Patents speak to the failure of qPCR to measure all DNA: CEO Stephane Bancel is an inventor.



US010077439B2

(12) **United States Patent**
Issa et al.

(10) **Patent No.: US 10,077,439 B2**
(45) **Date of Patent: Sep. 18, 2018**

(54) **REMOVAL OF DNA FRAGMENTS IN MRNA PRODUCTION PROCESS**

(71) Applicant: **ModernaTX, Inc.**, Cambridge, MA (US)

(72) Inventors: **William Joseph Issa**, Roslindale, MA (US); **Yuxun Wang**, Cambridge, MA (US); **Stephane Bancel**, Cambridge, MA (US)

(73) Assignee: **ModernaTX, Inc.**, Cambridge, MA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **14/777,301**

(22) PCT Filed: **Mar. 13, 2014**

(86) PCT No.: **PCT/US2014/026838**
§ 371 (c)(1),
(2) Date: **Sep. 15, 2015**

(87) PCT Pub. No.: **WO2014/152030**
PCT Pub. Date: **Sep. 25, 2014**

(65) **Prior Publication Data**

US 2016/0024492 A1 Jan. 28, 2016

Related U.S. Application Data

(60) Provisional application No. 61/799,872, filed on Mar. 15, 2013.

(51) **Int. Cl.**
C12Q 1/68 (2018.01)
C07H 21/00 (2006.01)
C12N 15/10 (2006.01)
C12Q 1/6806 (2018.01)

(52) **U.S. Cl.**

6,248,268 B1 6/2001 Cook
6,423,492 B1 7/2002 Harbron
6,511,832 B1 1/2003 Guarino et al.
6,881,314 B1 4/2005 Wang et al.
7,691,569 B2 4/2010 Wohlgemuth et al.
7,745,391 B2 6/2010 Mintz et al.
8,093,367 B2 1/2012 Kore et al.
8,664,194 B2 3/2014 de Fougerolles et al.
8,680,069 B2 3/2014 de Fougerolles et al.
8,710,200 B2 4/2014 Schrum et al.
8,822,663 B2 9/2014 Schrum et al.
8,898,864 B1 12/2014 Porter
8,980,864 B2 3/2015 Hoge et al.
8,999,380 B2 4/2015 Bancel et al.
(Continued)

FOREIGN PATENT DOCUMENTS

CA 2028849 A1 9/1991
CA 2473135 A1 6/2003
(Continued)

OTHER PUBLICATIONS

Crain, "Preparation and enzymatic hydrolysis of DNA and RNA for mass spectrometry," Methods in Enzymology, 193:782-790, 1990. Cited by third Party under 37 CFR 1.290.*

Krieg et al., Functional messenger RNA are produced by SP6 in vitro transcription of cloned cDNAs. Nucleic Acids Research 12(18) : 7057 (1984).*

Liu et al., "In vitro Transcription on DNA Templates Immobilized to Streptavidin MagneSphere(r) Paramagnetic Particles," Promega Notes, No. 64 : 21(1997). Cited by third Party under 37 CFR 1.290.*

Melton et al., Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. Nucleic Acids Research 12(18) : 7035 (1984).*

Pomerantz and McCloskey, "Analysis of RNA Hydrolyzates by liquid chromatography-mass spectrometry," Methods in Enzymology, 193:796(1990). Cited by third Party under 37 CFR 1.290.*
(Continued)

Primary Examiner — Ethan C Whisenant
(74) *Attorney, Agent, or Firm* — Clark & Elbing LLP

(57) **ABSTRACT**

extraction is based on the differential partitioning of DNA and RNA into organic and aqueous phases.

DNase I is an endonuclease that cleaves DNA by breaking phosphodiester bonds and produces smaller DNA fragments and/or di-, tri- and oligonucleotides which are subsequently removed by size-based separation methods. However, it is challenging to quantitatively determine the DNase I digestion efficiency and DNase I itself requires to be inactivated or removed in the subsequent process. Quantitative PCR is often applied to measure the residual DNA but it only detects the DNA molecules that contain both qPCR primers thus does not measure all other smaller DNA molecules that are partially digested. To overcome this challenge, a liquid chromatography-tandem mass spectrometry (LC/MS/MS) approach can be used where a total nuclease digestion is performed on the RNA drug substance sample following the DNA removal step. The presence of individual residual deoxynucleotides is quantitatively assayed against deoxynucleotide standards using MS/MS and the abundance is reported.

SV40 plasmids are known to integrate Used in Gene Therapy

> Mol Ther. 2002 Aug;6(2):227-37. doi: 10.1006/mthe.2002.0657.

Durability of transgene expression and vector integration: recombinant SV40-derived gene therapy vectors

David Strayer [1], Francisco Branco, Mark A Zern, Priscilla Yam, Sandra A Calarota, Carmen N Nichols, John A Zaia, John Rossi, Haiting Li, Bhupesh Parashar, Siddhartha Ghosh, J Roy Chowdhury

Affiliations + expand

PMID: 12161189 DOI: 10.1006/mthe.2002.0657

Free article

Abstract

Many applications of gene delivery require long-term transgene expression. In dividing cells, this result necessitates vector genome persistence, usually by integrating into cellular DNA. Since recombinant gene delivery vectors derived from tag-deleted, replication-incompetent simian virus-40 (SV40) provide for long-term transgene expression in resting and dividing cells, we tested whether such enduring transgene expression reflected integration into cellular genomes. Several lines of evidence suggested this likelihood. After transduction in vitro, continuously dividing cell lines and continuously stimulated primary cells uniformly showed transgene expression for many months. Mice whose livers were transduced in vivo, partially resected, and allowed to regenerate showed comparable levels of transgene expression in regenerated and preoperative livers. Thus, replicationincompetent SV40 vectors (rSV40) persist in vitro and in vivo despite extensive cell division. We tested the possibility that this persistence reflected integration directly. Southern blot analyses of genomic DNA from transduced 293 cells showed that vector genome incorporation into cell DNA happened within days of transduction. Episomal vector DNA was barely detectable 96

1000s integrations/patient 10% with cancer

ORIGINAL ARTICLE

Hematologic Cancer after Gene Therapy for Cerebral Adrenoleukodystrophy

C.N. Duncan, J.R. Bledsoe, B. Grzywacz, A. Beckman, M. Bonner, F.S. Eichler, J.-S. Kühl, M.H. Harris, S. Slauson, R.A. Colvin, V.K. Prasad, G.F. Downey, F.J. Pierciey, M.A. Kinney, M. Foos, A. Lodaya, N. Floro, G. Parsons, A.C. Dietz, A.O. Gupta, P.J. Orchard, H.L. Thakar, and D.A. Williams

ABSTRACT

BACKGROUND

Gene therapy with elivaldogene autotemcel (eli-cel) consisting of autologous CD34+ cells transduced with lentiviral vector containing *ABCD1* complementary DNA (Lenti-D) has shown efficacy in clinical studies for the treatment of cerebral adrenoleukodystrophy. However, the risk of oncogenesis with eli-cel is unclear.

METHODS

We performed integration-site analysis, genetic studies, flow cytometry, and morphologic studies in peripheral-blood and bone marrow samples from patients who received eli-cel therapy in two completed phase 2–3 studies (ALD-102 and ALD-104) and an ongoing follow-up study (LTF-304) involving the patients in both ALD-102 and ALD-104.

RESULTS

Hematologic cancer developed in 7 of 67 patients after the receipt of eli-cel (1 of 32 patients in the ALD-102 study and 6 of 35 patients in the ALD-104 study): myelodysplastic syndrome (MDS) with unilineage dysplasia in 2 patients at 14 and 26 months; MDS with excess blasts in 3 patients at 28, 42, and 92 months; MDS in 1 patient at 36 months; and acute myeloid leukemia (AML) in 1 patient at 57 months. In the 6 patients with available data, predominant clones contained lentiviral vector insertions at multiple loci, including at either *MECOM–EVI1* (MDS and EVI1 complex protein EVI1 [ecotropic virus integration site 1], in 5 patients) or *PRDM16* (positive regulatory domain zinc finger protein 16, in 1 patient). Several patients had cytopenias, and most had vector insertions in multiple genes within the same clone; 6 of the 7 patients also had somatic mutations (*KRAS*, *NRAS*, *WT1*, *CDKN2A* or *CDKN2B*, or *RUNX1*), and 1 of the 7 patients had monosomy 7. Of the 5 patients with MDS with excess blasts or MDS with unilineage dysplasia who underwent allogeneic hematopoietic stem-cell transplantation (HSCT), 4 patients remain free of MDS without recurrence of symptoms of cerebral adrenoleukodystrophy, and 1 patient died from presumed graft-versus-host disease 20 months after HSCT (49 months after receiving eli-cel). The patient with AML is alive and had full donor chimerism after HSCT; the patient with the most recent case of MDS is alive and awaiting HSCT.

CONCLUSIONS

Hematologic cancer developed in a subgroup of patients who were treated with eli-cel; the cases are associated with clonal vector insertions within oncogenes and clonal evolution with acquisition of somatic genetic defects. (Funded by Bluebird Bio; ALD-102, ALD-104, and LTF-304 ClinicalTrials.gov numbers, NCT01896102, NCT03852498, and NCT02698579, respectively.)

99% of patients got integrations

INTEGRATION-SITE ANALYSIS AND CLONAL ANALYSES

All Patients

As of April 25, 2024, among all 67 patients involved in both studies, the median highest total number of unique mappable insertion sites was 6973 (range, 582 to 15,683) (Table 3). Of the 10 genes with the highest frequency of insertions reported in patients, *SMG6*, *MECOM*, *CCND2-AS1*, *MPL*, and *C6ORF10* were the most abundant in the study population (Table S2). The total number of unique mappable insertion sites over time and genes with the greatest number of unique insertions across the two ALD trials and all the related Bluebird Bio–sponsored studies involving lentiviral vector therapy can be found in Table S3 and Figure S3, respectively. Across the ALD-102

Genomic Analyses of MECOM *and* PRDM16 *Loci*

A detailed genomic analysis was conducted with the use of data that were collected until April 3, 2024, with a specific focus on the *MECOM* locus (Fig. S5) and a more limited focus on its close homologue, *PRDM16* (Fig. S6). In total, 99% of the patients (66 of 67) who were treated with eli-cel had integrations in *MECOM*, with an average of 47 integration sites present in cells from the peripheral blood (range, 0 to 180). The pattern of integrations did not differ between patients with maintained polyclonality and those with persistent oligoclonality, MDS, or both (Fig. S5A).

Cytosolic DNA is an Oncogenic Risk

Review > Cancer Discov. 2020 Jan;10(1):26-39. doi: 10.1158/2159-8290.CD-19-0761.
Epub 2019 Dec 18.

The Cytosolic DNA-Sensing cGAS-STING Pathway in Cancer

John Kwon [1], Samuel F Bakhoum [2 3]

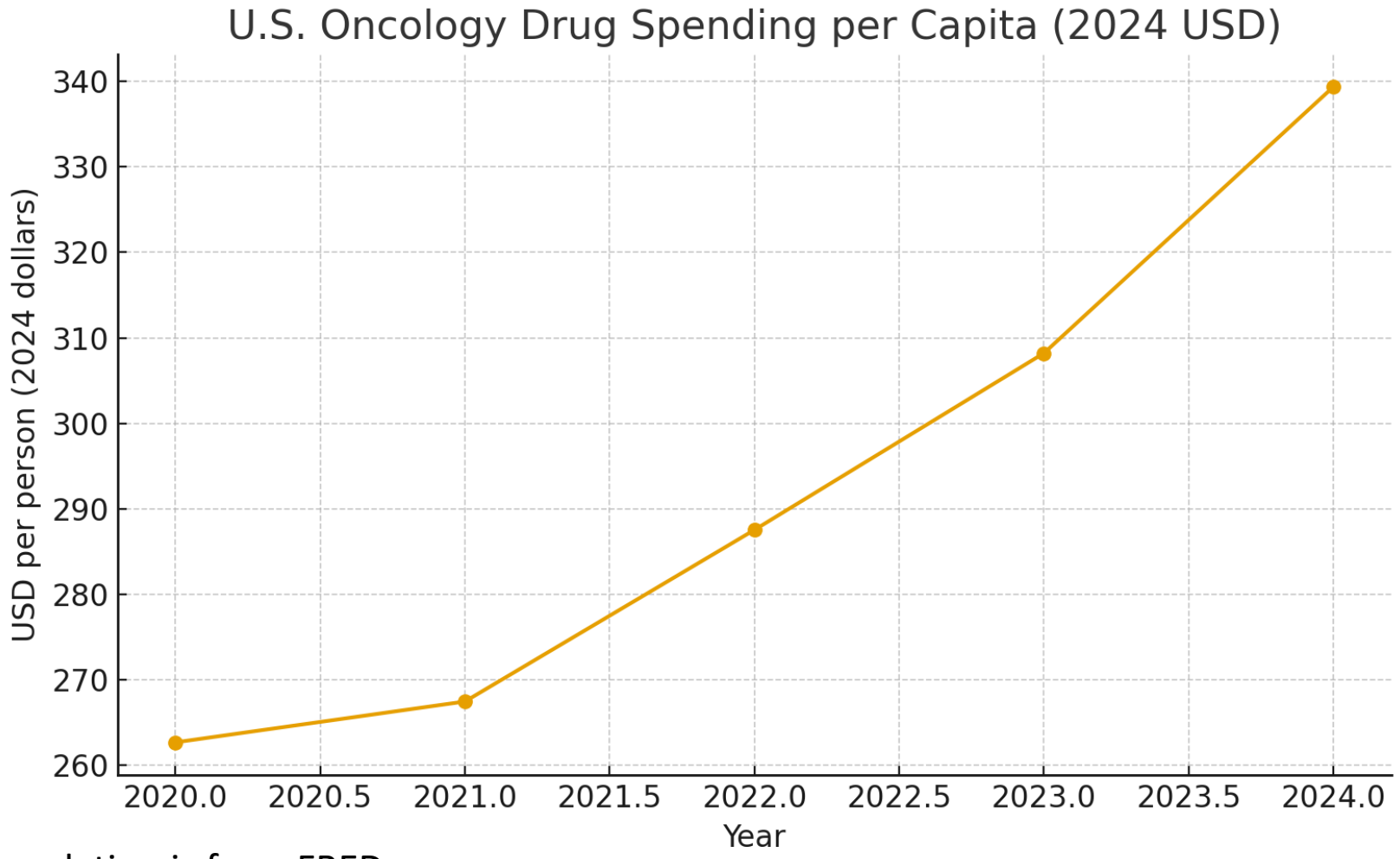
Affiliations + expand

PMID: 31852718 PMCID: PMC7151642 DOI: 10.1158/2159-8290.CD-19-0761

Abstract

The recognition of DNA as an immune-stimulatory molecule is an evolutionarily conserved mechanism to initiate rapid innate immune responses against microbial pathogens. The cGAS-STING pathway was discovered as an important DNA-sensing machinery in innate immunity and viral defense. Recent advances have now expanded the roles of cGAS-STING to cancer. Highly aggressive, unstable tumors have evolved to co-opt this program to drive tumorigenic behaviors. In this review, we discuss the link between the cGAS-STING DNA-sensing pathway and antitumor immunity as well as cancer progression, genomic instability, the tumor microenvironment, and pharmacologic strategies for cancer therapy. SIGNIFICANCE: The cGAS-STING pathway is an evolutionarily conserved defense mechanism against viral infections. Given its role in activating immune surveillance, it has been assumed that this pathway primarily functions as a tumor suppressor. Yet, mounting evidence now suggests that depending on the context, cGAS-STING signaling can also have tumor and metastasis-promoting functions, and its chronic activation can paradoxically induce an immune-suppressive tumor microenvironment.

IQVIA Institute for human data sciences normalized for population increase and GDP/inflation



Population is from FRED

modRNA is found in Heart Tissue 30 days after vax (Krauson)
modRNA is found in Lymph Nodes 60 days after vax (Roltgen)

npj | vaccines

Download PDF

Article | Open Access |
Published: 27 September 2023

Duration of SARS-CoV-2 mRNA vaccine persistence and factors associated with cardiac involvement in recently vaccinated patients

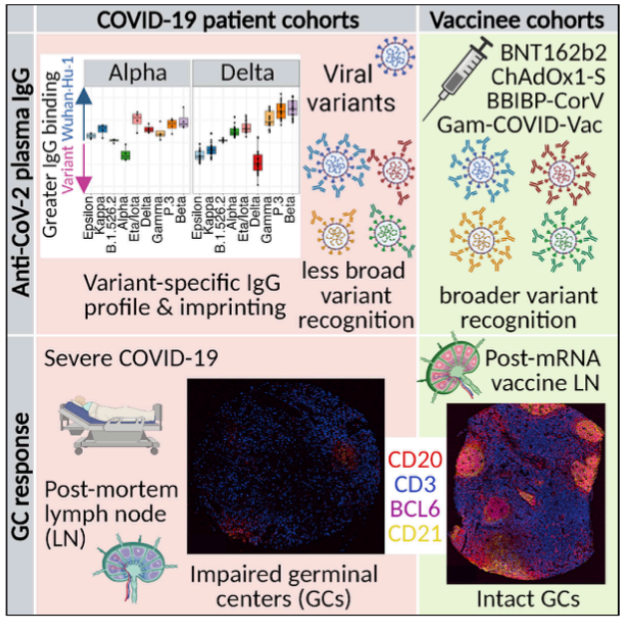
Aram J. Krauson, Faye Victoria C. Casimero, ... James R. Stone + Show authors

Cell

Article

Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination

Graphical abstract



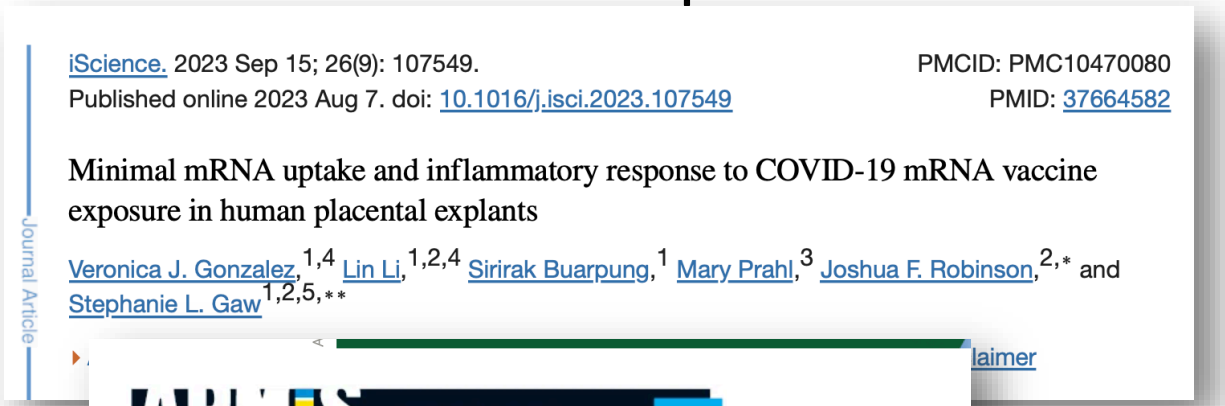
Authors
Katharina Röltgen, Sandra C.A. Nielsen, Oscar Silva, ..., Benjamin A. Pinsky, Kari C. Nadeau, Scott D. Boyd

Correspondence
publications_scott_boyd@stanford.edu

In brief

Human antibody responses to SARS-CoV-2 differ between vaccination and infection, with mRNA vaccination inducing more productive lymph node GC responses and several vaccine types stimulating IgG antibodies capable of recognizing a broader range of viral variants.

Spike nucleic acid persistence

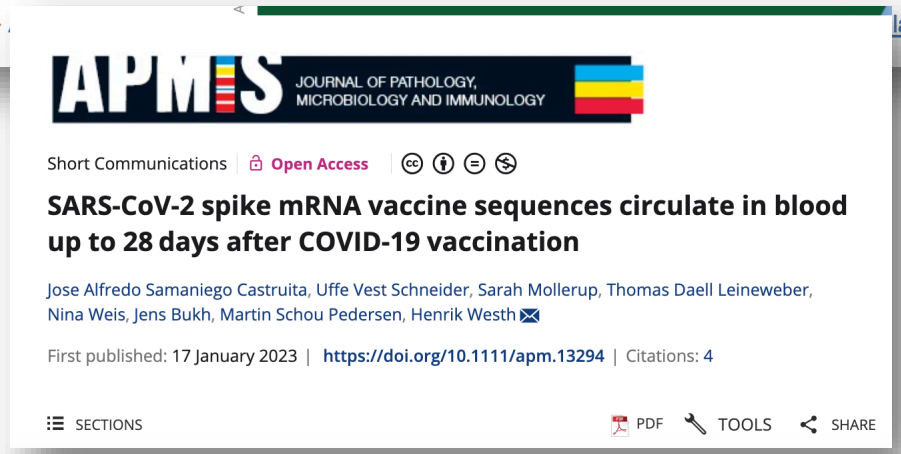


iScience. 2023 Sep 15; 26(9): 107549.
Published online 2023 Aug 7. doi: 10.1016/j.isci.2023.107549
PMCID: PMC10470080
PMID: 37664582

Minimal mRNA uptake and inflammatory response to COVID-19 mRNA vaccine exposure in human placental explants

Veronica J. Gonzalez,[1,4] Lin Li,[1,2,4] Sirirak Buarpung,[1] Mary Prahl,[3] Joshua F. Robinson,[2,*] and Stephanie L. Gaw[1,2,5,**]

Found in Placenta 2-10 days after (Gonzalez)



APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

Short Communications | Open Access

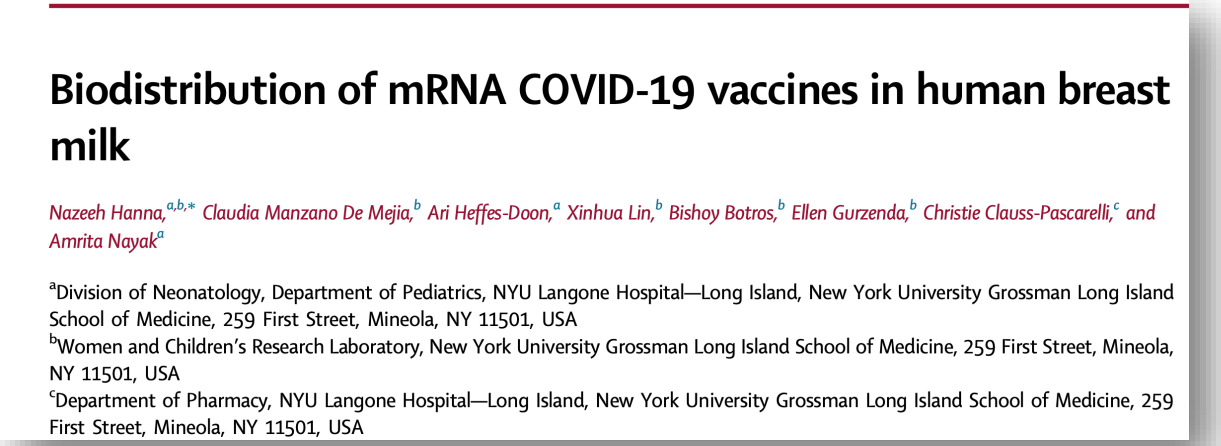
SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination

Jose Alfredo Samaniego Castruita, Uffe Vest Schneider, Sarah Mollerup, Thomas Daell Leineweber, Nina Weis, Jens Bukh, Martin Schou Pedersen, Henrik Westh

First published: 17 January 2023 | https://doi.org/10.1111/apm.13294 | Citations: 4

SECTIONS PDF TOOLS SHARE

Found in Plasma 28 days later (Castruita)



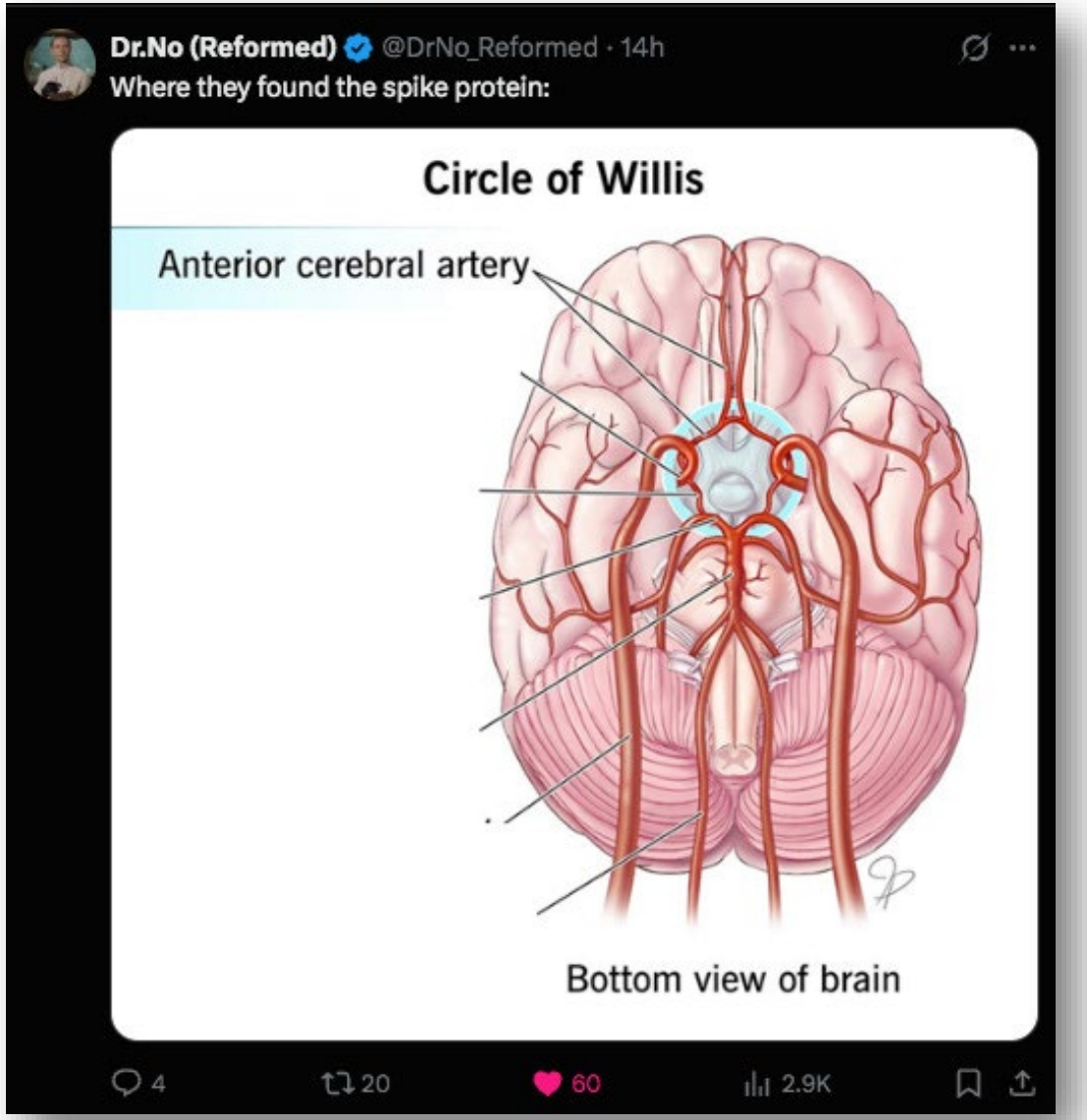
Biodistribution of mRNA COVID-19 vaccines in human breast milk

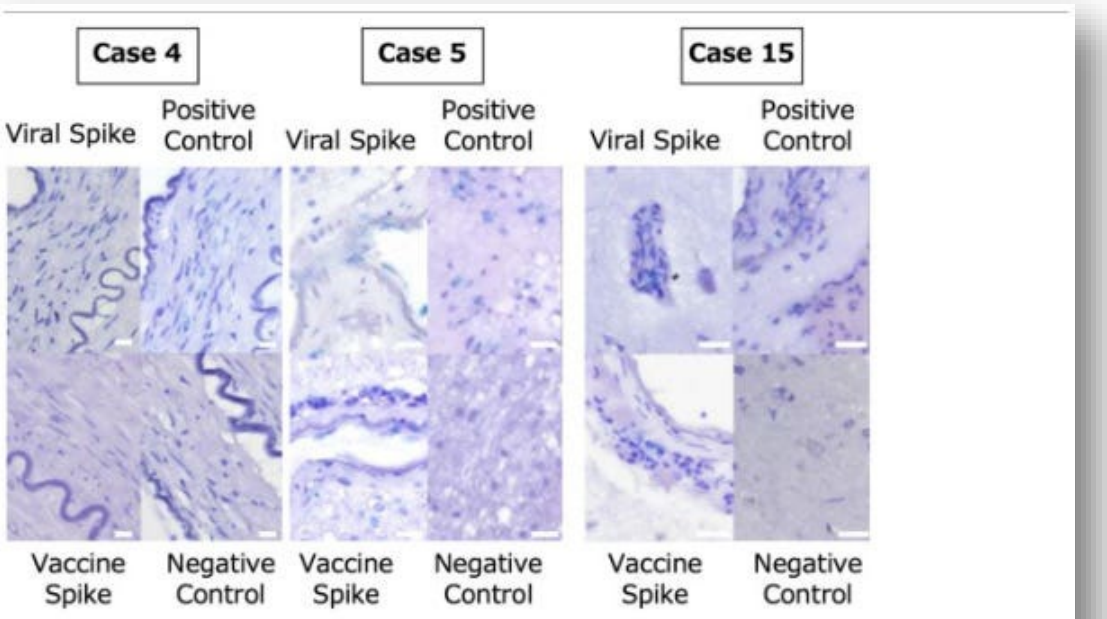
Nazeeh Hanna,[a,b,] Claudia Manzano De Mejia,[b] Ari Heffes-Doon,[a] Xinhua Lin,[b] Bishoy Botros,[b] Ellen Gurzenda,[b] Christie Clauss-Pascarelli,[c] and Amrita Nayak[a]*

[a]Division of Neonatology, Department of Pediatrics, NYU Langone Hospital—Long Island, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA
[b]Women and Children's Research Laboratory, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA
[c]Department of Pharmacy, NYU Langone Hospital—Long Island, New York University Grossman Long Island School of Medicine, 259 First Street, Mineola, NY 11501, USA

Found in breast milk 5 days later (Hanna)

Ota et al) 17 Months in Cerebral Arteries





Download: Download high-res image (325KB)

Download: Download full-size image

Fig. 5. **Result of in situ hybridization of the case 4, 5 and 15.** The specimens from cases 5 and 15 consist of brain tissue samples taken near the hematoma, while the specimen from case 4 is from the anterior cerebral artery, adjacent to the affected lesion, which was treated with trapping and excision of the aneurysm. Negative and positive controls were successfully validated for all cases. Furthermore, viral spike mRNA and vaccine-derived mRNA were detected as positive in all cases. A light blue luminescence indicates a positive result for mRNA specific to the probes. Scale bar indicates 20μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Spike Protein Persistence

- Yale Study (709 days)
- Patterson study (245 days)

HUMAN VACCINES & IMMUNOTHERAPEUTICS
2025, VOL. 21, NO. 1, 2494934
https://doi.org/10.1080/21645515.2025.2494934

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Detection of S1 spike protein in CD16+ monocytes up to 245 days in SARS-CoV-2-negative post-COVID-19 vaccine syndrome (PCVS) individuals

Bruce K. Patterson[a], Ram Yogendra[b*], Edgar B. Francisco[a], Jose Guevara-Coto[c], Emily Long[a], Amruta Pise[a], Eric Osgood[d], John Bream[e], Mark Kreimer[f], Devon Jeffers[g], Christopher Beaty[a], Richard Vander Heide[h], and Rodrigo A. Mora-Rodríguez[c]

[a]Research and Development Department, IncellDx Inc, Hayward, CA, USA; [b]Department of Anesthesiology, Lawrence General Hospital, Lawrence, MA, USA; [c]Lab of Tumor Chemosensitivity, CIET/DC Lab, Faculty of Microbiology, Universidad de Costa Rica, San Jose, Costa Rica; [d]Department of Medicine, St. Francis Medical Center, Trenton, NJ, USA; [e]Department of Emergency Medicine, Novant Health Kernersville Medical Center, Kernersville, NC, USA; [f]Department of Emergency Medicine, New York Presbyterian Hospital, Brooklyn, NY, USA; [g]Department of Anesthesiology, Stamford Hospital, Stamford, CT, USA; [h]Department of Pathology, Marshfield Medical Center, Marshfield, WI, USA

ABSTRACT

Despite over 13 billion SARS-CoV-2 vaccine doses administered globally, persistent post-vaccination symptoms, termed post-COVID-19 vaccine syndrome (PCVS), resemble post-acute sequelae of COVID-19 (PASC). Symptoms like cardiac, vascular, and neurological issues often emerge shortly after vaccination and persist for months to years, mirroring PASC. We previously showed the S1 subunit of the SARS-CoV-2 spike protein persists in CD16+ monocytes after infection, potentially driving PASC. Approved vaccines (Pfizer, Moderna, Janssen, AstraZeneca) deliver synthetic S1 to elicit immunity, suggesting a shared mechanism. We hypothesized that vaccine-derived S1 persistence in CD16+ monocytes sustains inflammation akin to PASC, contributing to PCVS. We studied 50 individuals with PCVS symptoms lasting over 30 days post-vaccination and 26 asymptomatic controls, using (1) machine learning-based immune profiling to compare cytokine signatures with PASC, (2) flow cytometry to detect S1 in CD16+ monocytes, and (3) LC-MS to confirm S1 across vaccine types. We correlated S1 persistence with symptom duration and inflammation. Prior infection was excluded via clinical history, anti-nucleocapsid antibody tests, and T-detect assays, though definitive tests are lacking. Preliminary findings suggest S1 persistence in CD16+ monocytes and an associated inflammatory profile may contribute to PCVS. Further studies are needed to confirm causality and prevalence.

SUMMARY

SARS CoV-2 S1 Protein in CD16+ Monocytes in Post-COVID-19 Vaccine Syndrome (PCVS).

ARTICLE HISTORY
Received 12 November 2024
Revised 2 April 2025
Accepted 15 April 2025

KEYWORDS
COVID-19; PASC; SARS CoV-2 S1 protein; non-classical monocytes; CCR5; fractalkine

Immunological and Antigenic Signatures Associated with Chronic Illnesses after COVID-19 Vaccination

Bornali Bhattacharjee[1,2#], Peiwen Lu[1#], Valter Silva Monteiro[1#], Alexandra Tabachnikova[1#], Kexin Wang[2,11#], William B. Hooper[1,2#], Victoria Bastos[1#], Kerrie Greene[1#], Mitsuaki Sawano[3], Christian Guirgis[1,4], Tiffany J. Tzeng[1,2], Frederick Warner[3,5], Pavlina Baevova[1,2], Kathy Kamath[6], Jack Reifert[6], Danice Hertz[7], Brianne Dressen[8], Laura Tabacof[9], Jamie Wood[9], Lily Cooke[9], Mackenzie Doerstling[9], Shadan Nolasco[9], Amer Ahmed[9], Amy Proal[9,10], David Putrino[9], Leying Guan[2,11*], Harlan M. Krumholz[2,3,12*], Akiko Iwasaki[1,2,13*]

Affiliations:

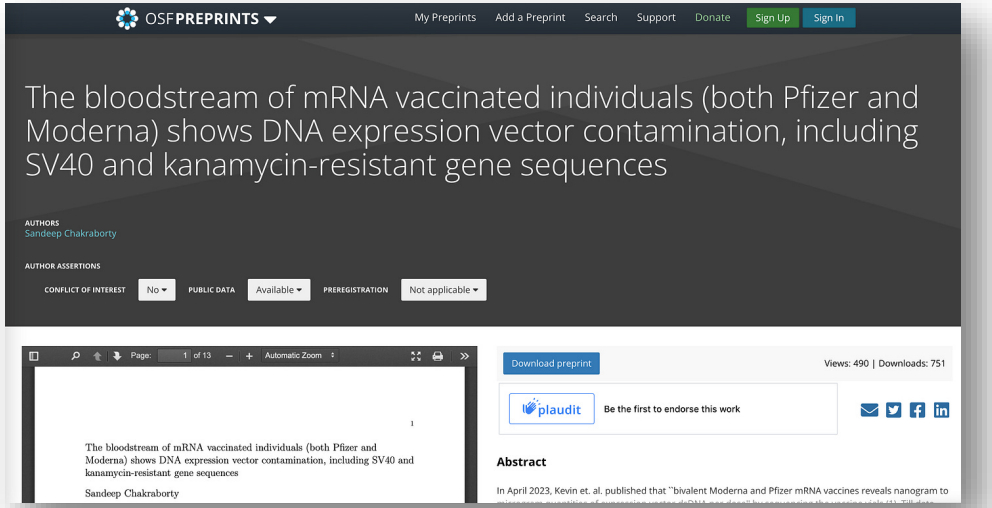
1. Department of Immunobiology, Yale University School of Medicine, New Haven, CT, USA
2. Center for Infection and Immunity, Yale School of Medicine, New Haven, CT, USA
3. Section of Cardiovascular Medicine, Department of Internal Medicine, Yale School of Medicine, Yale New Haven Hospital Center for Outcomes Research and Evaluation, Yale New Haven Hospital, CT, USA
4. Department of Molecular, Cellular, and Developmental Biology, Yale College, New Haven, CT, USA.
5. Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA
6. SerImmune, Goleta, CA USA
7. Independent Researcher, CA, USA
8. Independent Researcher, UT, USA
9. Cohen Center for Recovery from Complex Chronic Illness, Department of Rehabilitation and Human Performance, Icahn School of Medicine at Mount Sinai, New York City, NY, USA
10. Polybio Research Foundation, Boston, MA, USA
11. Department of Biostatistics, Yale School of Public Health, New Haven, CT, USA
12. Section of Cardiovascular Medicine, Department of Internal Medicine, Yale School of Medicine, New Haven, CT, USA
13. Howard Hughes Medical Institute, Chevy Chase, MD, USA

Not Just in the Vaccine. Now found in Patients!

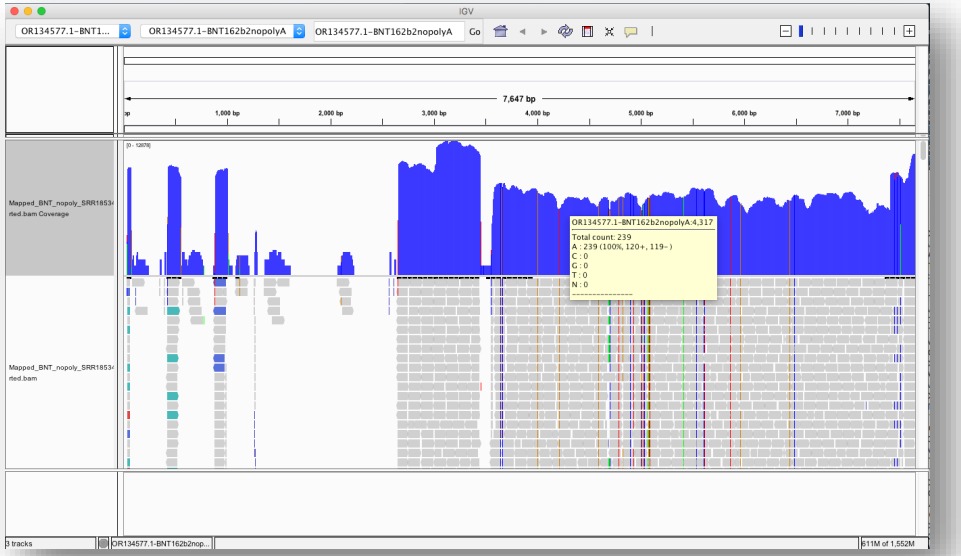
- In at least 5 Peer reviewed studies that submitted RNA-Seq data to the NCBI SRA, we can find Vaccine plasmid DNA
- Ryan et al
- Odak et al
- Lee et al
- Knabl et al
- Krawcyzk et al

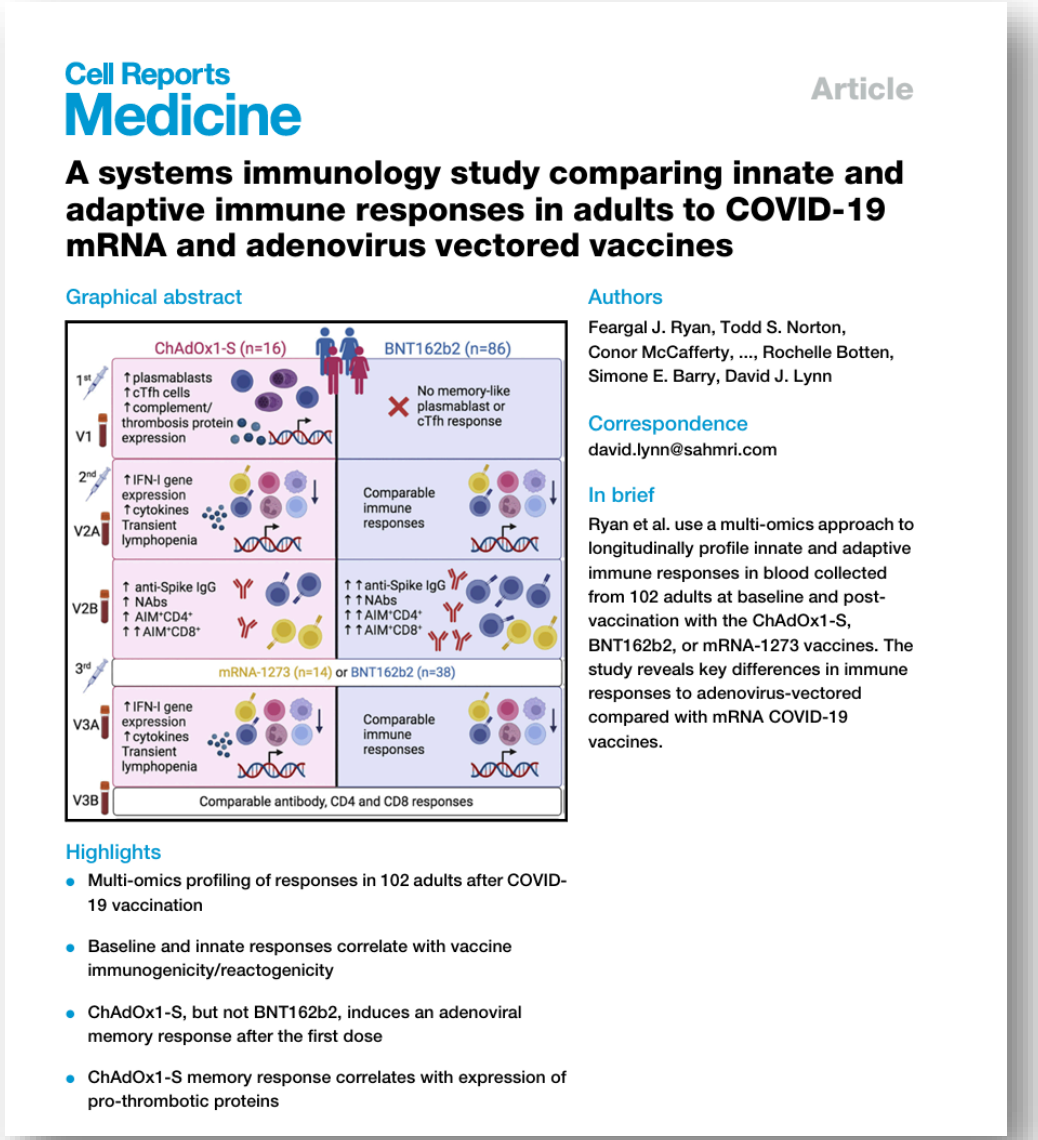
These studies were all looking at RNA with methods that suppress the DNA… Yet its still there

Now Found in the Blood Supply



Chakraborty et al



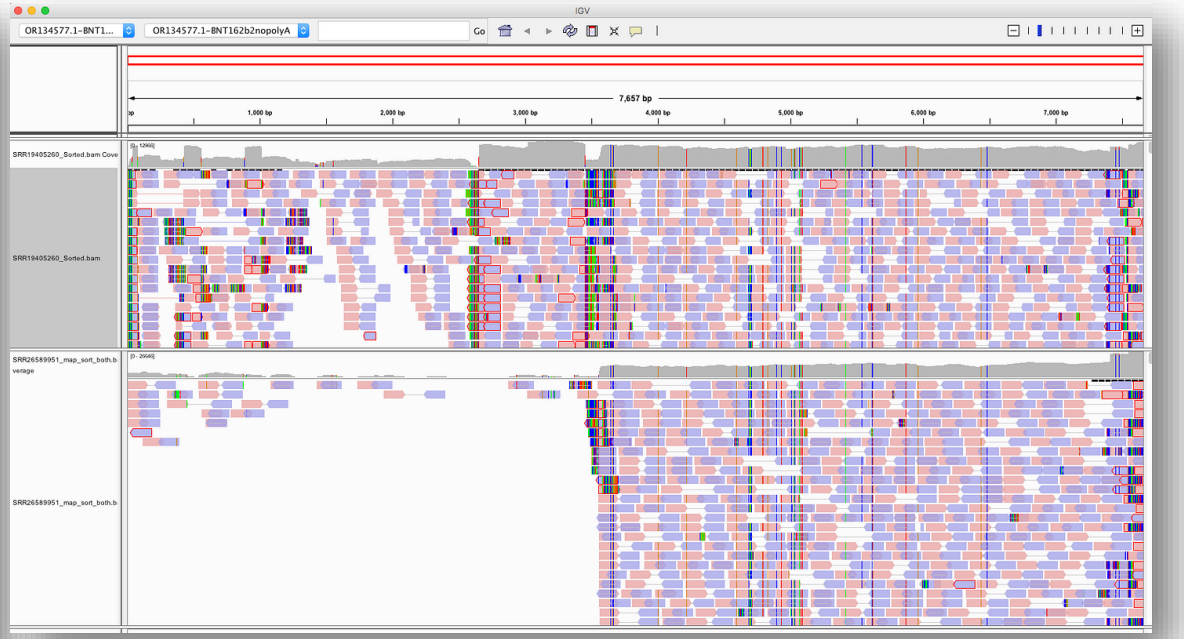


Ryan et al

Odak et al is contaminated

Plasmid

Spike



Systems biology analysis reveals distinct molecular signatures associated with immune responsiveness to the BNT162b COVID-19 vaccine

Ivan Odak,[a,n] Lennart Riemann,[a,b,c,n] Inga Sandrock,[a] Anne Cossmann,[d] Gema Morillas Ramos,[d] Swantje I. Hammerschmidt,[a] Christiane Ritter,[a] Michaela Friedrichsen,[a] Ahmed Hassan,[a] Alexandra Dopfer-Jablonka,[d,e] Metodi V. Stankov,[d] Leonie M. Weskamm,[f,g,h] Marylyn M. Addo,[f,g,h,i] Inga Ravens,[a] Stefanie Willenzon,[a] Anja Schimrock,[a] Jasmin Ristenpart,[a] Anika Janssen,[a] Joana Barros-Martins,[a] Gesine Hansen,[b,c,j,k] Christine Falk,[l] Georg M. N. Behrens,[d,e,m] and Reinhold Förster[a,c,h,j,k,*]

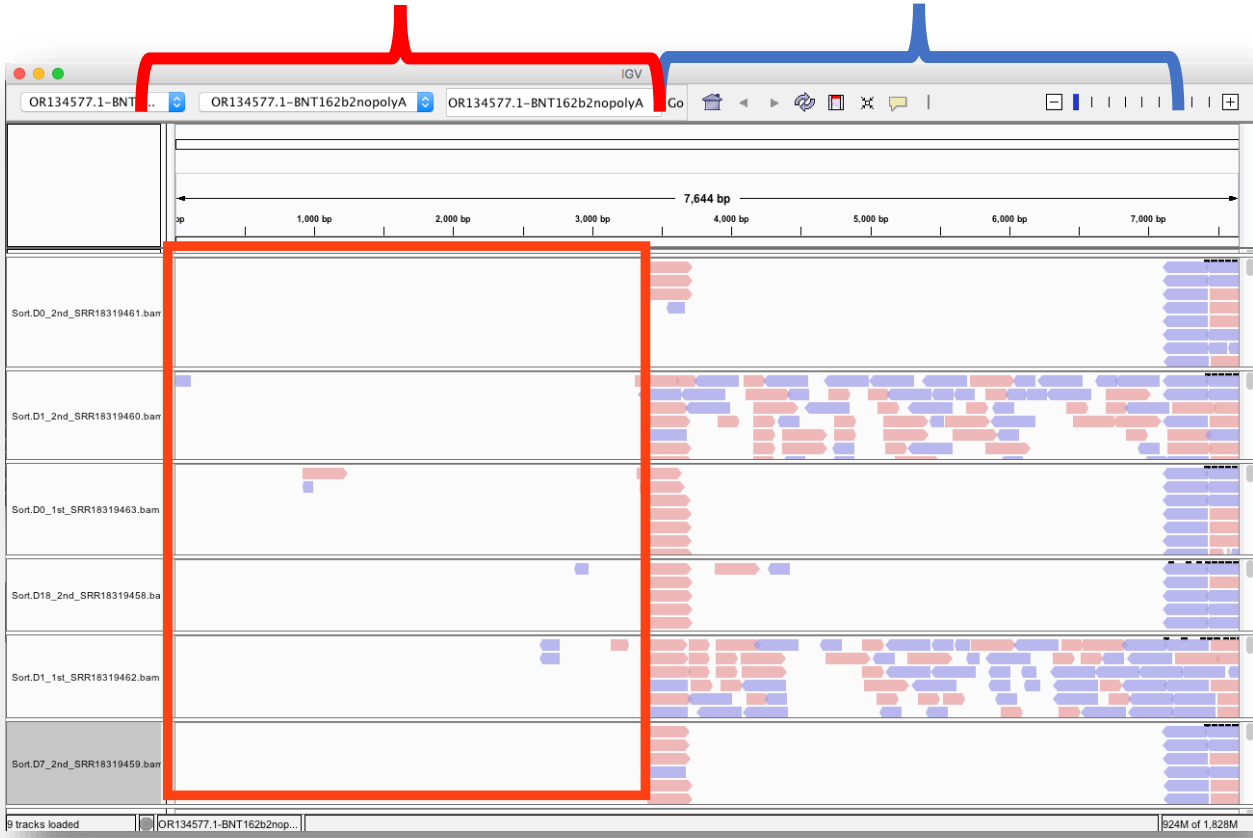
[a]Institute of Immunology, Hannover Medical School, Germany
[b]Department of Pediatric Pneumology, Allergology and Neonatology, Hannover Medical School, Germany
[c]Clinician Scientist Program TITUS, Else-Kröner-Fresenius Foundation, Hannover Medical School, Germany
[d]Department for Rheumatology and Immunology, Hannover Medical School, Germany
[e]German Center for Infection Research (DZIF), Partner Sites Hannover-Braunschweig, Germany
[f]Institute for Infection Research and Vaccine Development (IIRVD), University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
[g]Department for Clinical Immunology of Infectious Diseases, Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[h]German Centre for Infection Research, Partner Site Hamburg-Lübeck-Borstel-Riems, Hamburg, Germany
[i]First Department of Medicine, Division of Infectious Diseases, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany
[j]German Center of Lung Research (DZL), BREATH, Hannover, Germany
[k]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Germany
[l]Institute for Transplantation Immunology, Hannover Medical School, Hannover, Germany
[m]Centre for Individualized Infection Medicine (CiiM), Hannover, Germany

https://anandamide.substack.com/p/chakraborty-part-ii-odak-et-al

Knabl et al is Contaminated

Plasmid

Spike



RHEUMATOLOGY

Letter to the Editor (Case report)

Rheumatology 2022;61:e305–e307
https://doi.org/10.1093/rheumatology/keac281
Advance access publication 9 May 2022

Immune transcriptome and antibody response in adult-onset Still's disease with mild flare following administration of mRNA vaccine BNT162b2

Rheumatology key message

- Elevated immune response and high anti-Omicron-spike neutralizing activity in adult-onset Still's disease patient immunized with BNT162b2.

Dear Editor, Adult-onset Still's disease (AOSD) patients represent a population for which vaccination can induce disease flare [1], and little is known about the efficacy of coronavirus disease 2019 (COVID-19) vaccination in this group. The COVID-19 pandemic vaccination programs presented these patients and their healthcare providers with a critical decision as the safety and efficacy of the vaccination are of equal importance. Previously it has been described that AOSD patients can experience disease flare with COVID-19 vaccination [1–6] but no one has yet reported their immune transcriptional and antibody response. Here we present both safety and efficacy information on a 58-year-old male after vaccination who experienced a mild AOSD flare following second BNT162b2 vaccine. The clinical course, immune transcriptional response, and anti-spike antibody titres and neutralization activity were profiled following vaccination.

The patient was diagnosed with AOSD in 2013 when he developed classical symptoms following Frühsommer-Meningoenzephalitis (FSME) vaccination against tick-borne encephalitis. Treatment with prednisolone showed good efficacy and symptoms resolved after 3 months. In fall 2020 the patient suffered from another AOSD flare after receiving an influenza vaccination, manifesting as the systemic inflammation AOSD phenotype with lymph node swellings, myalgia, cervicalgia and recurrence of skin lesions from underlying psoriasis, but no synovitis. The disease flare was treated with prednisolone, which was gradually reduced and phased out in August 2021. The patient was infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in early March 2020 with symptoms limited to a mild sore throat and a cough. At the time of COVID-19 vaccination the patient was not receiving any immunosuppressive therapy. In fall 2021 the patient received two BNT162b2 vaccines separated by 4 weeks. Mild symptoms of AOSD started about 2 days after the second vaccination, characterized by myalgia, fever and fatigue with slightly elevated CRP and IL-1 levels measured. The patient was treated symptomatically with non-steroidal medications and the disease flare symptoms were attenuated. However, 3 months post-vaccination the patient still experienced myalgia in both thighs, which is managed with symptom-based application of non-steroidal antirheumatics.

The molecular immune status prior to and post-vaccination was measured through RNA-sequencing of buffy-coat cells at a sequencing depth of 190 million reads per sample (supplementary Table S1, available at *Rheumatology* online). Transcriptomes were generated prior to (D0) and 1 day (D1) after the first vaccination and prior to (D0) and days 1 (D1), 7 (D7) and 18 (D18) after the second vaccination, and compared with those from healthy naïve individuals [7] with similar ages (Fig. 1A–C; supplementary Tables S2 and S3, available at *Rheumatology* online). In addition, antibody titres and neutralization ability were assessed and compared with healthy naïve individuals (Fig. 1D and E; supplementary Table S4, available at *Rheumatology* online).

A strong IFN response in the AOSD patient was observed within 1 day following the second vaccination and expression of 194 genes was induced at least 2-fold (supplementary Table S2, available at *Rheumatology* online). Transcriptome comparison with healthy individuals at the time of the mild disease flare showed that the Still's patient exhibited several greater fold-induction of JAK-STAT regulated immune genes as well as higher levels of IFN-gamma pathway genes (Fig. 1B and C; supplementary Tables S2 and S3, available at *Rheumatology* online). These include *STAT1*, *ISG15*, *ETV7* and *BATF2*. Absolute higher levels were seen for *DDX60* and *GBP4*, genes controlling the immune response to viral infection, and *CIITA*, which controls class II histocompatibility genes. Of note, expression of the *TNFRSF14* gene, which encodes the herpesvirus entry protein and has been linked to autoimmune diseases, was induced only in the AOSD patient.

Comparison of the patient's antibody response with those from healthy individuals within the first 7 days after second BNT162b2 vaccination revealed a robust humoral immune response against the SARS-CoV-2 spike protein of the ancestral strain and the Alpha, Beta, Gamma and Delta variants (Fig. 1D; supplementary Table S4, available at *Rheumatology* online). The antibody titre against the Omicron variant was ~5-fold lower. Notably, the respective anti-spike antibody titres in healthy individuals [7] after the second vaccine were 3- to 5-fold lower than in the AOSD patient (Fig. 1E). Also, the neutralizing activities, as evaluated by the ACE2-binding interference assay, were similar between the different variants, including Omicron, and exceed those measured in the vaccinated healthy cohort (Fig. 1E; supplementary Table S4, available at *Rheumatology* online). RNA-

Ludwig Knabl[1], Hye Kyung Lee[2], Mary Walter[3], Priscilla A. Furth[4] and Lothar Hennighausen[2]

[1]TyrolPath, Obrist-Brunhuber GmbH, Hauptplatz 4, Zams, Austria, [2]National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, [3]Clinical Core, National Institute of Diabetes, Digestive and Kidney Diseases, Bethesda, MD and [4]Departments of Oncology & Medicine, Georgetown University, Washington DC, USA
Accepted 4 May 2022
Correspondence to: Ludwig Knabl, TyrolPath, Obrist-Brunhuber GmbH, Hauptplatz 4, Zams, 6511, Austria.
E-mail: Ludwig.knabl@tyrolpath.at

Lee et al is Contaminated

Plasmid

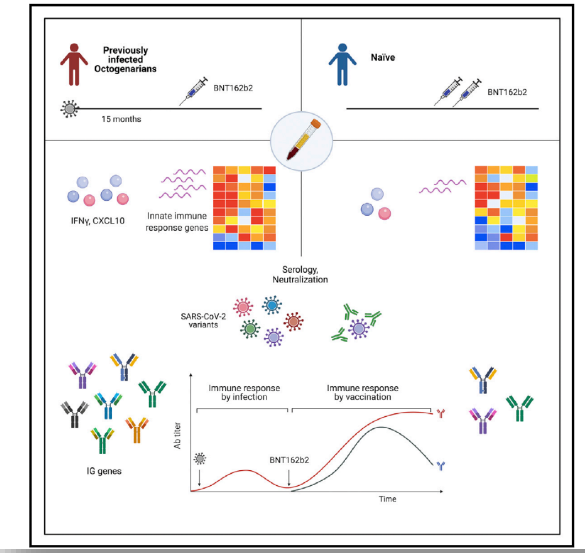
Spike

Article

Cell Reports

mRNA vaccination in octogenarians 15 and 20 months after recovery from COVID-19 elicits robust immune and antibody responses that include Omicron

Graphical abstract



Authors

Hye Kyung Lee, Ludwig Knabl, Juan I. Moliva, ..., Nancy J. Sullivan, Priscilla A. Furth, Lothar Hennighausen

Correspondence

hyekyung.lee@nih.gov (H.K.L.), ludwig.knabl@tyrolpath.at (L.K.), paf3@georgetown.edu (P.A.F.), lotharh@nih.gov (L.H.)

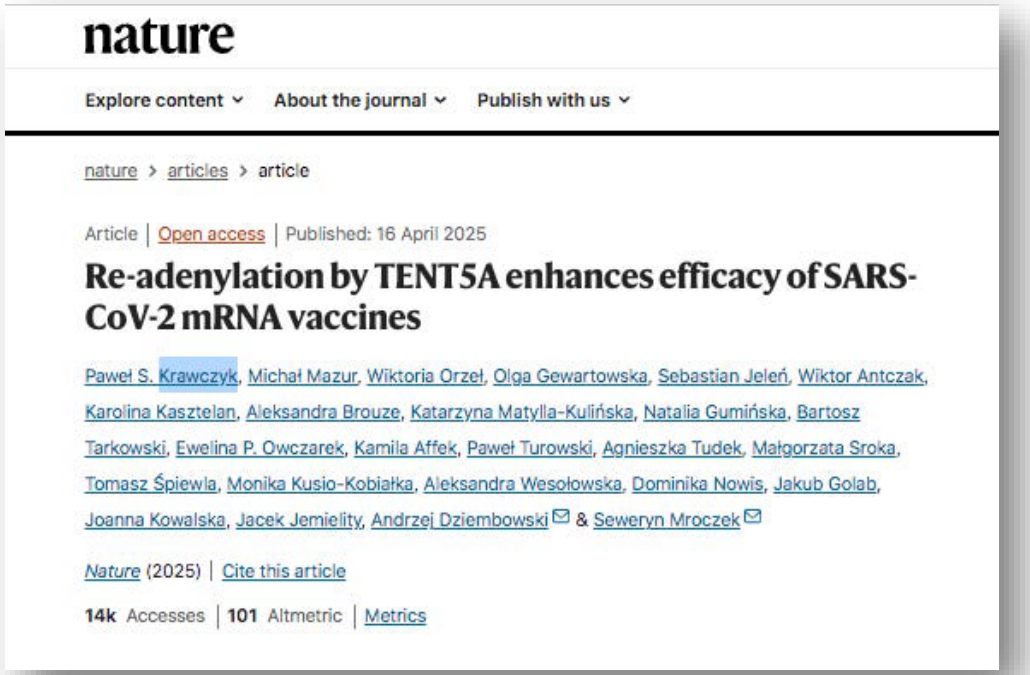
In brief

A data-driven approach for optimizing vaccination strategies in the very old population is needed. Lee et al. demonstrate that octogenarians mount a sustained antibody response following COVID-19 infection that is boosted upon receiving a single dose of BNT162b2 mRNA vaccine more than 1 year after recovery from COVID-19.

Krawczyk is contaminated

Spike

Plasmid



nature

Explore content ▾ About the journal ▾ Publish with us ▾

nature › articles › article

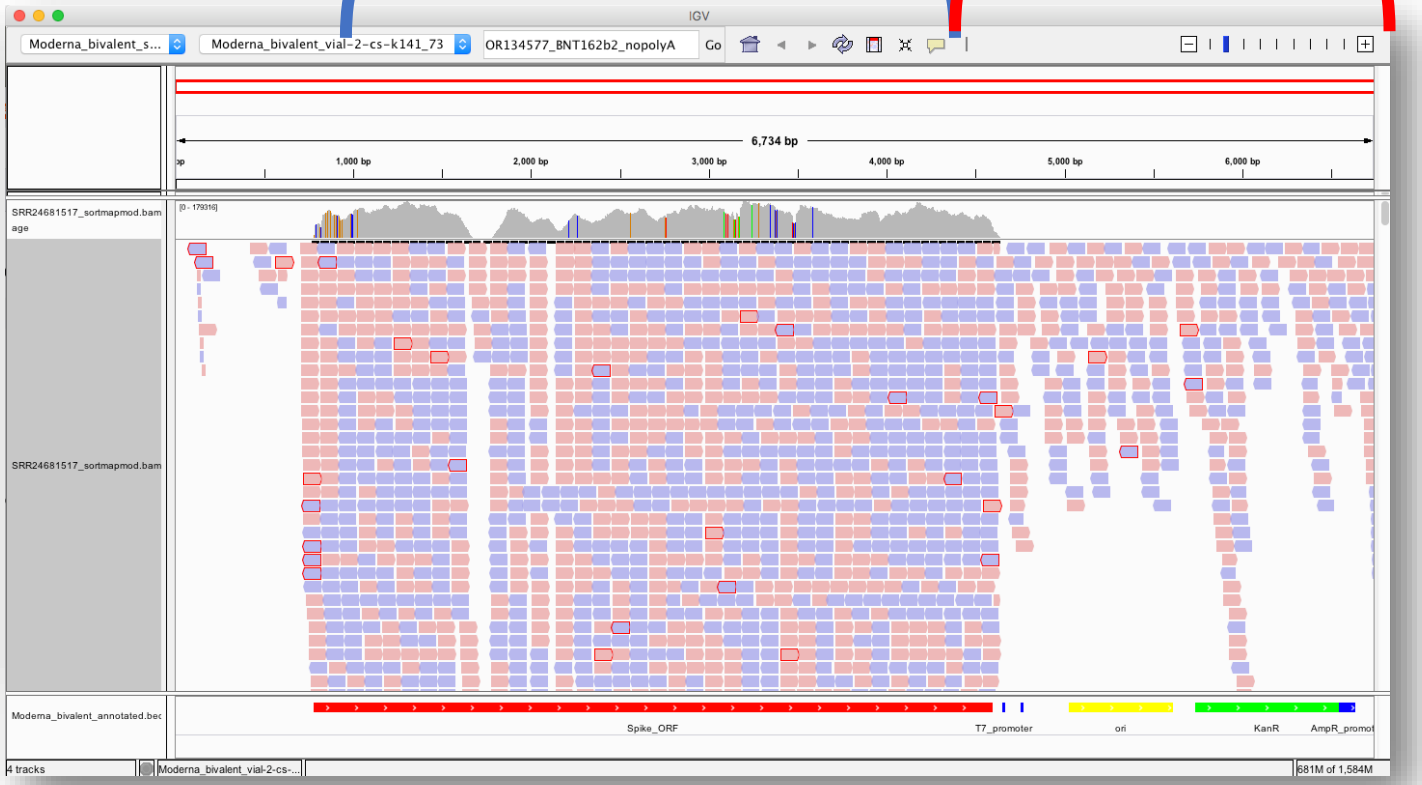
Article | Open access | Published: 16 April 2025

Re-adenylation by TENT5A enhances efficacy of SARS-CoV-2 mRNA vaccines

Paweł S. Krawczyk, Michał Mazur, Wiktoria Orzeł, Olga Gewartowska, Sebastian Jeleń, Wiktor Antczak, Karolina Kasztelan, Aleksandra Brouze, Katarzyna Matylla-Kulińska, Natalia Gumińska, Bartosz Tarkowski, Ewelina P. Owczarek, Kamila Affek, Paweł Turowski, Agnieszka Tudek, Małgorzata Sroka, Tomasz Śpiewla, Monika Kusio-Kobiałka, Aleksandra Wesołowska, Dominika Nowis, Jakub Golab, Joanna Kowalska, Jacek Jemielity, Andrzej Dziembowski ✉ & Seweryn Mroczek ✉

Nature (2025) | Cite this article

14k Accesses | **101** Altmetric | Metrics



Independent data on cancer mortality

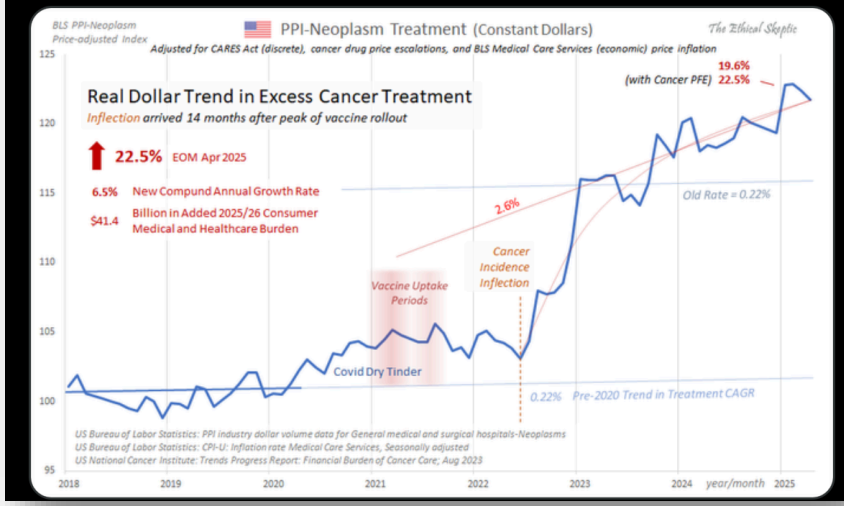
Death records show increase in ICD10 codes for various cancers

Ethical Skeptic ☀️ ✅
@EthicalSkeptic

🚨USA Cancer Treatment Expenditures Adjusted for Med Svc Inflation/Pricing (Constant $)

Up 22.5% over old trend
$41.4 billion in new cancer treatment costs per year

Inflection date = June 2022
Old Trend = 0.22% CAGR (real growth)
Novel Trend = 2.6% CAGR (real growth)



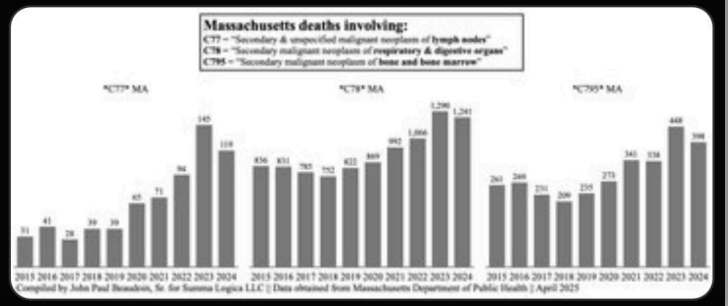
Cancer Treatments are up

John Beaudoin, Sr. aka, The ... ✅ @JohnBeau... · May 25
TURBOCANCER!

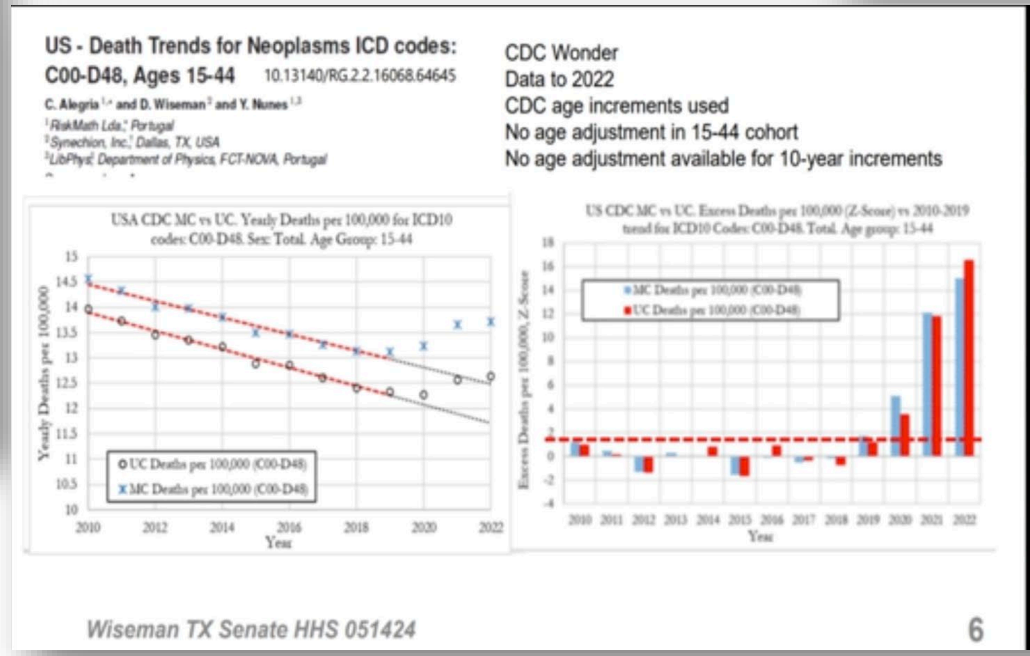
I made a fast look-up tool to check on some codes before I spend time generating large numbers of tables, statistics, and graphs

Example codes below

As bad as 2021 and 2022 are, 2023 and 2024 are certainly anomalous and EXCESSIVE



CDC data shows a Cancer wave
Ed Dowd, Carlos Alegria



Gibo et al Excess mortality in Japan
Cancer up 5-7% and they are different cancers

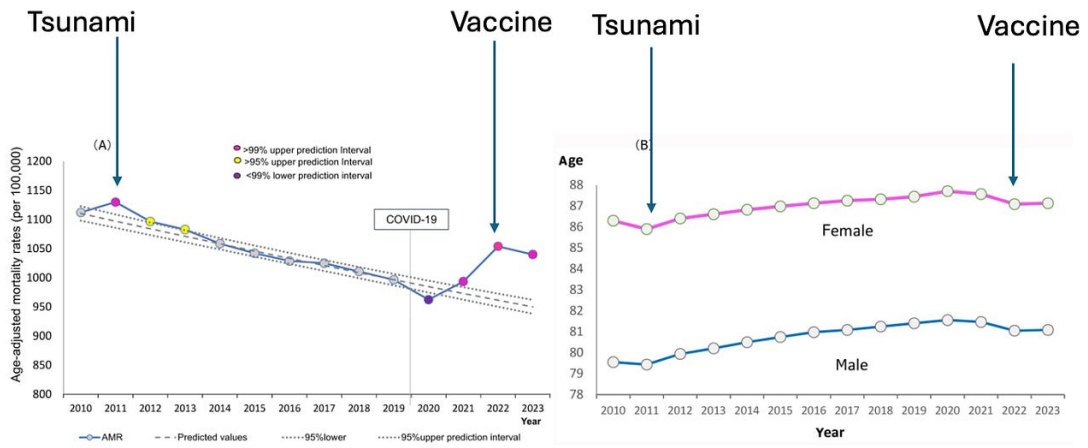
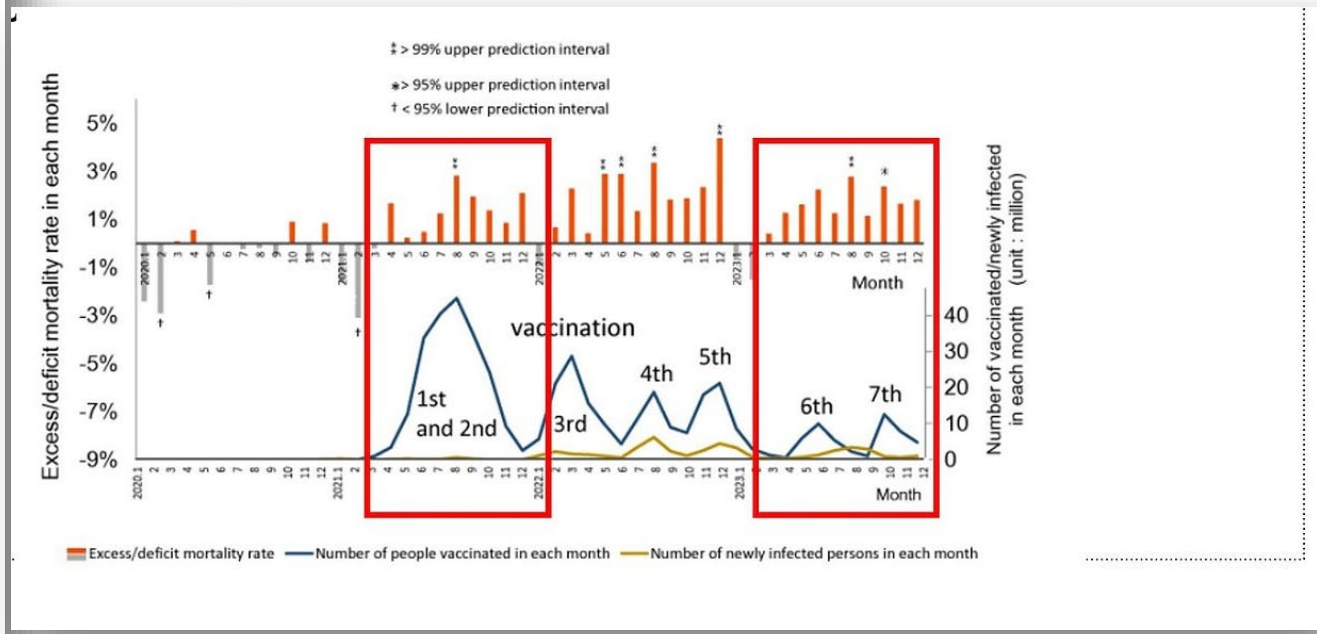


Figure 7 (A) Age-adjusted mortality rates (AMRs) over time: all causes. (B) Life expectancy in Japan [25]
(A) The predicted mortality rates and intervals from the pre-pandemic age-adjusted mortality (AMR) for all causes for 2010–2019, excluding 2011–2013, when the mortality rates were exceptionally high due to the major earthquake and tsunami, were compared with the AMR during the pandemic, from 2020–2023. The AMR in 2020 was under the 99% lower prediction interval (PI), but from 2021 it was above the 99% upper PI, with a further excess in 2022 and a slight downward trend in 2023, but still significantly above the 99% PI.
(B) Before the pandemic, with the exception of 2011, the year of earthquake and tsunami, life expectancy grew steadily, and the same was true in 2020, the first year of the pandemic. However, it began to turn downwards from 2021, shortened further in 2022, and remained almost the same in 2023.

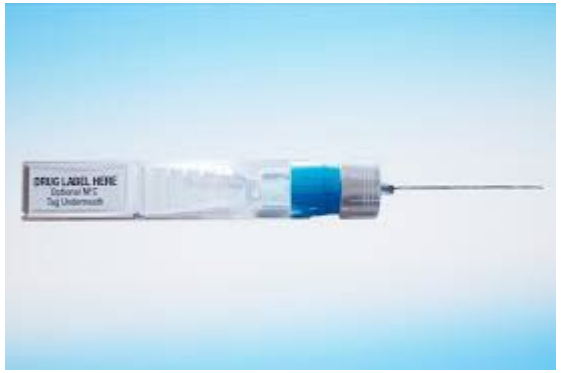
Even though 13% are taking 7th shot, same magnitude of excess mortality



Japan sees a shift in the types of cancers

Pre Jab

- Lung
- Colorectal
- Stomach
- Liver



Post Jab

Ovarian
Prostate
Leukemia
Pancreatic
Lip/oral/pharnygeal

Estrogen receptor related

They know something

$43 Billion

Pfizer Science Products Stories Newsroom About

Pfizer Completes Acquisition of Seagen

Thursday, December 14, 2023 - 07:39am

- *Further establishes Pfizer as a leading oncology company poised to accelerate the next generation of breakthrough treatments for people with cancer*
- *To address U.S. Federal Trade Commission concerns, Pfizer has chosen to irrevocably donate the rights of royalties from sales of Bavencio® (avelumab) in the U.S. to the American Association for Cancer Research (AACR)*

NEW YORK--(BUSINESS WIRE)-- Pfizer Inc. (NYSE: PFE) today announced the successful completion of its acquisition of Seagen Inc. (NASDAQ: SGEN), a global biotechnology company that discovers, develops and commercializes transformative cancer medicines. Pfizer completed its acquisition of all outstanding common stock of Seagen for $229 in cash per share, for a total enterprise value of approximately $43 billion.

$2.26 Billion

Pfizer Science Products Stories Newsroom About

Pfizer to Acquire Trillium Therapeutics Inc.

Monday, August 23, 2021 - 06:45am

Proposed acquisition strengthens Pfizer's category leadership in Oncology with addition of next-generation, investigational immuno-therapeutics for hematological malignancies

Expands innovative pipeline, potentially enhancing growth in 2026-2030 and beyond

Pfizer to host analyst and investor call at 10:00 a.m. ET today with Pfizer Oncology executives

NEW YORK & CAMBRIDGE, Mass.--(BUSINESS WIRE)-- Pfizer Inc. (NYSE: PFE) and Trillium Therapeutics Inc. (NASDAQ/TSX: TRIL) today announced that the companies have entered into a definitive agreement under which Pfizer will acquire Trillium, a clinical stage immuno-oncology company developing innovative therapies for the treatment of cancer. Under the terms of the agreement, Pfizer will acquire all outstanding shares of Trillium not already owned by Pfizer for an implied equity value of $2.26 billion, or $18.50 per share, in cash. This represents a 118% premium to the 60-day weighted average price for Trillium.

Integration is likely rare and delayed
The acute story is cGAS-STING

Hyperstimulatory N⁶-methyladenine (m6A) in residual SV40 plasmid DNA in mRNA vaccines.

Kevin McKernan
Medicinal Genomics, Beverly MA

Abstract

Many mRNA vaccine production pipelines rely on *Escherichia coli* to replicate plasmid DNA templates used in the in vitro transcription of modified RNA. However, *E. coli* DNA methylation patterns differ substantially from those of humans. In *E. coli*, DNA methylation is primarily mediated by DNA adenine methyltransferase (Dam), which introduces N⁶-methyladenine (m6A) within GATC motifs, whereas human methylation occurs predominantly at cytosines in CpG dinucleotides. Some *E. coli* strains also express Dcm methyltransferase, which methylates CCWGG sequences (CC[A/T]GG), further distinguishing bacterial from mammalian epigenetic marks.

Cytosolic DNA that lacks CpG methylation can potently activate Toll-like receptor 9 (TLR9), while m6A-modified DNA has been shown to stimulate the cGAS–STING pathway, leading to the induction of type I interferons and other inflammatory mediators.

Because the Pfizer mRNA vaccine plasmids are propagated in *E. coli*, and residual plasmid DNA has been detected in finished vaccine material, it is likely that this DNA bears bacterial-type methylation patterns that could be immunostimulatory through TLR9 and cGAS–STING signaling. To investigate this possibility, we applied Oxford Nanopore sequencing to examine the methylation status of plasmid DNA present in Pfizer lot FL8095.

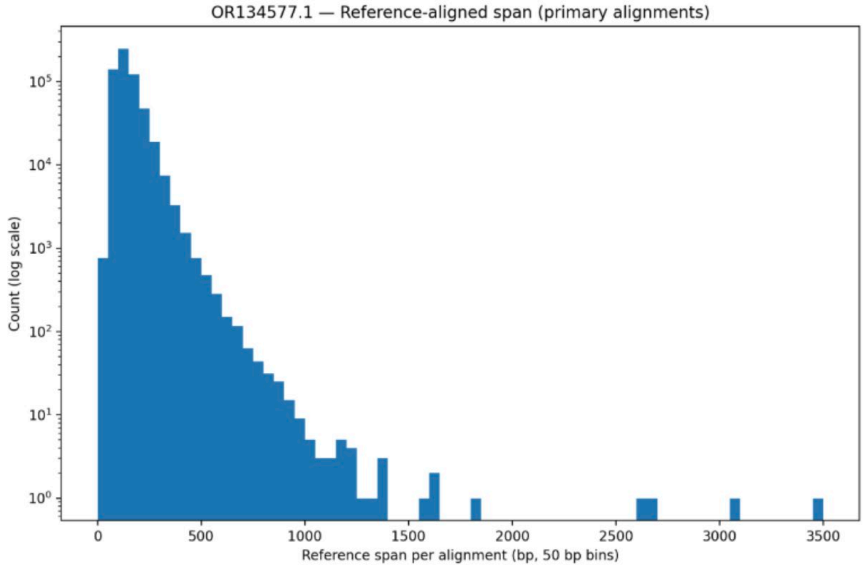


Figure 3. Aligned ONT Read length distributions. Oxford Nanopores cannot thread circular DNA through the pores and cannot be measured with these methods.

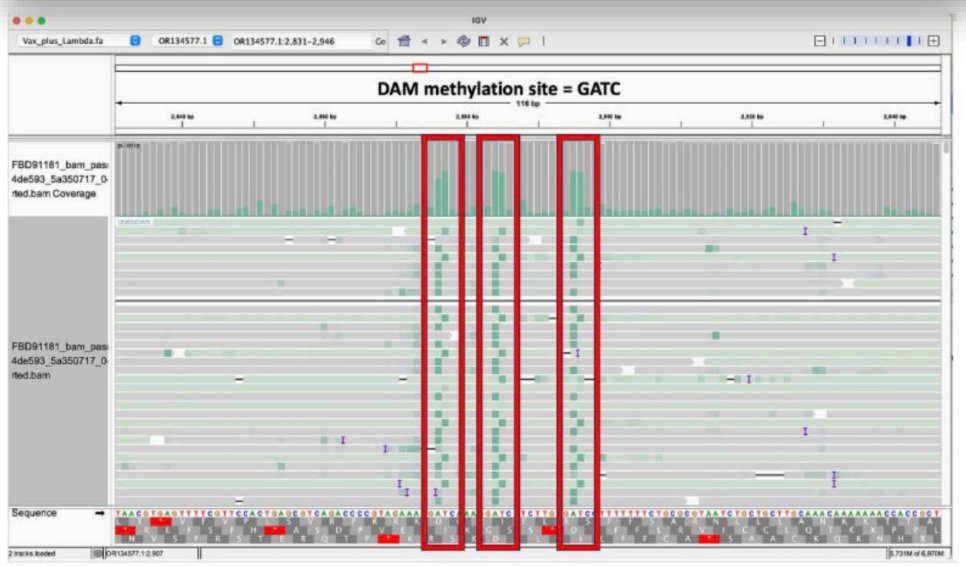
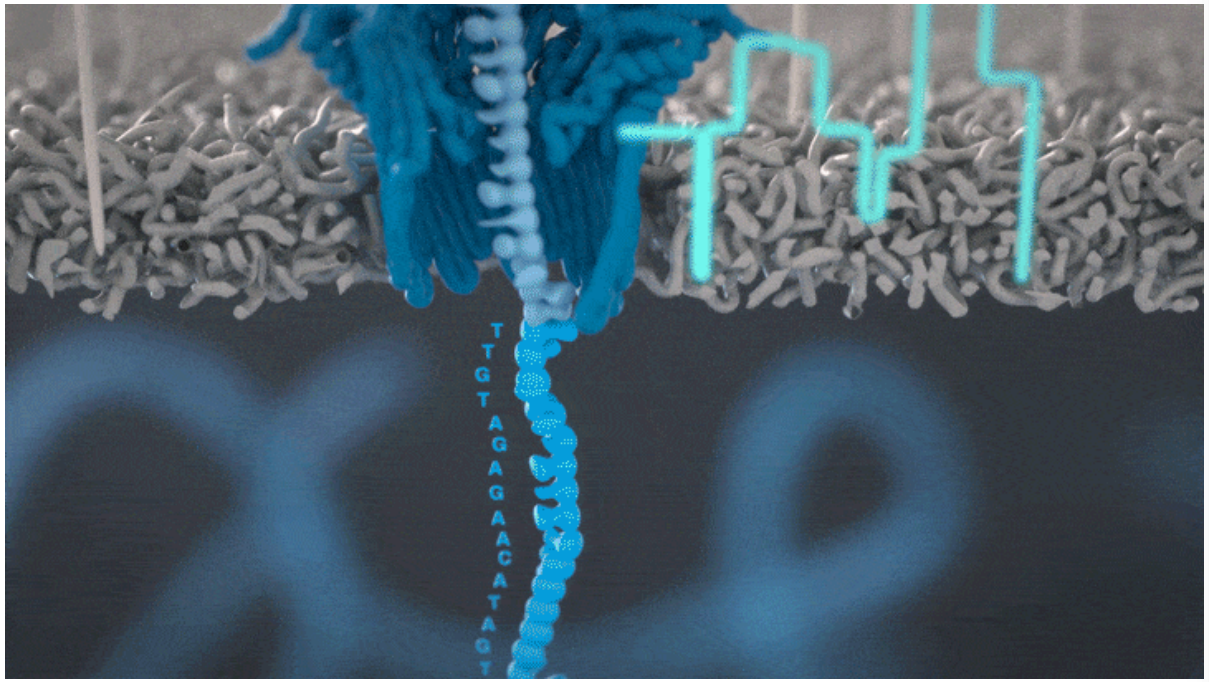


Figure 5. Tandem GATC sites heavily methylated

m6A hyperstimulates cGAS-STING and CD69 expression



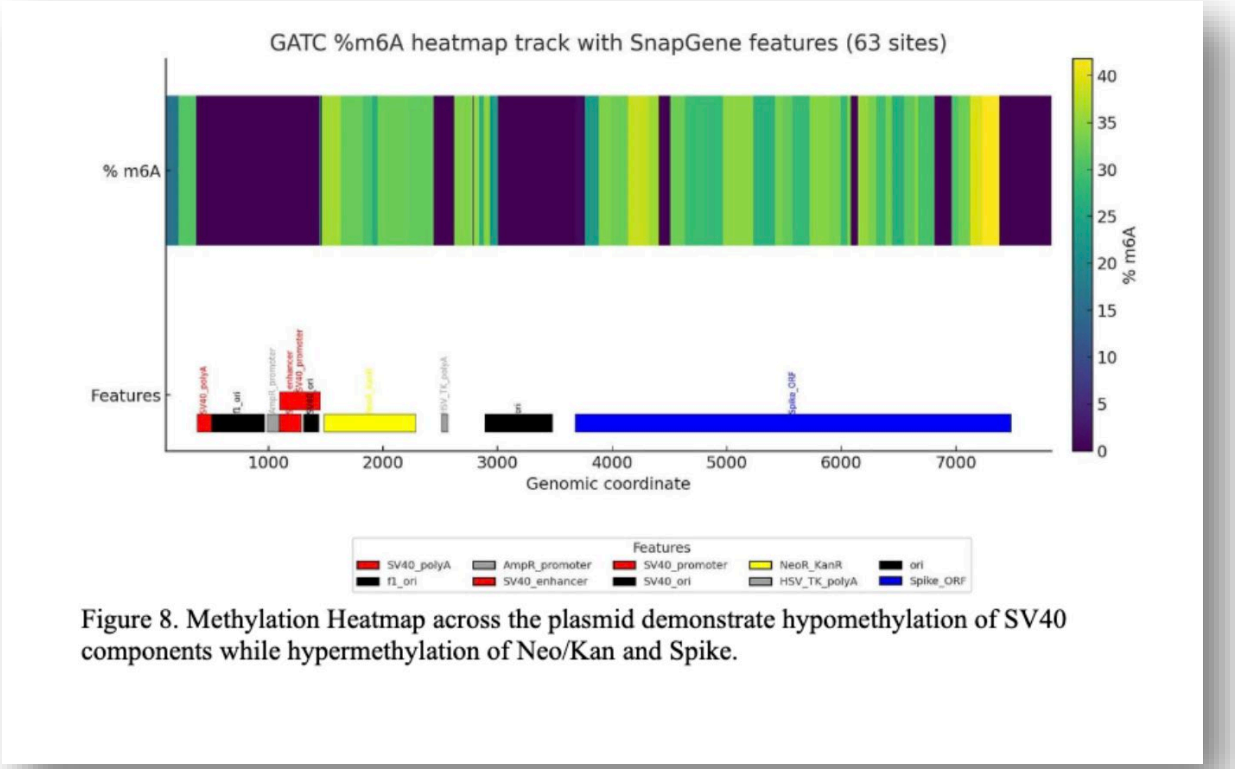
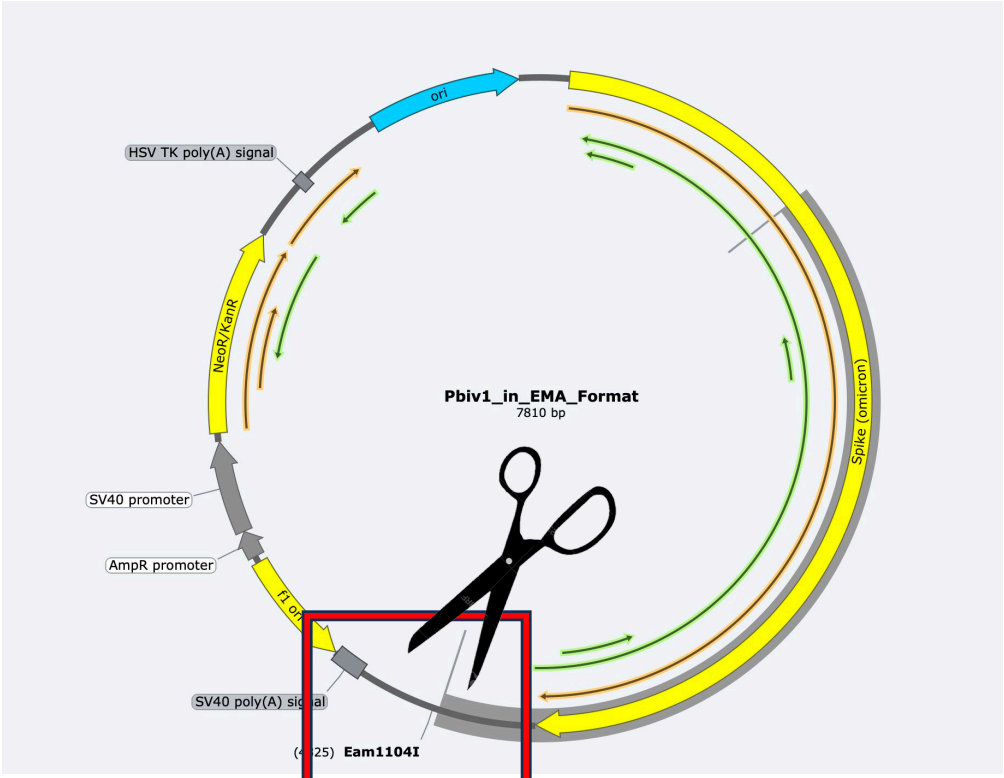
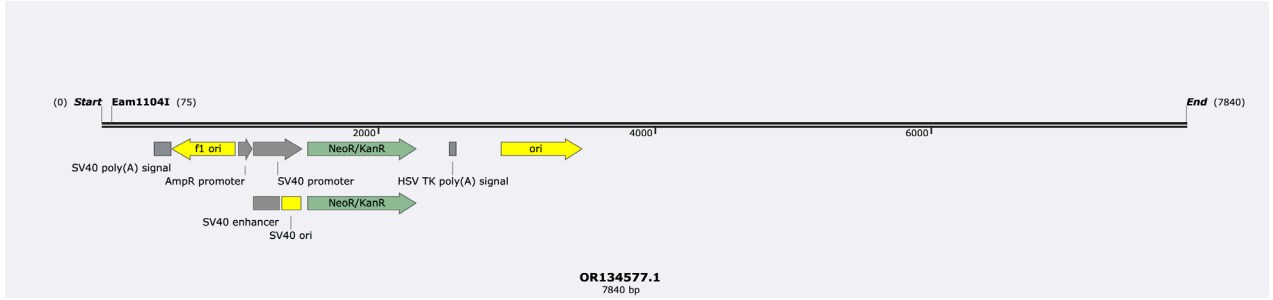


Figure 8. Methylation Heatmap across the plasmid demonstrate hypomethylation of SV40 components while hypermethylation of Neo/Kan and Spike.

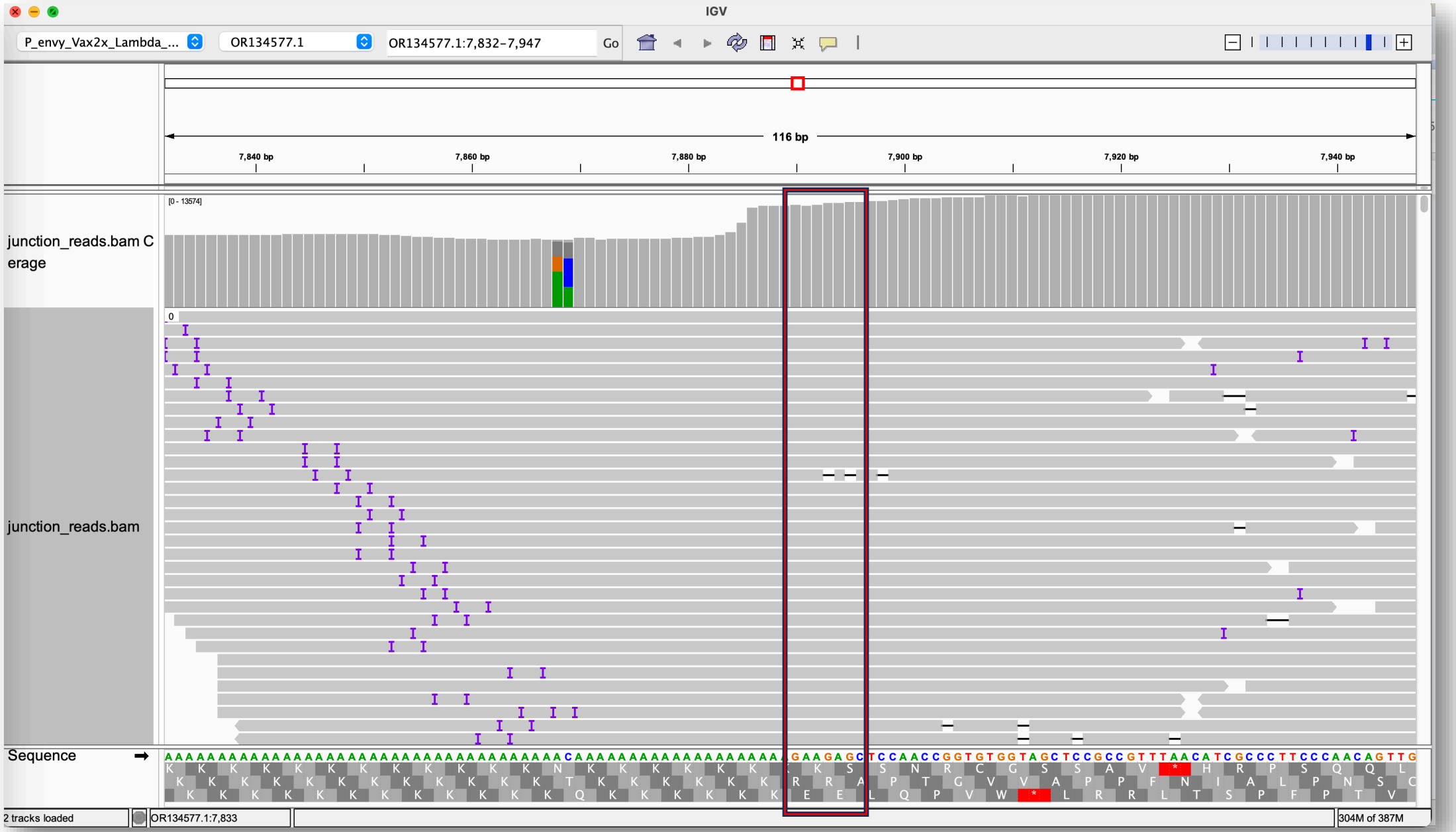
ONT sequencing to assess the Eam1104i linearization in Pfizer Monovalent vaccines



Digest with Eam1104i

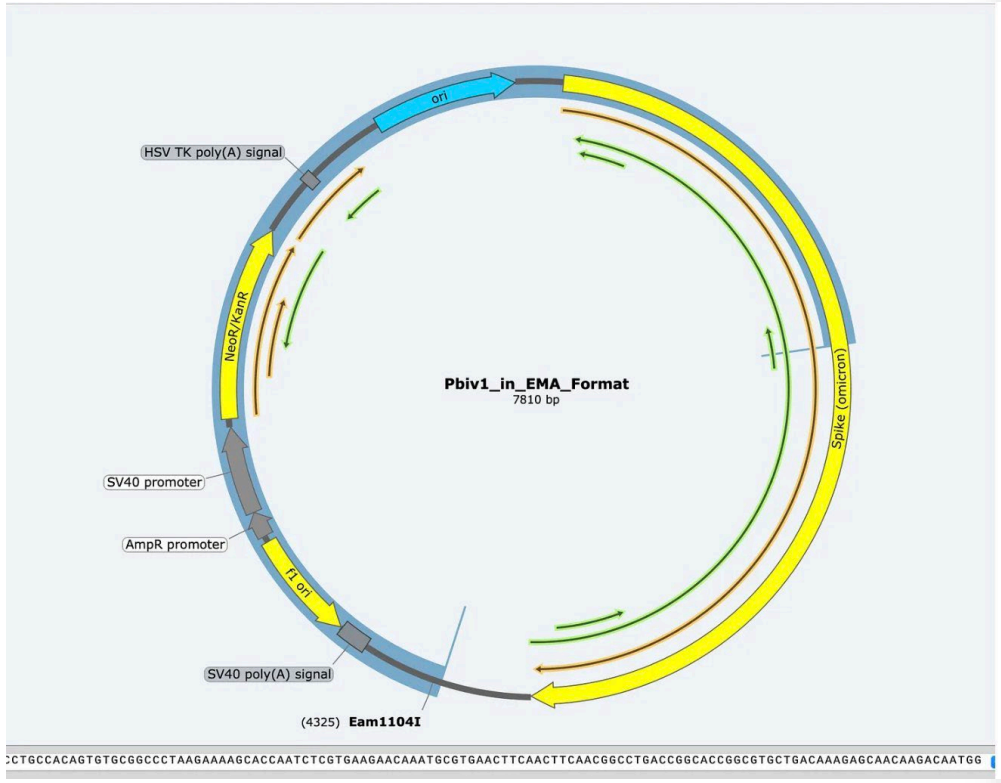


Failure to Digest can leave circular replication competent plasmids in the vials



Eam1104i cut site

5,283 base pair fragment in a Pfizer vaccine



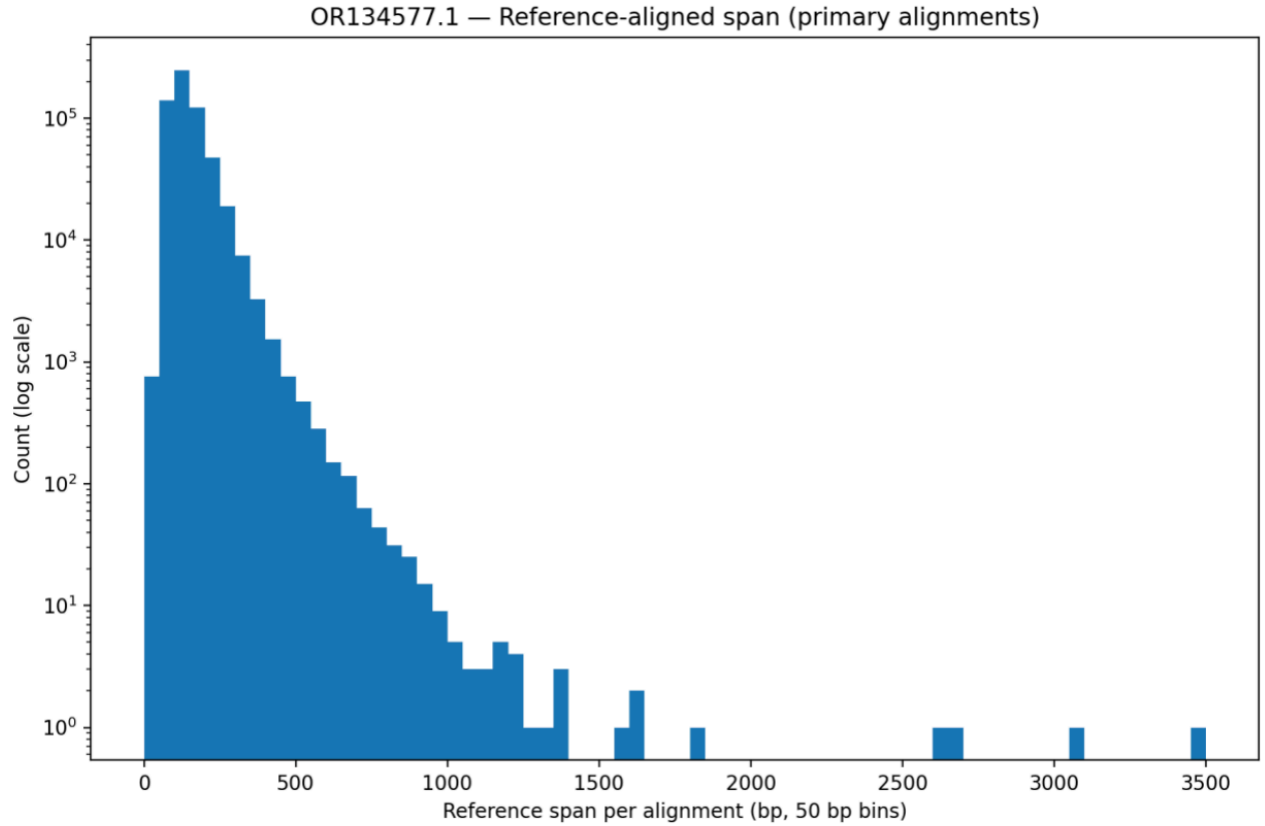
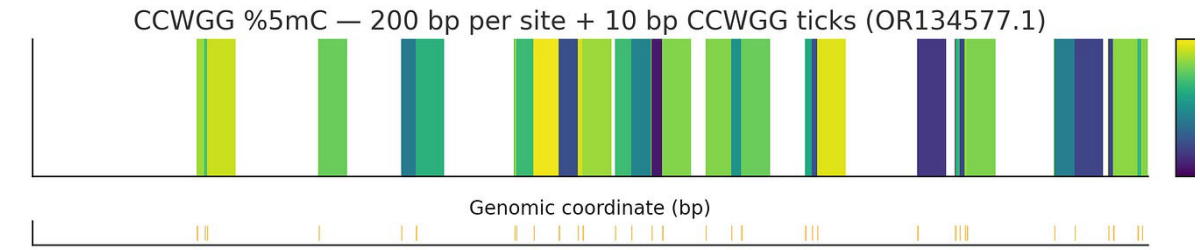
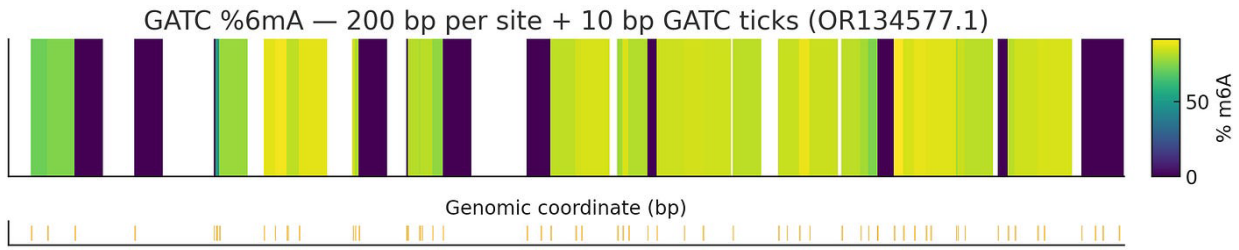


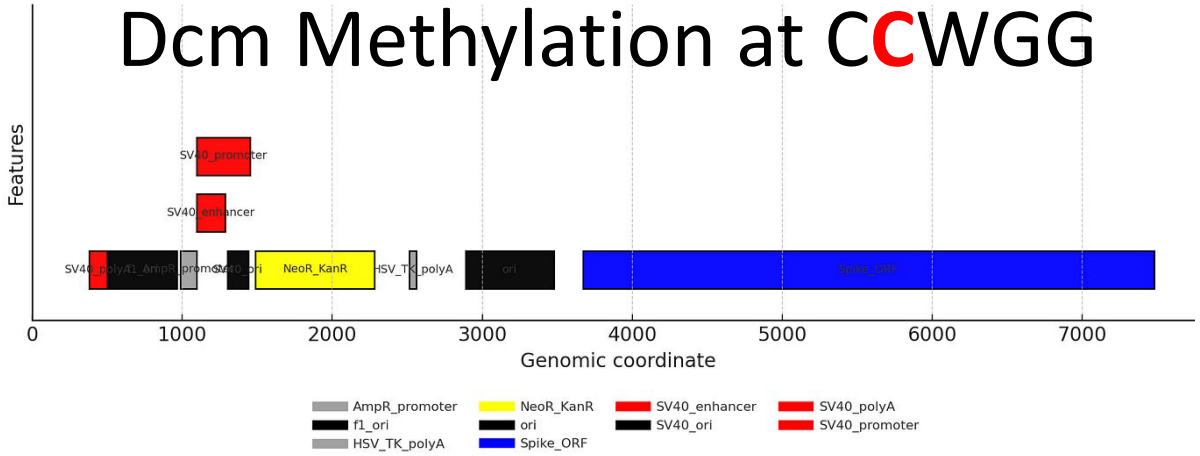
Figure 3. Aligned ONT Read length distributions. Oxford Nanopores cannot thread circular DNA through the pores and cannot be measured with these methods.

Wrong E.coli cell line for Plasmid manufacturing

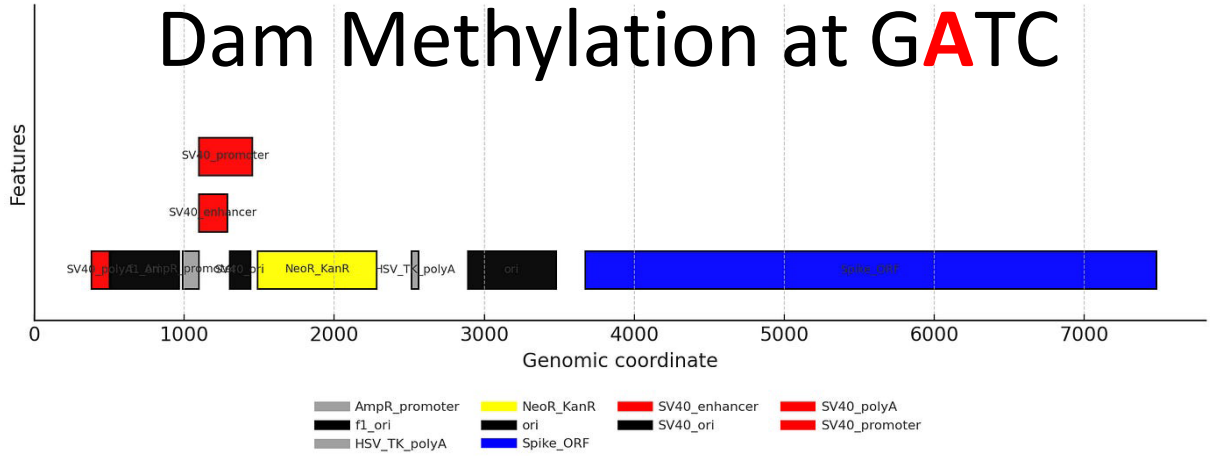


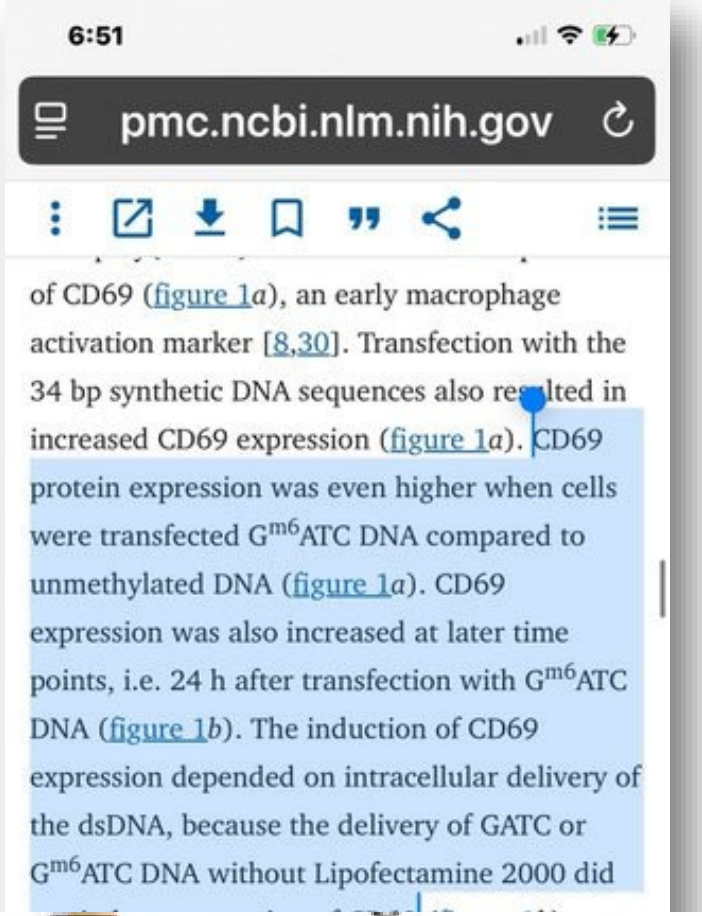


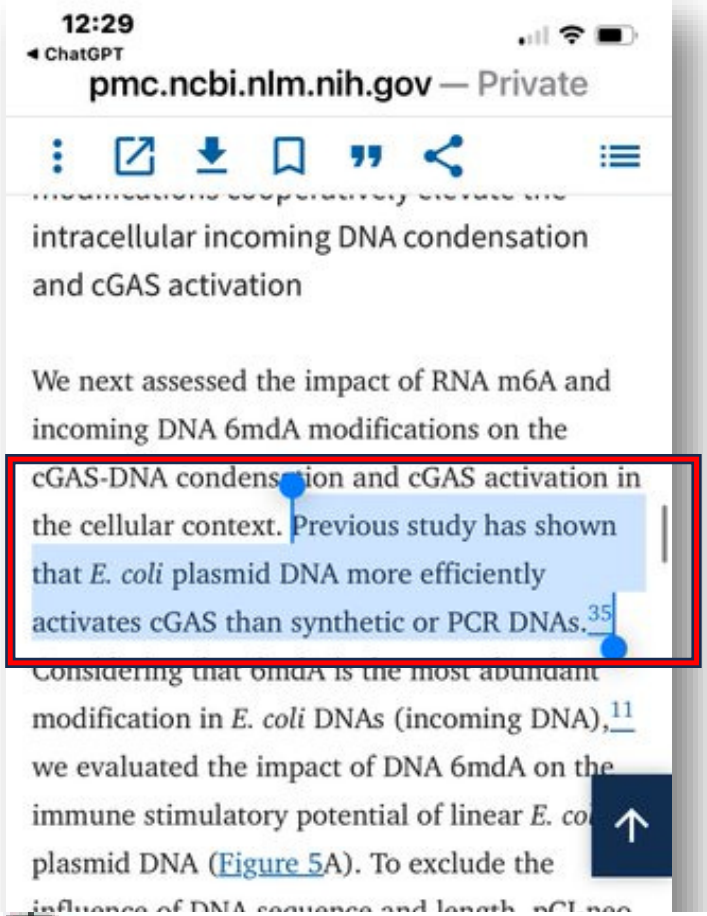
Dcm Methylation at C**C**WGG

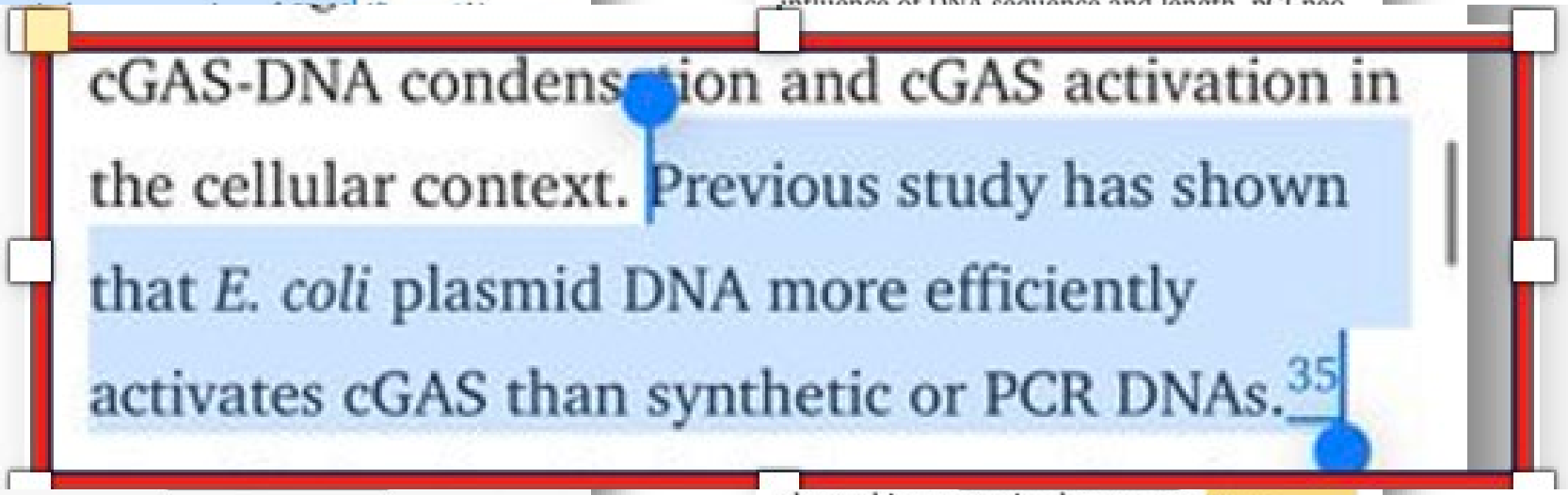


Dam Methylation at G**A**TC




6:51
pmc.ncbi.nlm.nih.gov
of CD69 (figure 1a), an early macrophage activation marker [8,30]. Transfection with the 34 bp synthetic DNA sequences also resulted in increased CD69 expression (figure 1a). CD69 protein expression was even higher when cells were transfected G^{m6}ATC DNA compared to unmethylated DNA (figure 1a). CD69 expression was also increased at later time points, i.e. 24 h after transfection with G^{m6}ATC DNA (figure 1b). The induction of CD69 expression depended on intracellular delivery of the dsDNA, because the delivery of GATC or G^{m6}ATC DNA without Lipofectamine 2000 did


12:29
ChatGPT
pmc.ncbi.nlm.nih.gov — Private
intracellular incoming DNA condensation and cGAS activation
We next assessed the impact of RNA m6A and incoming DNA 6mdA modifications on the cGAS-DNA condensation and cGAS activation in the cellular context. Previous study has shown that *E. coli* plasmid DNA more efficiently activates cGAS than synthetic or PCR DNAs.[35]
modification in *E. coli* DNAs (incoming DNA),[11] we evaluated the impact of DNA 6mdA on the immune stimulatory potential of linear *E. co*
plasmid DNA (Figure 5A). To exclude the


cGAS-DNA condensation and cGAS activation in the cellular context. Previous study has shown that *E. coli* plasmid DNA more efficiently activates cGAS than synthetic or PCR DNAs.[35]

- Wang et al
- https://pmc.ncbi.nlm.nih.gov/articles/PMC11638879
- Luecke et al
- https://www.embopress.org/doi/full/10.15252/embr.201744017
- Balzarolo et al
- https://pubmed.ncbi.nlm.nih.gov/33715389/
- Carnes et al
- https://pubmed.ncbi.nlm.nih.gov/20830679/

ARTICLE

BIOTECHNOLOGY and BIOENGINEERING

Plasmid DNA Fermentation Strain and Process-Specific Effects on Vector Yield, Quality, and Transgene Expression

Aaron E. Carnes, Jeremy M. Luke, Justin M. Vincent, Angela Schukar, Sheryl Anderson, Clague P. Hodgson, James A. Williams

Nature Technology Corporation, 4701 Innovation Drive, Lincoln, Nebraska 68521;
telephone: 402-472-6530; fax: 402-472-6532; e-mail: jim@natx.com

Received 10 June 2010; revision received 27 August 2010; accepted 2 September 2010
Published online 9 September 2010 on Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/bit.22936

ABSTRACT: Industrial plasmid DNA manufacturing processes are needed to meet the quality, economy, and scale requirements projected for future commercial products. We report development of a modified plasmid fermentation copy number induction profile that increases gene vaccination/therapy vector yields up to 2,600 mg/L. We determined that, in contrast to recombinant protein production, secretion of the metabolic byproduct acetate into the media had only a minor negative effect on plasmid replication. We also investigated the impact of differences in epigenetic *dcm* methylase-directed cytosine methylation on plasmid production, transgene expression, and immunogenicity. While *Escherichia coli* plasmid production yield and quality are unaffected, *dcm*− versions of CMV and CMV-HTLV-I R promoter plasmids had increased transgene expression in human cells. Surprisingly, despite improved expression, *dcm*− plasmid is less immunogenic. Our results demonstrate that it is critical to lock the plasmid methylation pattern (i.e., production strain) early in product development and that *dcm*− strains may be superior for gene therapy applications wherein reduced immunogenicity is desirable and for in vitro transient transfection applications such as AAV production where improved expression is beneficial.

Biotechnol. Bioeng. 2011;108: 354–363.

KEYWORDS: DNA vaccine; plasmid; *dcm* methylation; fermentation; gene therapy; non-viral vector

for example, transient transfection of production cells for adeno-associated virus (AAV) production. There are four licensed veterinary plasmid products (Cai et al., 2009) and over 150 plasmid-based vectors in Phase I and Phase II clinical trials on the NIH ClinicalTrials.gov website. In order to commercialize these DNA medicines, it is essential to devise industrial processes whereby plasmid DNA can be manufactured to meet the quality, economy, and scale requirements projected for future products.

In general, plasmid quality is higher from fed-batch rather than batch fermentation (reviewed in O'Kennedy et al., 2003). A few high yield fed-batch plasmid fermentation processes (500–2,200 mg/L) have been described (Carnes et al., 2006; Listner et al., 2006; Mairhofer et al., 2010; Phue et al., 2008; Singer et al., 2009; Williams et al., 2009c). These processes all couple reduced growth rate (which generally increases copy number) with high copy replication origins (reviewed in Carnes and Williams, 2007).

Plasmid DNA production is typically performed in *Escherichia coli* K12 strains such as DH5α (Carnes et al., 2006), DH5 (Listner et al., 2006), DH1 (Cooke et al., 2004), JM108 (Mairhofer et al., 2010), or DH10B (Lahijani et al., 1996). *E. coli* B strain BL21 *recA endA* is also a high yielding plasmid producer (Phue et al., 2008) (reviewed in Cai et al., 2009; Williams et al., 2009a). However, plasmid products produced from these strains are distinct due to strain-specific epigenetic nucleotide methylation.

(Caspeta et al., 2009).

We also reported the impact of differences in epigenetic *dcm* methylase-directed cytosine methylation on plasmid production, transgene expression, and immunogenicity. From a regulatory perspective, *dcm*+ and *dcm*− versions of a plasmid are different chemical entities, due to the presence or absence of multiple 5-methyl-cytosine residues. While plasmid yield and quality in the inducible fed-batch fermentation process was equivalent between isogenic *dcm*+ and *dcm*− strains, eukaryotic cell transgene expression was higher using *dcm*− plasmid DNA with both CMV-HTLV-I R (e.g., NTC8685) and CMV (gWIZ, pVAX1) promoters. Surprisingly, *dcm*− plasmid DNA for an influenza HA DNA vaccine vector elicited lower anti-HA antibody responses after low-dose naked DNA prime boost intramuscular immunization. While this effect may not occur with other deliveries or higher plasmid dosage, these results demonstrated that 5-methyl-cytosine modified bases are not functionally inert.

Reduced immunogenicity with *dcm*− plasmid is unlikely to be due to decreased expression in vivo.

approach was used to demonstrate that acetate accumulation only had a minor negative effect on plasmid replication. Using isogenic strains, we demonstrated that plasmid *dcm* methylation status differentially impacted vector expression and immunogenicity. These data demonstrated biological differences between *dcm*+ and *dcm*− plasmid DNA, highlighting the critical need to define the optimal product (i.e., with or without 5-methyl-cytosine) early to correctly lock the plasmid methylation pattern (i.e., production strain) for subsequent clinical development.

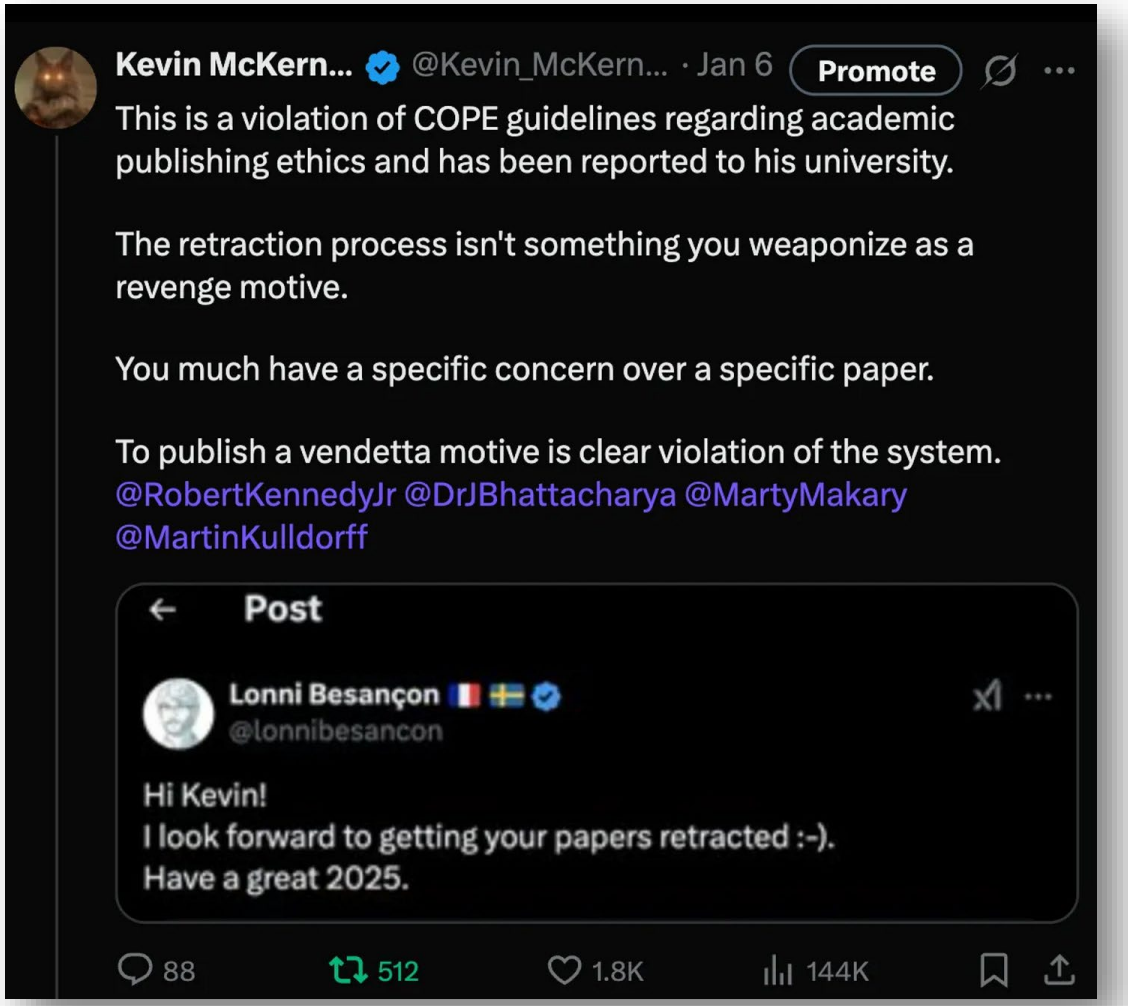
Wrong E.coli cell line for Plasmid manufacturing

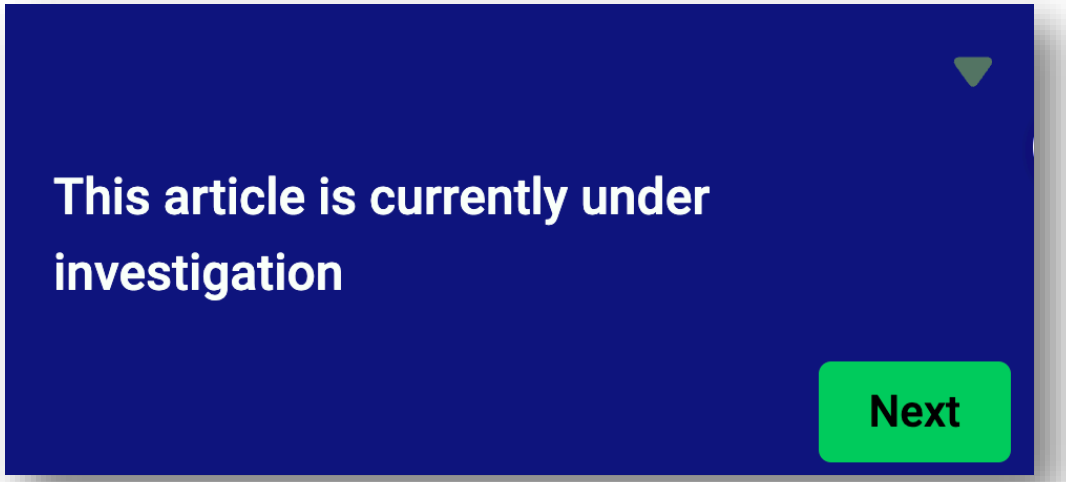
One hypothesis for reduced immunogenicity is that *dcm* methylation sites are a pattern recognized by the innate immune system. For example, Dam and K12 methylation product, N^6-methyladenosine, is a wide spread bacterial signature not present in mammalian cells. Oligonucleotides or plasmid DNA containing N^6-methyladenosine increased cytokine induction (interleukin 12; IL-12) in mice compared to unmethylated control DNA (Tsuchiya et al., 2005), and IL-12 induction was twofold lower with unmethylated plasmid (vs. *dam*+ *dcm*+ methylated) after IV lipofection delivery to mice (Ochiai et al., 2005). N^6-methyladenosine responses are cell-type specific, since *dam*− *dcm*− unmethylated or *dam*+ *dcm*+ methylated plasmid was equivalent for macrophage activation (Roberts et al., 2005) or TLR9 independent activation of human neutrophils (Trevani et al., 2003).

In conclusion, *dcm* methylation status affects plasmid-directed transgene expression and immunogenicity but not plasmid production. Our results demonstrated that it is critical to lock the plasmid methylation pattern (i.e., production strain) prior to product clinical development and that *dcm*+ plasmid is recommended for DNA vaccines and *dcm*− plasmid for DNA therapeutics and cell transfection reagents such as AAV helper plasmids.

Informed Consent is Critical cornerstone of medical ethics.

Weaponization of Science





The *Con*fession

VaccineMole ✅
@VaccineMole

🤐📢Regarding the comment below on PubPeer network by Actinopolyspora biskrensis (Kevin Patrick) criticizing Speicher et al. mRNA vaccine contamination study:

Patrick's citation of on Klinman et al. paper ***is completely FABRICATED. it does not appear in the Klinman et al. paper at all!***

Please read his citation on his claim 16:

"While it is theoretically possible for homologous recombination to occur at very short stretches of identity (as small as 7 bp), the frequency of such events in mammalian cells is extremely low, and multiple studies have failed to detect integration of plasmid DNA into host chromosomes above background levels following immunization with DNA vaccines."

Patrick's citation above was totally fabricated, the Klinman et al. Section 3.4 ends exactly after the examples of sequences (VDJ, ALU, etc.), with no additional sentence about "theoretically possible," "extremely low frequency," or "failed to detect."

Cheshire @Thatsregrettab1 · 7h
Good catch, and I agree with you that the quote was entirely made up. I apologize. 😋 I used ChatGPT to clean up my text, but it decided to make shit up and I didn't notice.

The point is still valid: Klinman (2010) does not support the statements made in Speicher (2025).

#26 *Actinopolyspora biskrensis* comment accepted September 2025

#16

As was p
inaccura

"Res
parti
plasr

"Ove

"This
will i

"Bas
insu
spon

Taken to
guidelin
the impr

Jikkyleaks 🐭 ✅
@Jikkyleaks

"We charge $5000 for retracting an article. PayPal me".

@SecKennedy you have the power to initiate this prosecution.

#pubpeergate

On Fri, June 3, 2022 at 10:55 AM Alexander Magazinov <alexander.magazinov@gmail.com> wrote:
Dear [redacted]

My team manages several Pubpeer blue accounts, we generally charge 500$.
We charge 5000$ for retracting an article. Please send me the DOI of the papers and Paypal me.

Regards,
Alexander Magazinov

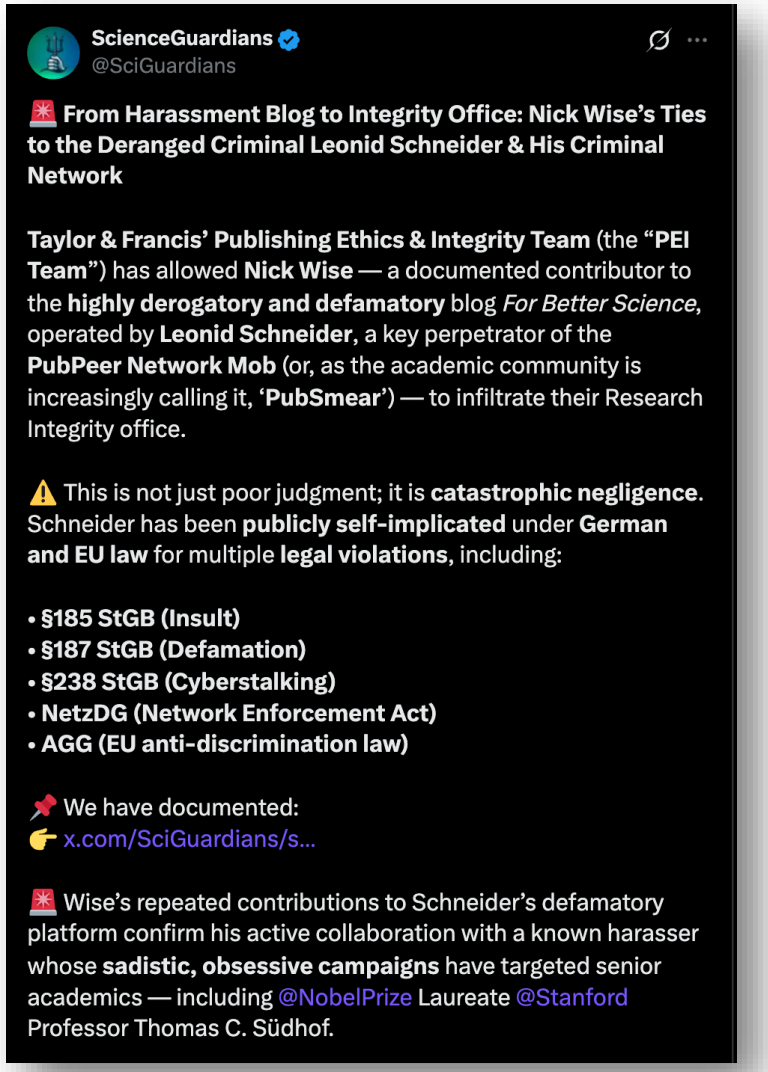
On Fri, June 3, 2022 at 10:11 AM [redacted] wrote:
Dear Alexander Magazinov,

M[redacted] recommended I contact you. How much would you charge us for five Pubpeer comments on the article
Prof[redacted]?

Thanks,
[redacted]

Again, I apologize for the misquote, however I think the concern is still valid.

State of Peer Review



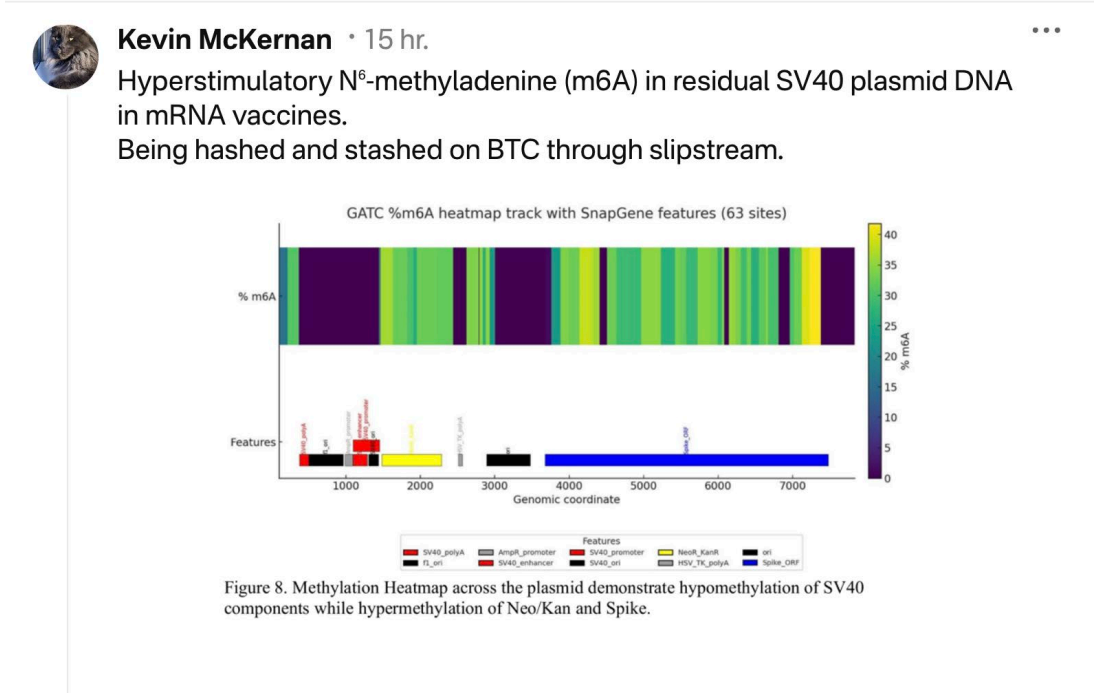
Taylor and Francis Research Integrity office = Nick Wise
Wise is a PubSmear participant
PubSmear complaints do not have to disclose their conflicts of interest.
Kevin Patrick declares himself an "investor"

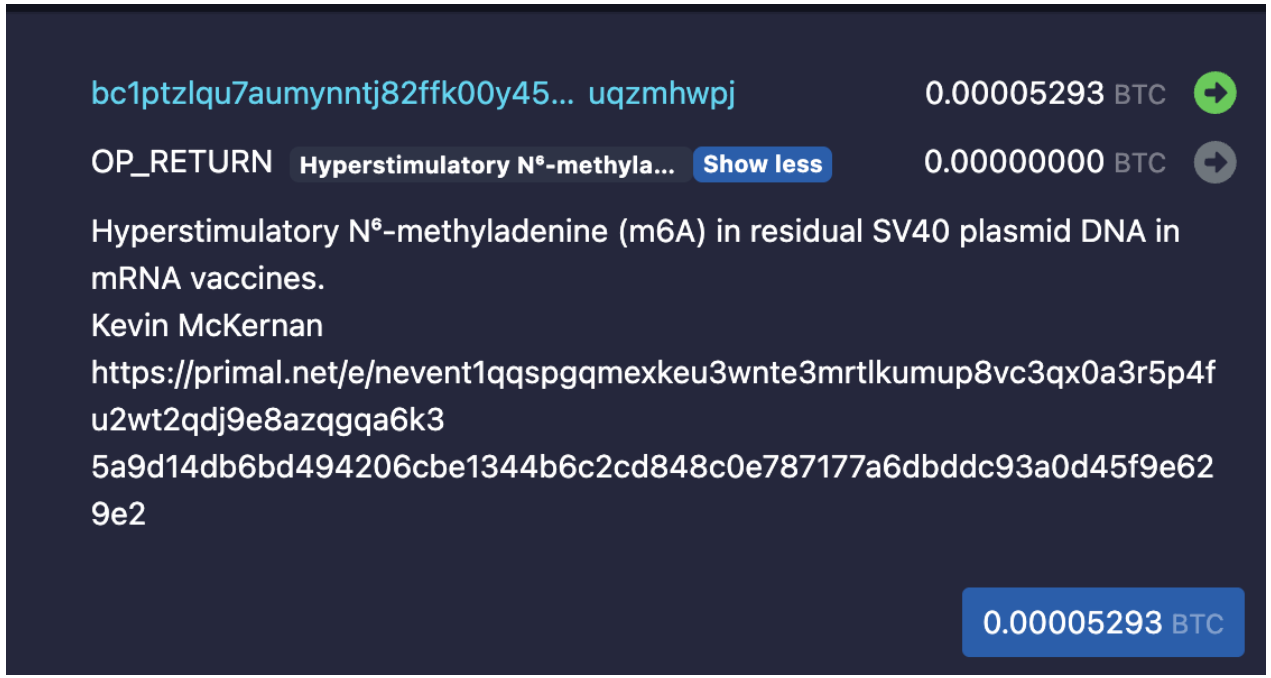
The Journal charges you $3K per paper. The reviewers are free.
They would rather have your paper pass peer review to collect you $3K and then retract it later, keeping your copyright and taking your money

Asking for 3 more reviewers to Re-Review your paper costs them $0.
Retracting your paper damages dissidents more than Anon Desk Rejections

Disintermediate the Journals.
“Peer to Peer” review with decentralized networks

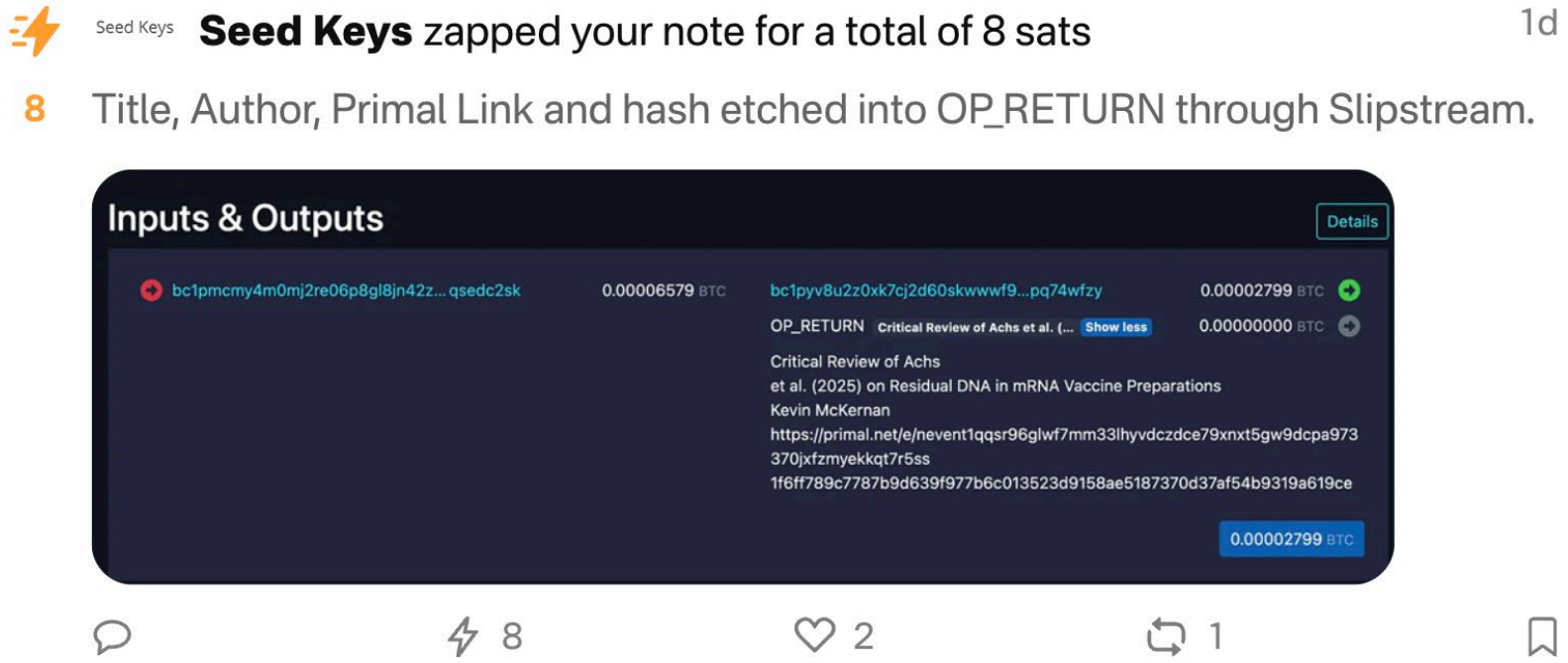
- Nostr/Primal = Relay network with Lightening payments integrated
- Turn your PDF into a PNG file. Post on Nostr
- Etch a Title, Author, Nostr Link and Hash of paper onto Bitcoin
- No one has ever censored a Bitcoin transaction since inception. Immutable





Lightening Network integration is Key!

In order to **“Uberize”** the Peer review market, we need Pricing signals.
We need transparent Review Bounties.
Pricing signals confer urgency and scarcity. You cannot optimize supply and demand in any market without prices.
Free review invites antagonistic reviewers with specious motivations.



Nostr/primal is a lightening network.
You can post review bounties with transparency conditions.

Reviewers disclose CV, Bounty, COIs and all review communication.
Payment rails are international.

Don’t give the Journals $3K.
Post it as a review bounties.
Record everything on Immutable ledgers

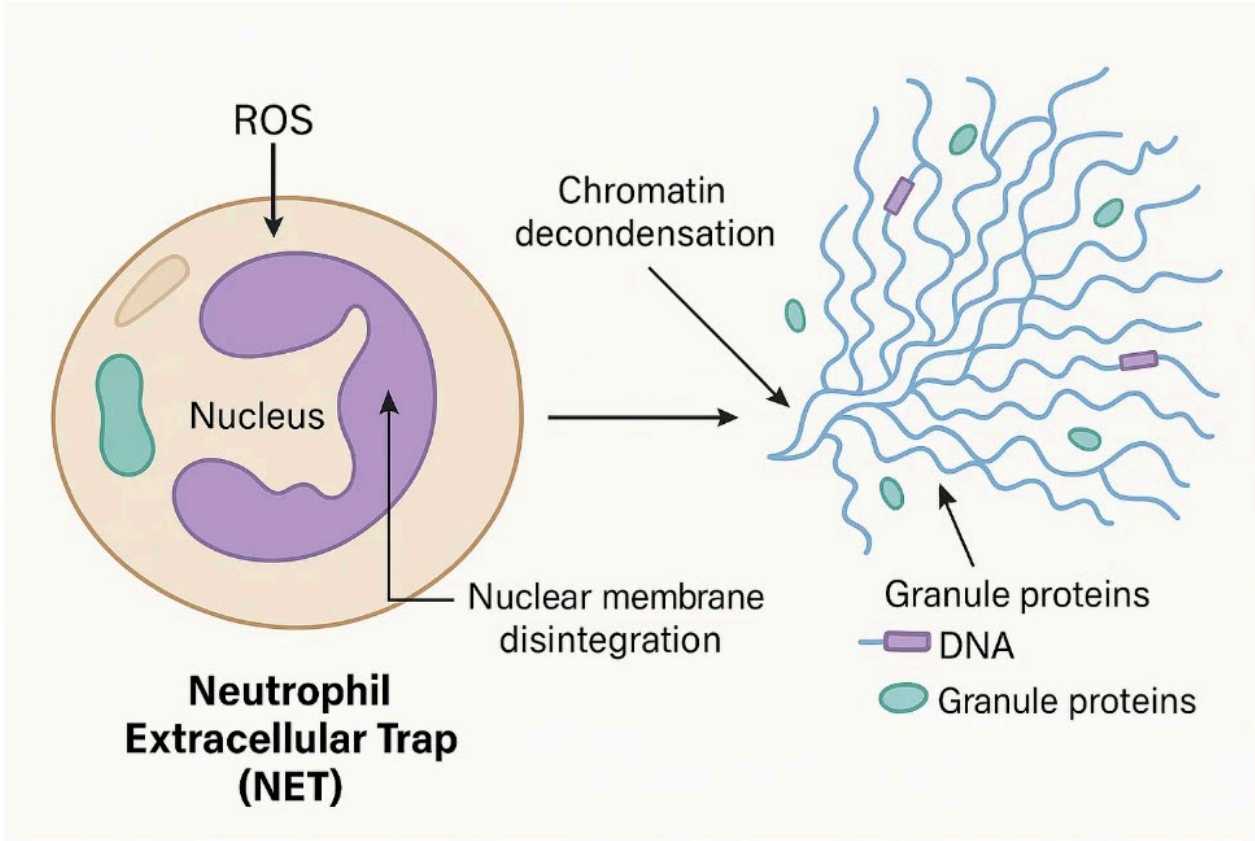
Summary

- The Pfizer vaccines on the market are not the same formulation as what was tested in clinical trials! Bait and Switch. Fraud!
- Significant DNA contamination has been found in 10/11 studies
- Significant DNA contamination is now found in 5 peer reviewed studies looking at people's blood and tissue.
- Cancer is on the rise!
- Several papers report cancer post vaccination.
- Wrong E.coli cell line used for Manufacturing
- Liability free mandates of the largest carcinogen to ever hit the population.
- We need to modernize Peer Review

Whole Genome Sequencing of Fibrin Clots

Neutrophil Extracellular Traps, SERPINs and amyloidogenic variants of concern

ANANDAMIDE
MAY 23, 2025



Learn about decentralized medicine at CannMed, Primal and Substack