Twitter Thread by rubic3n

rubic3n
@rubic3n

80% of diseases not treatable with 'conventional medicine'. Protein treatment as an alternative with drawbacks.
An 80% market for mRNA. Yet, the vector is the bottleneck causing the limitation.
Viral V is almost a 'use once only' bcs of antibody formation.
LNP sole way to push.

> READ THE INTRODUCTION... https://t.co/1k4qZeutit
>
> — Not Your Mom (@Notyour28981739) December 24, 2021

mRNA Therapy - with a gargantuan market - in dire need for a vector.
Viral vectors as a dead ally until 2015.
mRNA Therapy meets enormous resentments.

Luckily, billions of people have now got first hand experience. Obviously, AZ was not that useful in the general drift.

Gene therapy appears simple in principle but involves identification of affected gene(s), cloning and loading of a wild type or recombinant healthy version in a suitable vector for optimal delivery and expression in the target cells or tissue and thus has seen its fair share of hurdles. Because it often uses repurposed viruses to deliver therapeutic genes, gene therapy has been caught in a vicious cycle for nearly two decades owing to immune response, insertional mutagenesis, viral tropism, off-target activity, unwanted clinical outcomes (ranging from illness to death of participants in clinical trials), and patchy regulations (23, 28–31). This led to a sharp decline in research funding for basic, preclinical development and vector production via individual investigators grants such as R01 and program grants. Thus, with limited information of preclinical data and vector production, the number of clinical trials conducted worldwide did not rise steadily from 1999 to 2015 (32). Furthermore, funding of the actual clinical trial was not guaranteed even vectors have been produced and certified for human use at significant cost. The American Society of Gene Therapy has taken lead in fixing this fragmented funding method by making many recommendations including the elimination of redundant regulatory processes and establishment of the National Gene Vector Laboratories (NGVL) to review vector production and toxicology. Now, with new technological advances in gene delivery and editing methods, increased enthusiasm of clinicians and drug companies, the advent of several viral-based drugs in the market, and the potential to provide a one-time treatment option without corrupting the genetic code, gene therapy is breaking free of this cycle. Undoubtedly, the resurgent interest in offering gene therapy-based treatments is one of the most defining developments in the pharmaceutical industry and is expected to have far-reaching implications on curing dangerous diseases in the future. With an estimated US $11 billion market in the next 10 years, both clinical trials and pharmaceutical industry are anticipated to benefit immensely from gene therapy. Here, we describe popular viral vectors used in gene therapy and gene therapy drugs available in the market.

LNP instead of a vector
PFIZER BioNtech

ALC-0315
ALC-0159, DSPC
"This product is for research use only and not for human use."
https://t.co/tNsG5wxVCT

'EMA Zulassung ohne Daten'
https://t.co/cWW1PDMzW3

> https://t.co/rSOxRFTZwHhttps://t.co/gi40e7mUqIhttps://t.co/3yAElKnXgshttps://t.co/QXYZbFxHRqhttps://t.co/Nb0q026Zvj
>
> — Lexa W. \U0001f1e9\U0001f1ea (@rebew_lexa) December 22, 2021

Official statement by Echelon
Paraphrased - "we do produce it, but only for use in labs.
Pfizer get theirs from other companies. With them it is safe in humans."

No study data presented
A reference to GMP as 'safe in humans'

ALC-0315
ALC-0159, DSPC

We have recently become aware that our website has been used to falsely call into question the safety of the vaccines developed for COVID-19 and want to address this misrepresentation.

While ALC-0315 and ALC-0159 are being safely used in the BioNTech/Pfizer vaccine, the material that Echelon Biosciences is making and selling is not being used in vaccine production and is only for research use in laboratories. When sold as laboratory products, the manufacturing and testing processes do not need to be as strict as they do when the same product is being administered to people. That is the reason why we state on our website that the material we make is for research use only and not for human use. It is not a statement that ALC-0315 and ALC-0159 are unsafe.

The companies who manufacture ALC-0315, ALC-0159, and other components for the vaccine are following Good Manufacturing Practices (GMP) standards (as required by the FDA) and their facilities are inspected to ensure that their manufacturing processes are controlled and safe for human use.

We hope this clarifies any confusion regarding our products and the language on our website. Additional questions and requests for information can be directed to echelon@echelon-inc.com

PFIZER LNP

This paper claims "It is just like another fatty compound. You eat salad oil and chips as well"

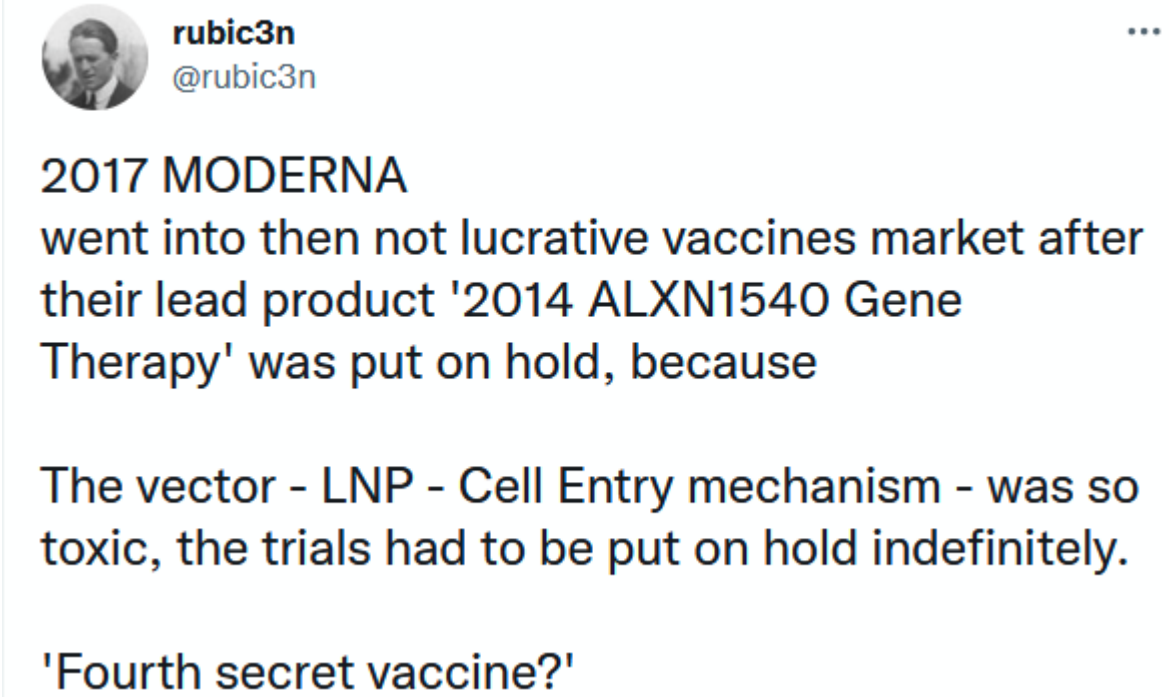
https://t.co/uncdUpkFnI

No, it is not.

LNP is far from being 'just another salad oil'.

It is
-a recent development
-proprietary
-highly specialized
-very expensive and
-It was one of the limiting factors in delivering the mRNA and not killing people.
https://t.co/VXAK5RNTAs

https://t.co/wC3nGyo7wp
.



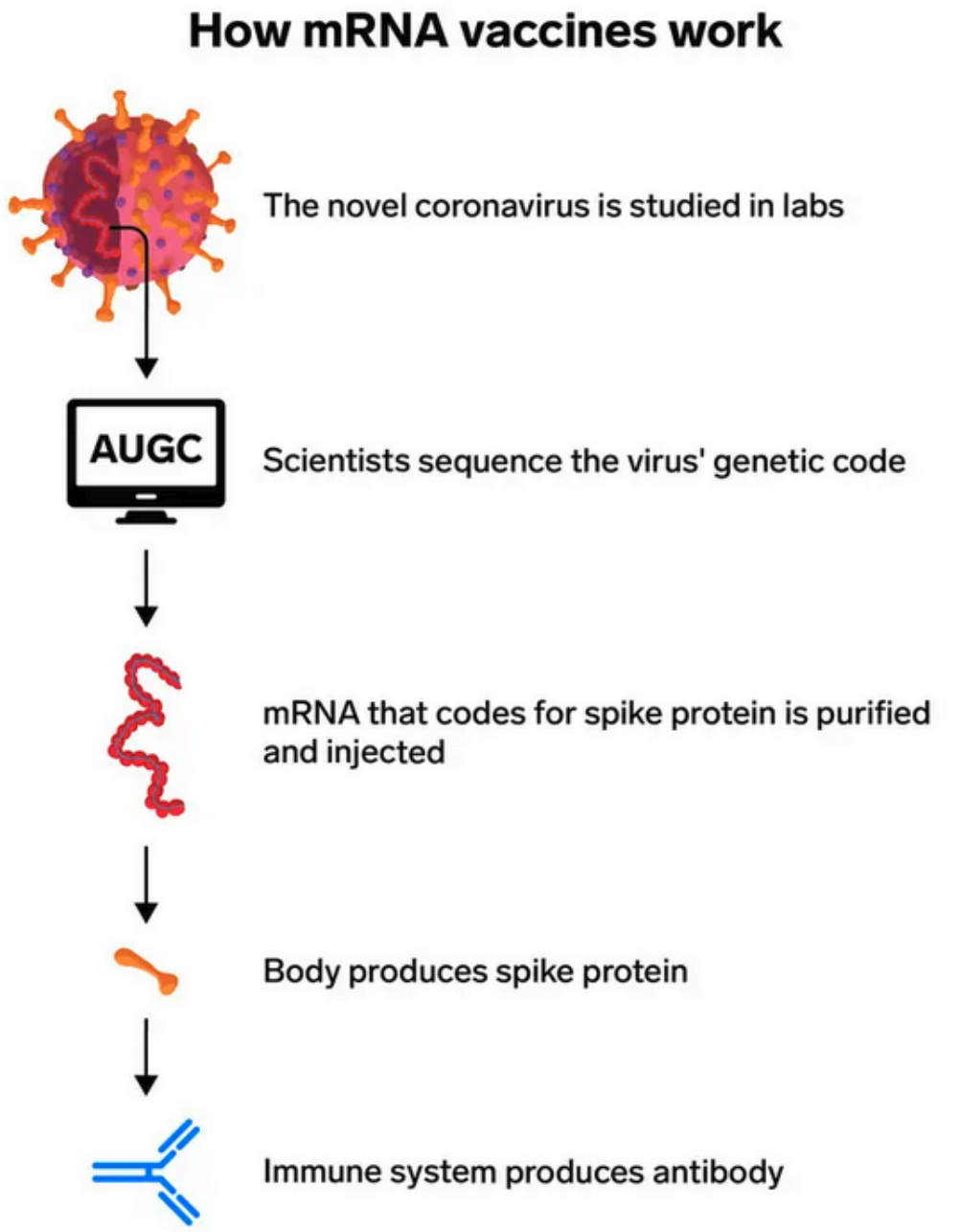
2017 MODERNA
went into then not lucrative vaccines market after their lead product '2014 ALXN1540 Gene Therapy' was put on hold, because

The vector - LNP - Cell Entry mechanism - was so toxic, the trials had to be put on hold indefinitely.

'Fourth secret vaccine?'@arkmedic https://t.co/eG40SsMWdk

— rubic3n (@rubic3n) July 12, 2021

The LNP is the hidden 'trouble maker'
It is often left out of explanatory drawings such as this one.
Not because it is not important, but bcs it poses questions:
- how stable is it?
- how is made sure that it only reaches desired target cells - and at the injections site?



An infographic showing how mRNA vaccines are developed. Shayanne Gal/Insider

As mentioned above, it is a fairly recent field of interest

https://t.co/UH0h4StiXR

> 2019
> NIH's vaccine centered interest in LNPhttps://t.co/JvDFSrWObz
>
> Pick your favourite sentence.

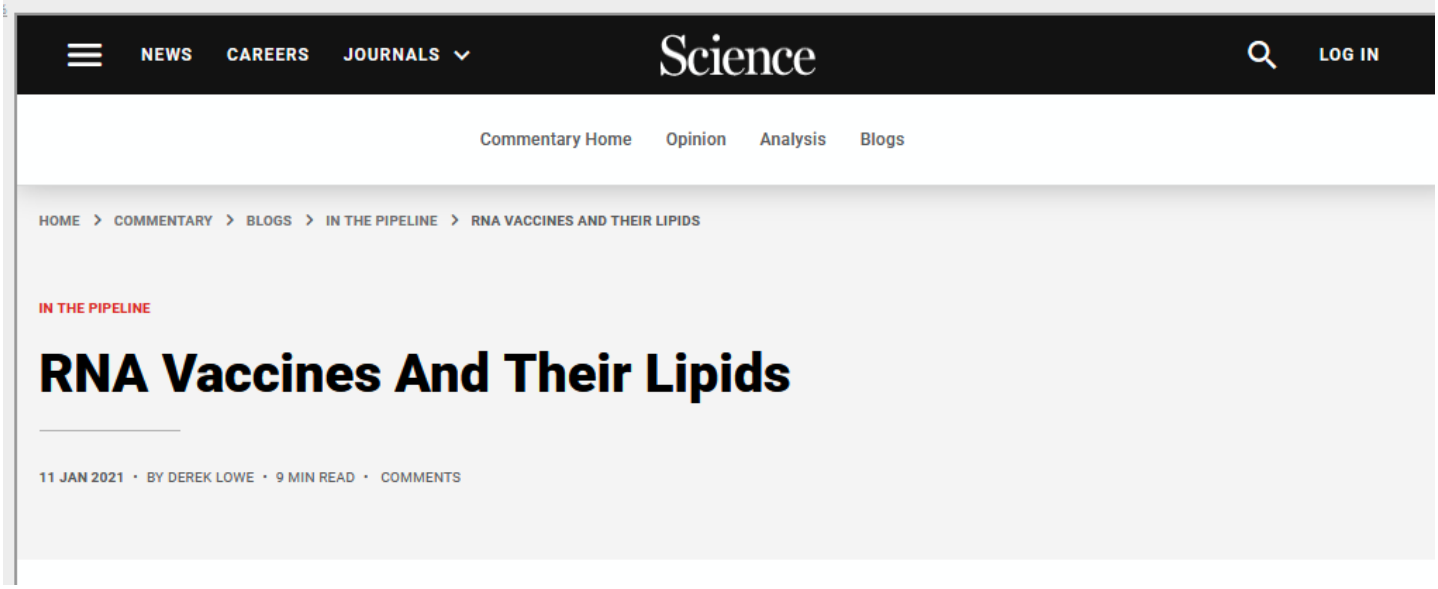
I liked these:
"Nanoparticle mists could be inhaled"
"genome editing using the CRISPR-Cas9 system, which permanently modifies DNA for some beneficial purpose"

— rubic3n (@rubic3n) December 13, 2021

A vast area - far from 'just being another salad oil'
- different compositions
- charge
- qualities that allow it to be injected again

Some interesting comments beneath
https://t.co/AImm2MMdOV

Wide use of applications - and specific formulations
https://t.co/1JNg2iWXMJ
.

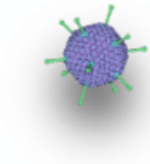
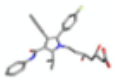


Are Lipid Nano Particles LNP organ specific?

A discussion for a POTENTIAL FUTURE development. 2021/August Paper
https://t.co/t5yiijYlCr

by
- cationic charge
- embedding target cell specific antibodies

Table 1. Tools for therapeutic manipulation of protein levels in tissues.

	AAV	Modified mRNA	miRNA/siRNA	Small Molecules	Protein
					
Route of Administration	Local or I.V	Local or I.V	Local or I.V	I.V or oral	Local
Limitation of Gene Size	Yes (4.5 Kb)	No	N/A	N/A	No
Pharmacokinetics	Long term	Short term	Short term	Long or short term	Short term
Multiple Administration	No	Yes	Yes	Yes	Yes
Compromised DNA Integrity	Yes	No	No	No	No
Controlled Expression	No	Yes	Yes	Yes	Yes
Gene Expression Regulation	Up or down	Up or down	Mostly down	Mostly down	Mostly up
Organ Specificity	Yes	No/possibly	No	No	No
Cell Specificity	Yes	No/possibly	No	No	No

Do LNP stay at the injection site?

The cationic charge is the property that makes it stay longer at the site of injection - however, 'longer' is relative. Animal models?
Any other mechanisms?
https://t.co/TnqGqSSSSs
“Everyone is trying to figure out the next big ionizable lipid"

The Effect of Electric Charge on Lipid Nanoparticles in Vaccine Efficacy

Controlling the electric charge of lipid nanoparticles is a real asset in vaccine production, allowing vaccine manufacturers to dictate how the vaccine is distributed through the body. To demonstrate this, we explored the circulation rate of four different lipid nanoparticle delivery system formulations, each with different electric charges.

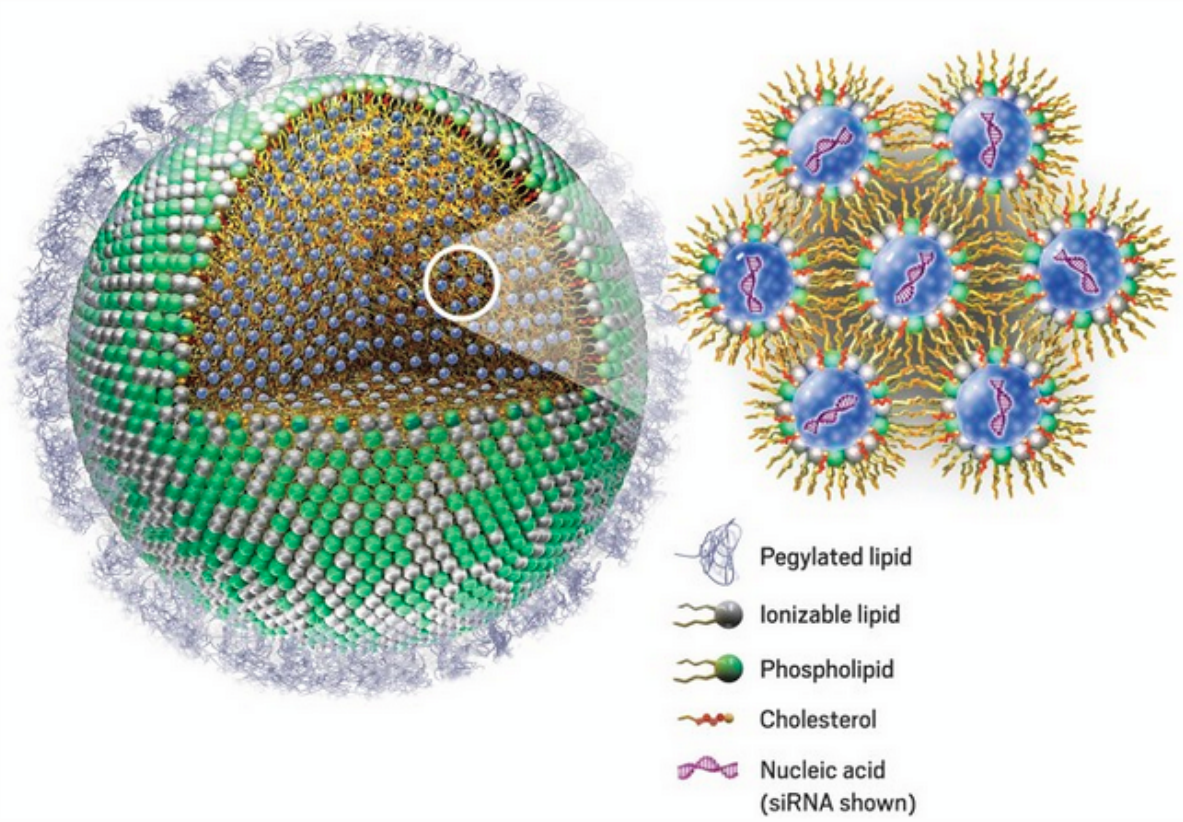
Anionic formulations move away from the injection site more quickly than neutrally charged formulations. Conversely, cationic formulations remain at the injection site for much longer, which can form a depot effect: where the antigen is slowly released from the injection site.

For vaccine manufacturers, this depot effect can be helpful for delivery systems where a slow, steady release of antigen is desired. This can translate into fewer doses required to create immunity in the patient.

However, a depot effect can also be counterproductive if a high concentration of antigen in the blood serum is needed to cause immunogenicity. To achieve this, the active will need to be released and circulated as quickly as possible. Negatively charged lipid nanoparticles are the best way to achieve a quick release of the antigen.

LNP 'Parts List'
https://t.co/TnqGqSSSSs
https://t.co/1JNg2iWXMJ

PARTS LIST



Credit: Genevant Sciences

A lipid nanoparticle (LNP) contains hundreds of small interfering RNA (siRNA) molecules, each surrounded by ionizable lipids, phospholipids, and cholesterol. The outside of the particle is coated in pegylated lipids. LNPs for messenger RNA (mRNA) are made with similar ingredients but contain only a few mRNA strands.

LNP seem to have their natural tissue affinity.
Heart muscle cells.
Among others.

https://t.co/phOW7BsMrf

https://t.co/DIWgG5P5SY

https://t.co/lnfOoIKua5

ELSEVIER

Metabolism Open

Volume 13, March 2022, 100159

Potential implications of lipid nanoparticles in the pathogenesis of myocarditis associated with the use of mRNA vaccines against SARS-CoV-2

Dimitrios Tsilingiris, Natalia G. Vallianou, Irene Karampela, Junli Liu, Maria Dalamaga[1]

https://doi.org/10.1016/j.metop.2021.100159

Abstract

Although mRNA-based vaccines BNT162b2 and mRNA-1273 exhibit a remarkable efficacy and effectiveness in preventing particularly severe Covid-19 with an overall favorable adverse event profile, their use has been associated with rare cases of acute myocarditis. These occur most commonly after the second dose, with the highest incidence among young male recipients. This complication has not been frequently observed among adenoviral vector vaccine receivers, and its clinical, laboratory and imaging features resemble those of other common causes of acute myocarditis. The pathogenesis of mRNA-vaccine associated myocarditis has not yet been elucidated,

"The vaccine is directly there. Lipid Nano Particles LNP go right into the heart, the heart expresses the spike protein, the body attacks the heart [] vaccine induced myocarditis is a big deal.
And in children in is way more serious, more prominent than a post Covid myocarditis." https://t.co/r5xUlNY4qE

— rubic3n (@rubic3n) October 30, 2021

Some producers of vaccines have gone ahead with their own special LNP formulations - which read like a scifi book in part.

Just to name select few:
Acuitas
(Trudeau)

https://t.co/Gv5XFbm6Qj

ACUITAS, Vancouver Canada, Justin Trudeau

LNP

Dr Madden describes how the mRNA carrier works
BioNtech, Curevac, othershttps://t.co/tuoQ23FkJY

— rubic3n (@rubic3n) November 2, 2021

Moderna has been very inventive in the area of modulating their LNP - it is even designed to enter stem cells, ...

You may want to have a look for yourself.

Moderna LNP - Route of administration

https://t.co/ykXuTk5MhR
.

LNP construction at MODERNA
"Each new route of administration (ROI) or target type requires consideration and optimization of all paramaters"
And at the start, you just do not know at all how some cell types under certain conditions and gene expressions will behave.
You never may pic.twitter.com/JKiJJc1KMQ

— rubic3n (@rubic3n) November 13, 2021

Moderna LNP modifying cell uptake.

A bit short sighted, when taking into consideration in vivo parameters not present in the reduced model.
Such as:
- Genes can be switched on and of in patterns, diurnally
- with age
- nutrition
- other medication ...

https://t.co/EpGIOcQimJ
.

MODERNA
To adapt LNP and mRNA and other aspects of the platform - is this why they have been frantically searching for the whole world genome?

Problem is: Genes can be switched on and of in patterns, diurnally, with age, nutrition, medicine taken
...https://t.co/CuvA6XIY3P pic.twitter.com/gCzLhL3flX

— rubic3n (@rubic3n) November 13, 2021

Moderna LNP
In their high gloss material they were already envisaging serial application

https://t.co/bB2pHGA2CX
.

> Repeated injections with different LNP formulations and repeated dosing can enhance transfection of [the cells it can transfect] [in the lineage]
>
> Is that what the boosters are doing?
> Transfecting lineages of cells in our body - for the rest of our lives and our 'lineages'? pic.twitter.com/gs6kFCd7Ak
>
> — rubic3n (@rubic3n) November 13, 2021

Moderna LNP

Stem Cell - 'transfecting bone marrow cells'
Going that deep actually would require looking around for other influencing factors more

https://t.co/Wy47iMEWGP

> MODERNA
> Anyone, who is willing to have an mRNA injected that alters the stem cells for their entire blood system - for the rest of their lives?
> With unclear epigenetic changes?
> In a gene expression environment that is 'in flux'
> Fabricating these LNP - could these be the BW? pic.twitter.com/a9LHFHr3nm
>
> — rubic3n (@rubic3n) November 13, 2021

Other studies - already in 2017 - looking specifically at
"repeated applications"
To find the right balance between toxicity and therapeutic value (tv).
Is this what we are observing in the third dose and no tv for children at the moment?

https://t.co/fvlE7lbyIl

> 2018 mRNA multi shot study
> LNP might not be really up for 'chronic application' - i.e. the 'multi shot' situation.
>
> They were still looking for the right amino lipids and had done studies in animals.
>
> Has there been any progress to the multi shot able amino lipid LNP? pic.twitter.com/WQKlAevV41
>
> — rubic3n (@rubic3n) December 11, 2021

Just a reminder - PFAEZER's LNP

https://t.co/YXltgTZaKE

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> ALC-0315 LNP https://t.co/7Lsy8okQCD
>
> — rubic3n (@rubic3n) December 26, 2021

LNP
The mRNA-LNP platform’s lipid nanoparticle component used in preclinical vaccine studies is highly inflammatory

https://t.co/gXZ8fCZ5H9

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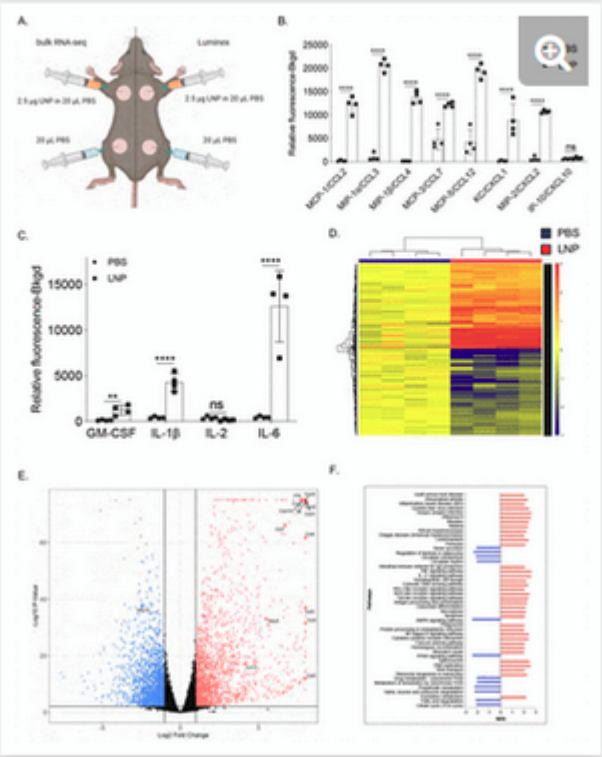

Figure 2. Download figure | Open in new tab

Intradermal inoculation with LNPs complexed with non-coding poly-cytosine mRNA leads to an inflammatory milieu.
A. Experimental design. The mice were treated as indicated, and 24 hours later, the skin samples were prepared for Luminex® and bulk RNA-seq analyses. **B. and C**. Luminex® data summarizing inflammatory chemokines and cytokines induced by the LNPs. **D**. Heatmap of gene expression changes triggered by the LNPs (FDR < 0.05, log2 FC > 1 – 4091 genes). **E**. Volcano plot summarizing the up and downregulated genes upon LNP injection. **F**. GSEA analyses of the KEGG pathways and displayed as normalized enrichment score (NES). FDR<0.05. Pathways with NES less than ±2 are not displayed. N=4.

In summary, using different techniques, we show that LNPs, alone or complexed with control non-coding poly-cytosine mRNA, are highly inflammatory in mice, likely through the engagement and activation of various distinct and convergent inflammatory pathways.

Intranasal inoculation with 10 µg of LNPs causes a high mortality rate in mice

For further reading (uncategorized)

https://t.co/GbyRljCNpi

https://t.co/2dMkuO7dyQ

https://t.co/O9MRNhz3ul

https://t.co/4dAMzuGqD8

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Preparation of lipid nanoparticles

The diagram below demonstrates a common technique used to produce LNPs. The phospholipid, carrier oil and actives are dissolved in a solvent, which is then evaporated. This precipitate has a buffer added to it and is then warmed and vortexed to hydrate the phospholipids. The antigen is then added wherein multi-layer vesicles (MLVs) are generated. This solution is processed through a Microfluidizer® processor to reduce particle size to small uni-lamellar vesicles.

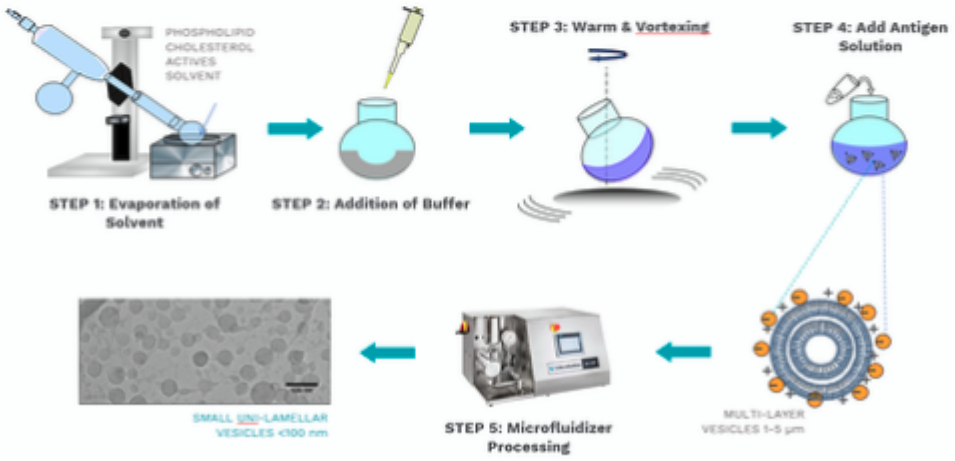

Image Ref: University of Strathclyde research

Mix your LNP yourself

https://t.co/crNb4sLyUa

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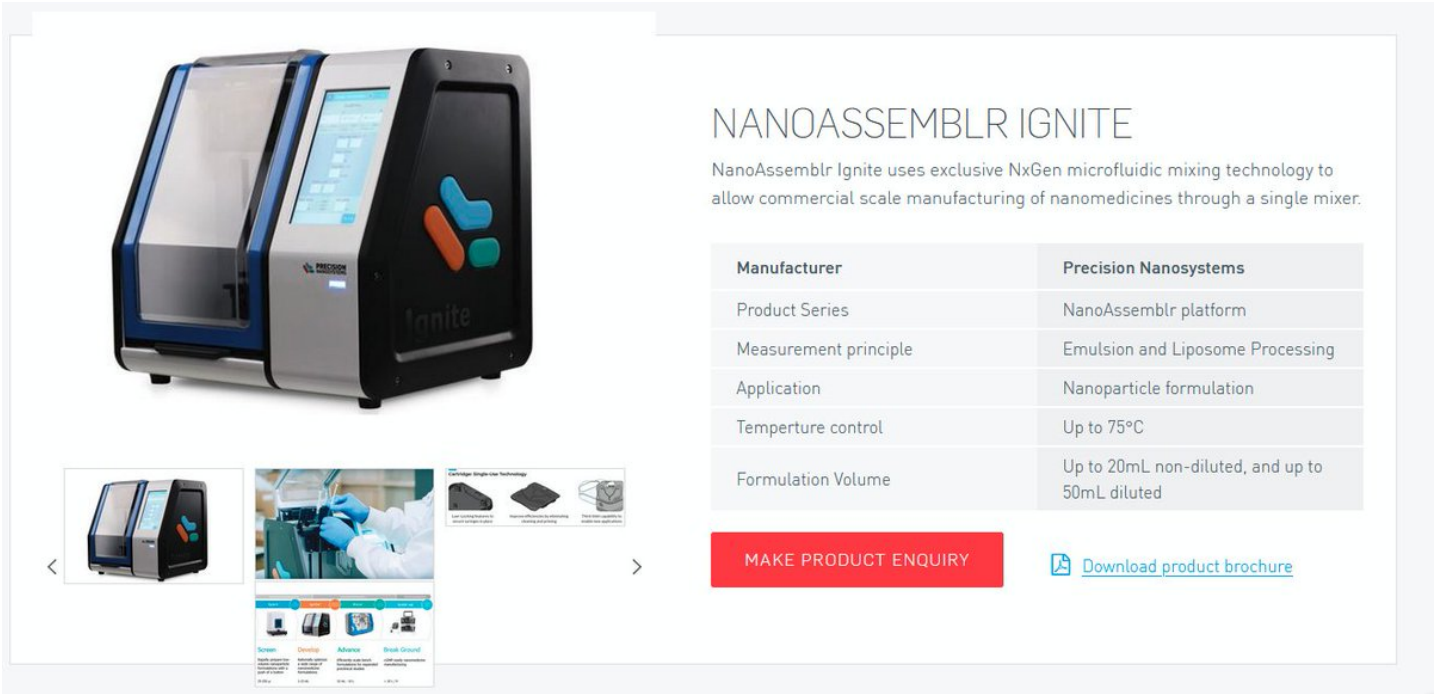

Short overview of who is involved in LNP production for PF and Mode RNA

https://t.co/LinrJ8g858

Different LNPs for different applications in clinical trials

Also SPION tech
Magnetic
Carbon

and other weird and wonderful

TABLE 1. Clinically approved nanoparticle therapies and diagnostics, grouped by their broad indication

Name	Particle type	Payload	Approved application/indication	Approval (year)
New additions				
mRNA-1273 (Moderna)	Lipid nanoparticle	mRNA	COVID-19 vaccine	FDA, Emergenc Use Authoriza (2020)
Tozinameran/BNT162b2 (Pfizer-BioNTech)	Lipid nanoparticle	mRNA	COVID-19 vaccine	FDA, Emergenc Use Authoriza (2020)
Cancer				
Doxil Caelyx (Janssen)	PEGylated liposome	Doxorubicin	Ovarian cancer, HIV-associated Kaposi's sarcoma, Multiple myeloma	FDA (1995) EMA (1996)
DaunoXome (Galen)	Liposome (non-PEGylated)	Daunorubicin	HIV-associated Kaposi's sarcoma	FDA (199

Note: Recent nanoparticles that have received Emergency Use Authorization are separately listed in the first rows.

Link LNP SPION et al
2021 August

Nanoparticles in the clinic: An update post COVID-19 vaccines

https://t.co/tXRJdt8RfO
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