EPSRC
Engineering and Physical Sciences Research Council

Department of Health & Social Care

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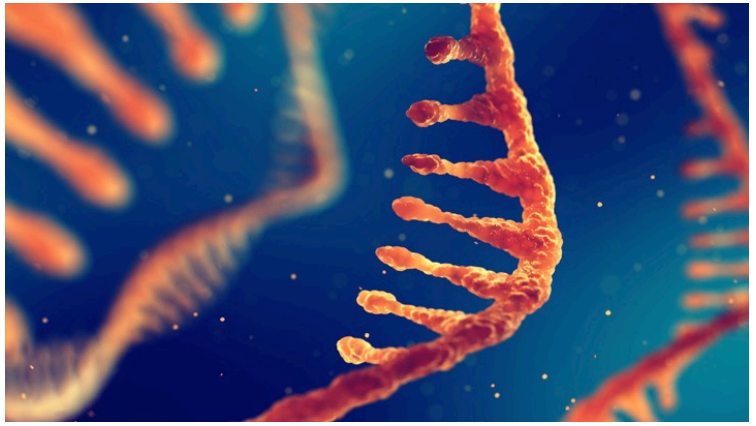
Imperial College London

The Department of Health and EPSRC Imperial Future Vaccine Manufacturing Research Hub

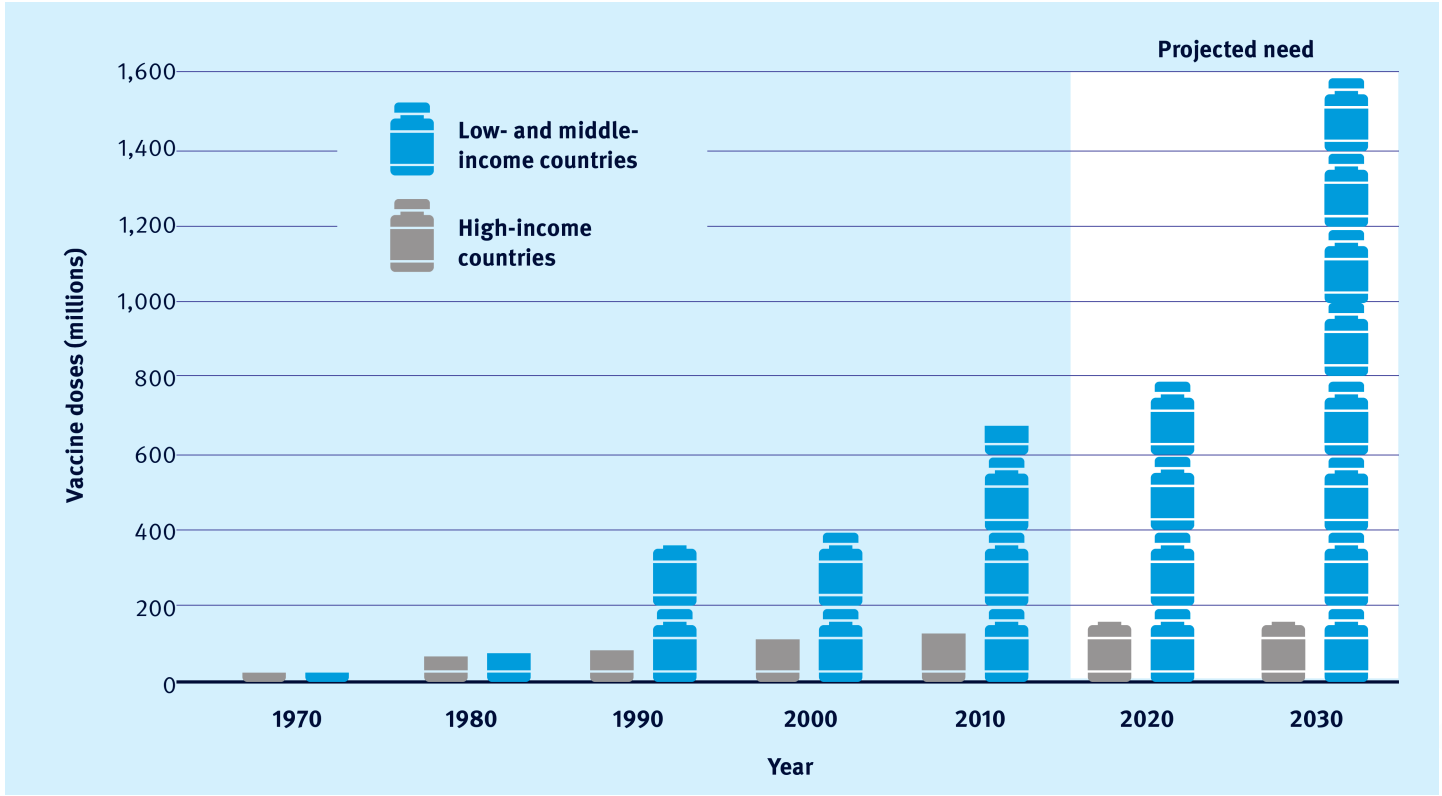
RNA Vaccines

Dr Benjamin F Pierce



This research is funded by the Department of Health and Social Care using UK Aid funding and is managed by the Engineering and Physical Sciences Research Council (EPSRC, grant number: EP/R013764/1). The views expressed in this presentation are those of the author(s) and not necessarily those of the Department of Health and Social Care.

Introduction to the Imperial Future Vaccine Manufacturing Research Hub



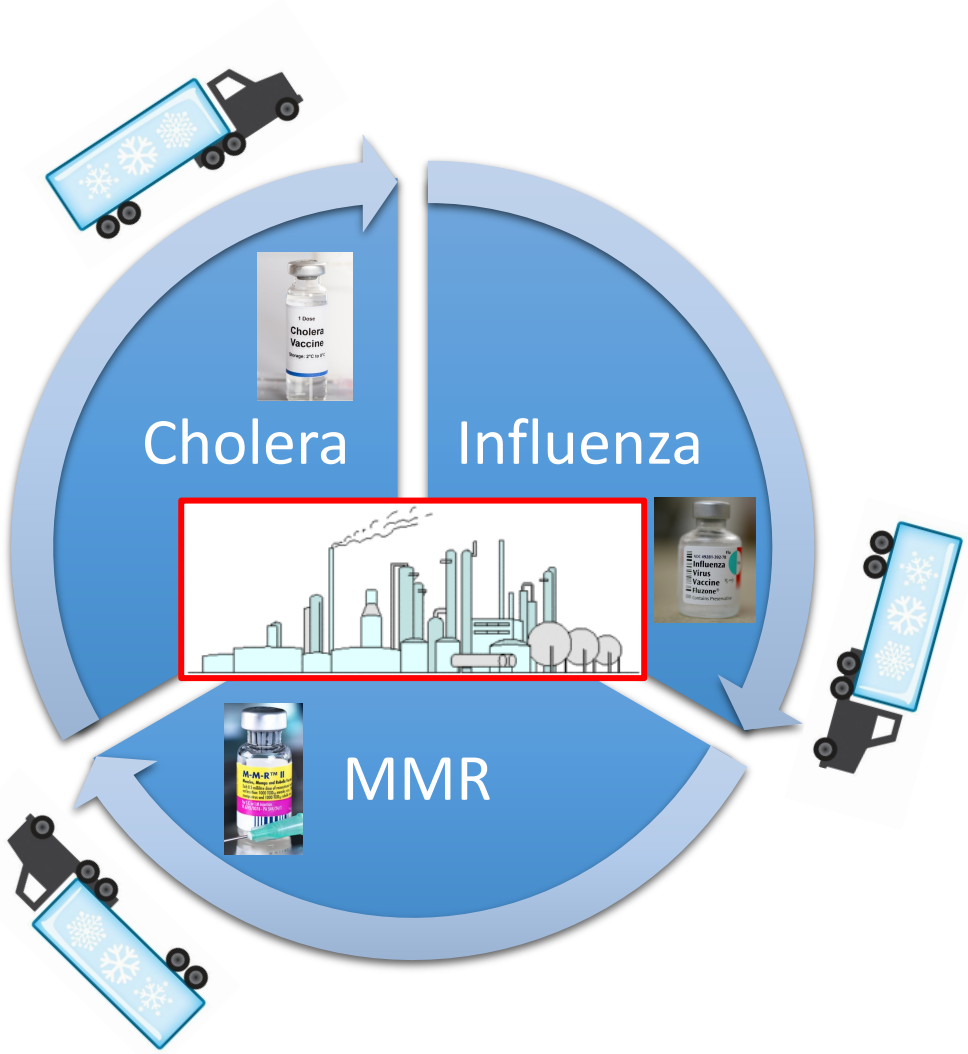


Figure (left) adapted from Rino Rappuoli, Steven Black, and David E Bloom. *Science Translational Medicine.* **2019**. 11, eeaw2888.

Imperial Future Vaccine Manufacturing Research Hub and DCVMN

Quality by Design and Supply Chain Modelling Workshop organised / hosted by FVMR Hub

DCVMN Members from Developing Countries

Hanoi, Vietnam

24 – 27 November 2019

Already 40 registered attendees!

Please check DCVMN website – Events tab

Imperial College London

NIBSC Confidence in Biological Medicines

dcvmn Developing Countries Vaccine Manufacturers Network

CALL FOR EXPRESSION OF INTEREST

THE DEVELOPING COUNTRIES VACCINE MANUFACTURERS NETWORK's (DCVMN) OPEN CALL FOR EXPRESSION OF INTEREST IN QC/QA TRAINING WITH IMPERIAL COLLEGE LONDON'S FUTURE VACCINE MANUFACTURING RESEARCH HUB (FVMR)

GUIDANCE

DCVMN International periodically sponsors technical assistance for its member companies. This assistance comes from internationally-reputable expert consultants and/or service firms

QC training at NIBSC – 2 page EoI by 30 Nov 2019.
(see email from DCVMN late on 17 Oct or early on 18 Oct)

Imperial College London

dcvmn Developing Countries Vaccine Manufacturers Network

SECOND CALL FOR PROPOSALS

THE DEVELOPING COUNTRIES VACCINE MANUFACTURERS NETWORK's (DCVMN) OPEN CALL FOR EXPRESSION OF INTEREST IN COLLABORATIVE PROJECTS WITH FUTURE VACCINE MANUFACTURING RESEARCH HUB (FVMR)
GUIDANCE FOR APPLICANTS

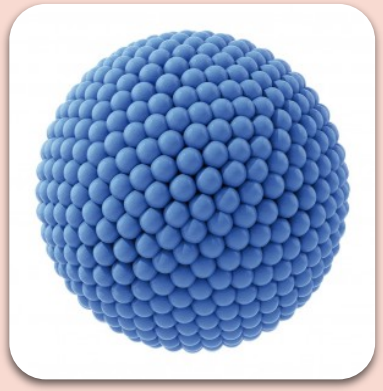
DCVMN International periodically sponsors technical assistance provided to member companies by internationally-reputable expert consultants and/or service firms, to improve manufacturing technology, processes or quality control systems. The objective is to increase availability of high-quality vaccines globally.

1. Purpose

As announced on the DCVMN website in January 2018, a novel partnership has been launched to support responsible innovation for manufacturing in emerging countries and to improve the response to

DCVMN member companies will be notified once call is announced.

Hub vaccine innovative technologies



GMMA

Easy scale-up

Mature

Slow

Human glycosylation challenging

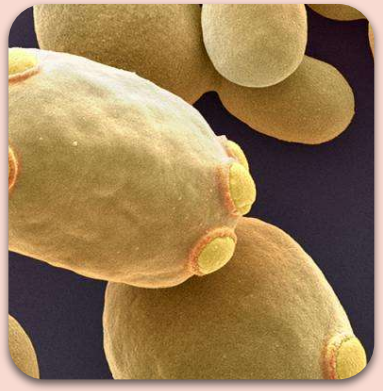
Baculovirus

Thermostable

Rapid

Feasible scale-up

Technologically complex

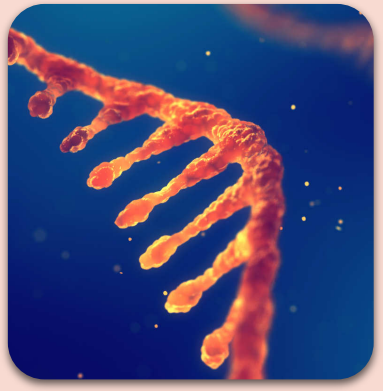


Yeast

Easy scale-up and high yield

Low risk of contamination

Downstream purification challenging



RNA

Rapid

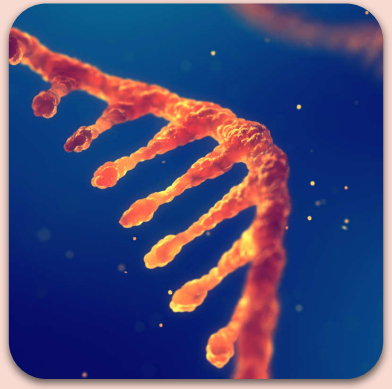
Low cost

Synthetic and cell-free

Immature

Decreasing risk

RNA Vaccines Background



RNA

Rapid

Low cost

Synthetic and cell-free

Immature

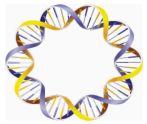
The ‘anti-hype’ vaccine Nature Biotechnology. 35, 193 (2017).

Timeline of mRNA vaccine research

1983	Birth of recombinant RNA. J. Mol. Biol. 171, 281 (1983).
1987	*In vitro* transcription of RNA. Nucleic Acids Research. 15, 8783 (1987).
1990	Direct gene transfer of mRNA in mice. Science. 247, 1465 (1990).
1994	Self-replicating RNA. Vaccine. 12, 1510 (1994).
2012	60 days antigen expression from non-viral RNA vaccine. PNAS. 109, 14604 (2012).

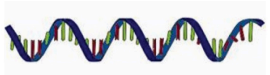
Nucleic acid vaccines

DNA-based vaccines

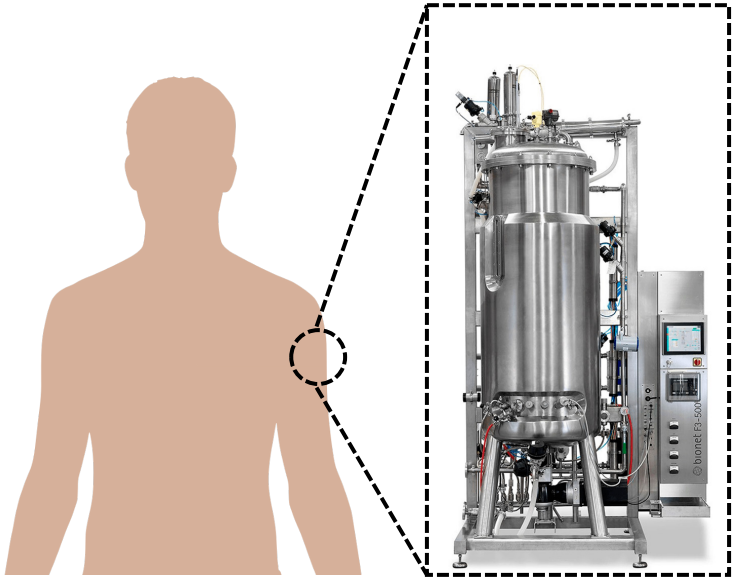


- Facile and rapid synthesis
  - Sequence obtained, transfected cells with synthetic HA/NA genes in <1 d
- Activation of $CD8^+$ and $CD4^+$ T cells
- Vaccines licensed for veterinary use
- Entry required to nucleus
- Risk of integration in genome
- Potency issues in humans

RNA-based vaccines



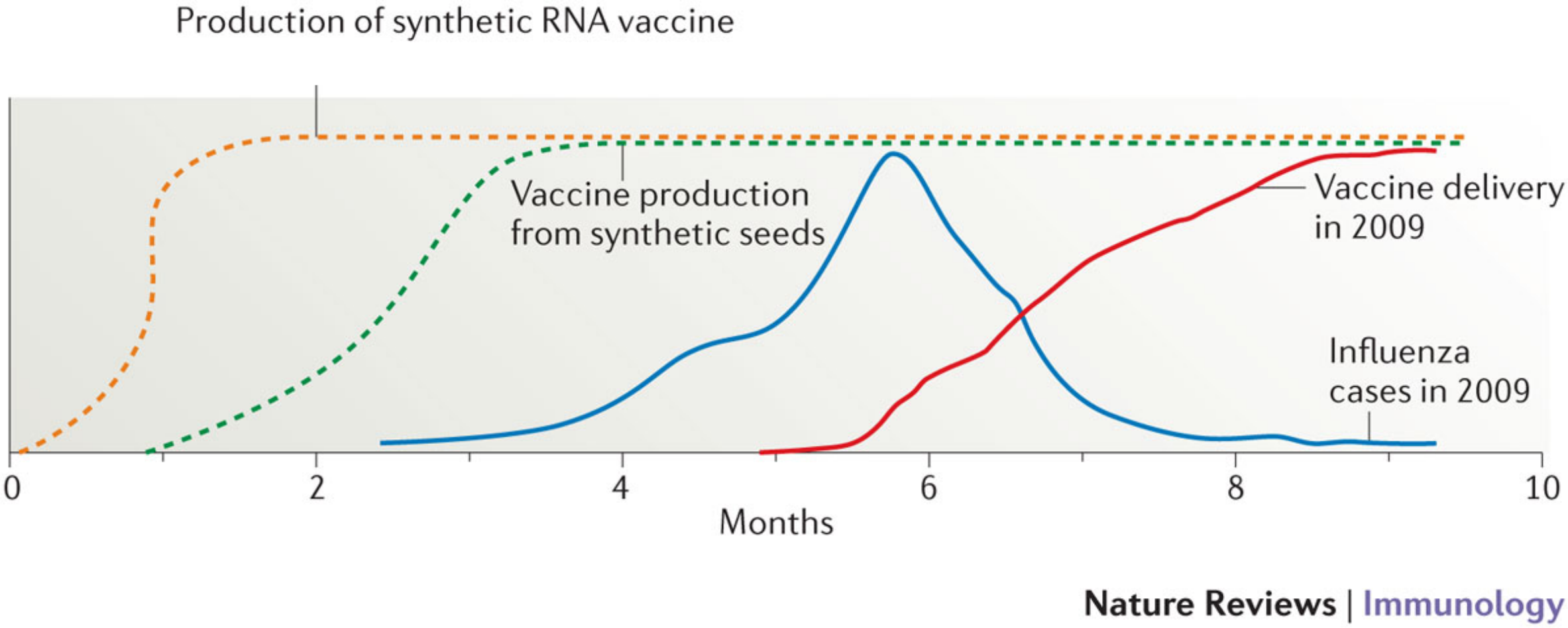
- Facile and rapid synthesis – IVT
- Entry to cytosol sufficient
- No risk of genome integration
- Industry focusing more on cancer / therapeutic applications
  - Moderna, Inc.; CureVac AG; Argos Therapeutics; BioNTech; Tiba Biotech; TriLink (manufacturer)
- Active and passive (mAbs) immunization
- Further clinical trials required



Prof Robin Shattock
Director of ICL's FVMR Hub

Large scope progress on RNA-based vaccines needed

Synthetic biology may provide for rapid response vaccines and meet the needs of regional manufacture



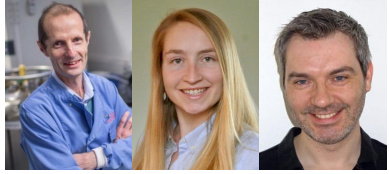
Ennio De Gregorio and Rino Rappuoli. *Nature Reviews Immunology.* **2014**. 14, 505-514.

RNA synthesis enables rapid manufacturing

Our objective is to demonstrate the utility and flexibility of an innovative, broadly applicable synthetic saRNA vaccine platform that enables tailored "just in time" vaccine production to improve regional and global preparedness for foreseeable recurrent outbreaks with viral pathogens.

We acknowledge the financial support provided by the Coalition for Epidemic Preparedness Innovations (CEPI) for our work under an award agreement entitled RapidVac.

RNA as a Platform Technology



Optimisation and generation of suitable DNA template encoding the antigen of interest

Optimisation and generation of suitable DNA template encoding the antigen of interest

Optimisation and generation of suitable DNA template encoding the antigen of interest → *In vitro* RNA transcription followed by hydrolysis of the DNA template and purification of the RNA → Release of bulk RNA → Formulation, fill and finish → Performance of preclinical toxicology → Release of product for distribution

Optimisation and generation of suitable DNA template encoding the antigen of interest

Optimisation and generation of suitable DNA template encoding the antigen of interest

Optimisation and generation of suitable DNA template encoding the antigen of interest

Ebola*, Marburg, Lassa Fever*, Rabies**, Rift Valley Fever§, Chikungunya§**

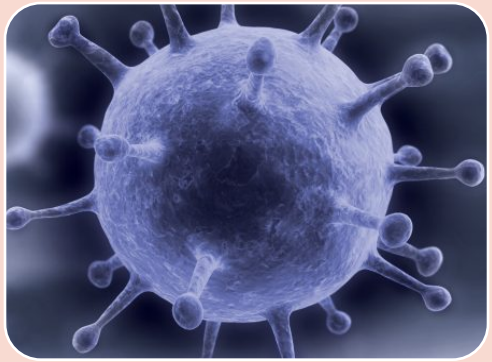
Considering Crimean–Congo hemorrhagic fever

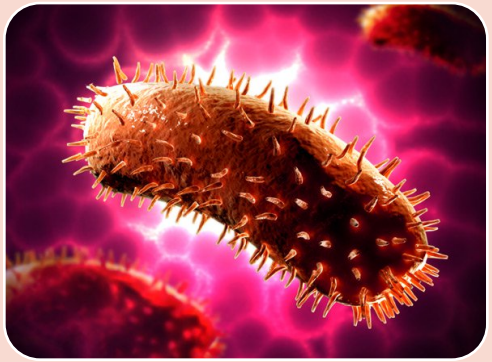
*Clinical development now being driven by Innovate UK

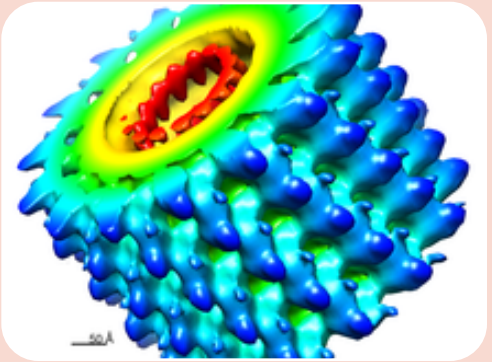
**Clinical development now being driven by CEPI

§Preclinical/process development – FVMR Hub (UVRI/ICL)

RNA – Professor Robin Shattock







Influenza	Rabies	Marburg
Ferret studies	Mice studies	Guinea pig studies

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Clinical Trials of RNA Vaccines for Infectious Diseases

Product, Company/Institution	Indication (disease)	Antigen	Formulation	Phase	Status	Results	National Clinical Trial Identifier
RNActive® CureVac	Rabies	Rabies virus glycoprotein [55]	None	1	Active, Not Recruiting	Generally safe, but some significant adverse events (AEs); boostable functional antibodies	NCT02241135
RNActive® CureVac	Rabies	Rabies virus glycoprotein	None	1	Recruiting	New construct versus prior trial	NCT03713086
mRNA-1851 Moderna	Influenza H7N9	Influenza Hemagglutinin H7N9 A/Anhui/1/2013 [94]	Lipid Nano-particles	1	Active, Not Recruiting	Moderna website says 1° and 2° endpoints met, but no published data	NCT03345043
mRNA-1440 Moderna	Influenza H10N8	Influenza Hemagglutinin H10N8 (A/Jiangxi-Donghu/346/2013) [94]	Lipid Nano-particles	1	Active, Not Recruiting	Interim: AEs: Majority mild moderate; A few: severe; Seroconversion rates high	NCT03076385
mRNA-1653 Moderna	Human Metapneumo-virus + Parainfluenza virus 3	Fusion proteins of each virus	Lipid Nano-particle	1	Active, Not Recruiting	Announced via press release safe and immunogenic; no publications found	NCT03392389
mRNA-1388 Moderna/DARPA	Chikungunya	Not Disclosed (ND)	ND	1	Active, Not Recruiting	Primary Completion: March 2019; no results posted at time of publication	NCT03325075
RNA-1325 Moderna/BARDA	Zika	prM and E [95,96]	Lipid Nano-particles	1	Active, Not Recruiting	Primary Completion: February 2019; no results posted at time of publication	NCT03014089
mRNA-1647 and mRNA-1443 Moderna	Cytomegalovirus	mRNA-1647 is gB, pentameric complex, and mRNA-1443 is pp65 [97]	Lipid Nano-particles	1	Recruiting	Primary Completion: February 2020	NCT03382405
mRNA-1777 Moderna/Merck-V171	Respiratory Syncytial Virus	ND	ND	1	ND	Moderna press release says 1° and 2° endpoints met, but no published data	Not listed on clinicaltrials.gov

Vaccines. 7, 37 (2019).

Recent exciting clinical results for RNA vaccines

moderna

Phase I

Chikungunya virus

mRNA-1944, which encodes antibodies against for an antibody (CHKV-24) with activity against chikungunya virus, administered via intravenous infusion in healthy adults at escalated doses.

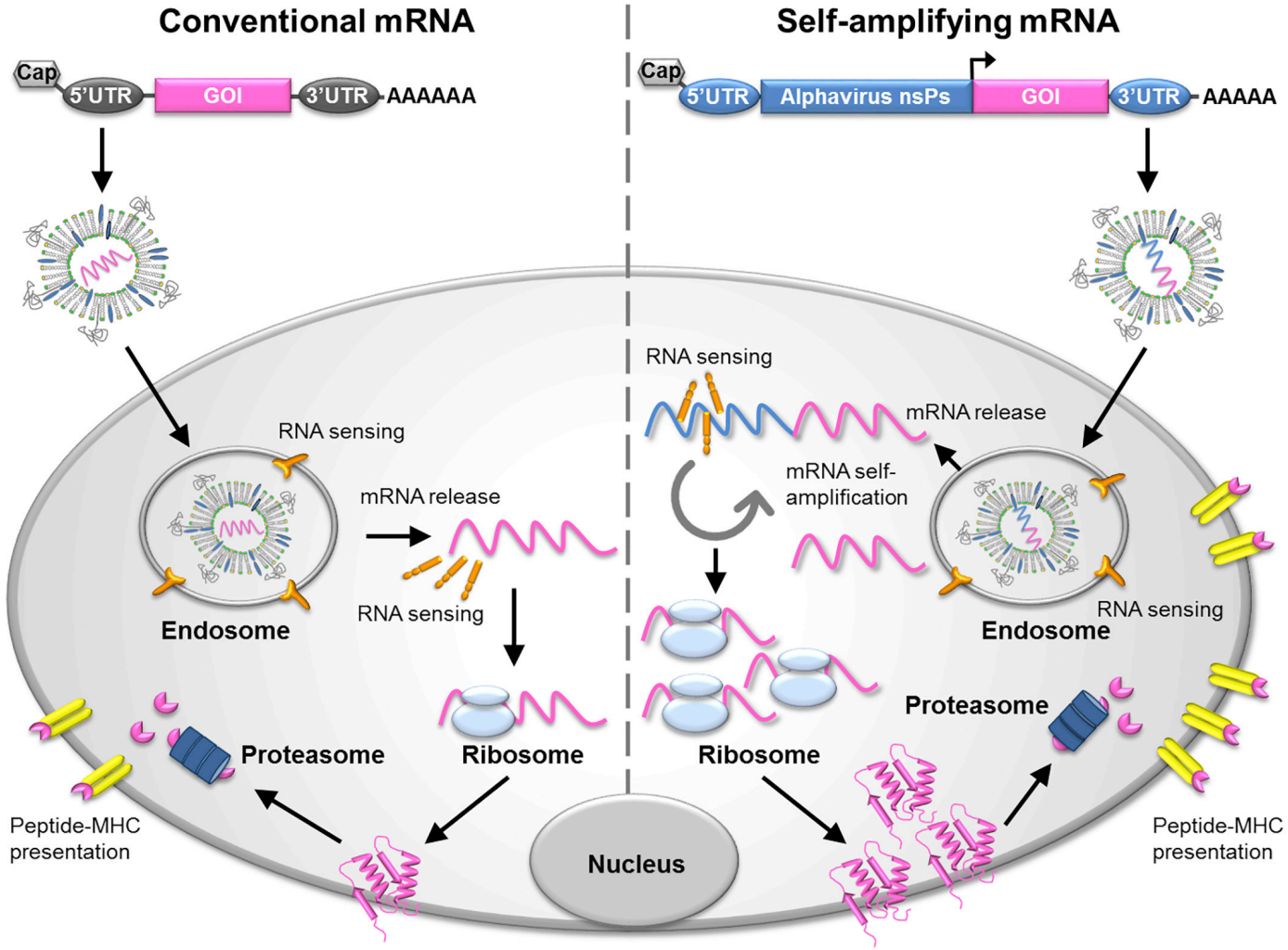
These results mark the **first systemic mRNA therapeutic to show production of a secreted protein in humans**.

Phase II planning and Phase III preparations

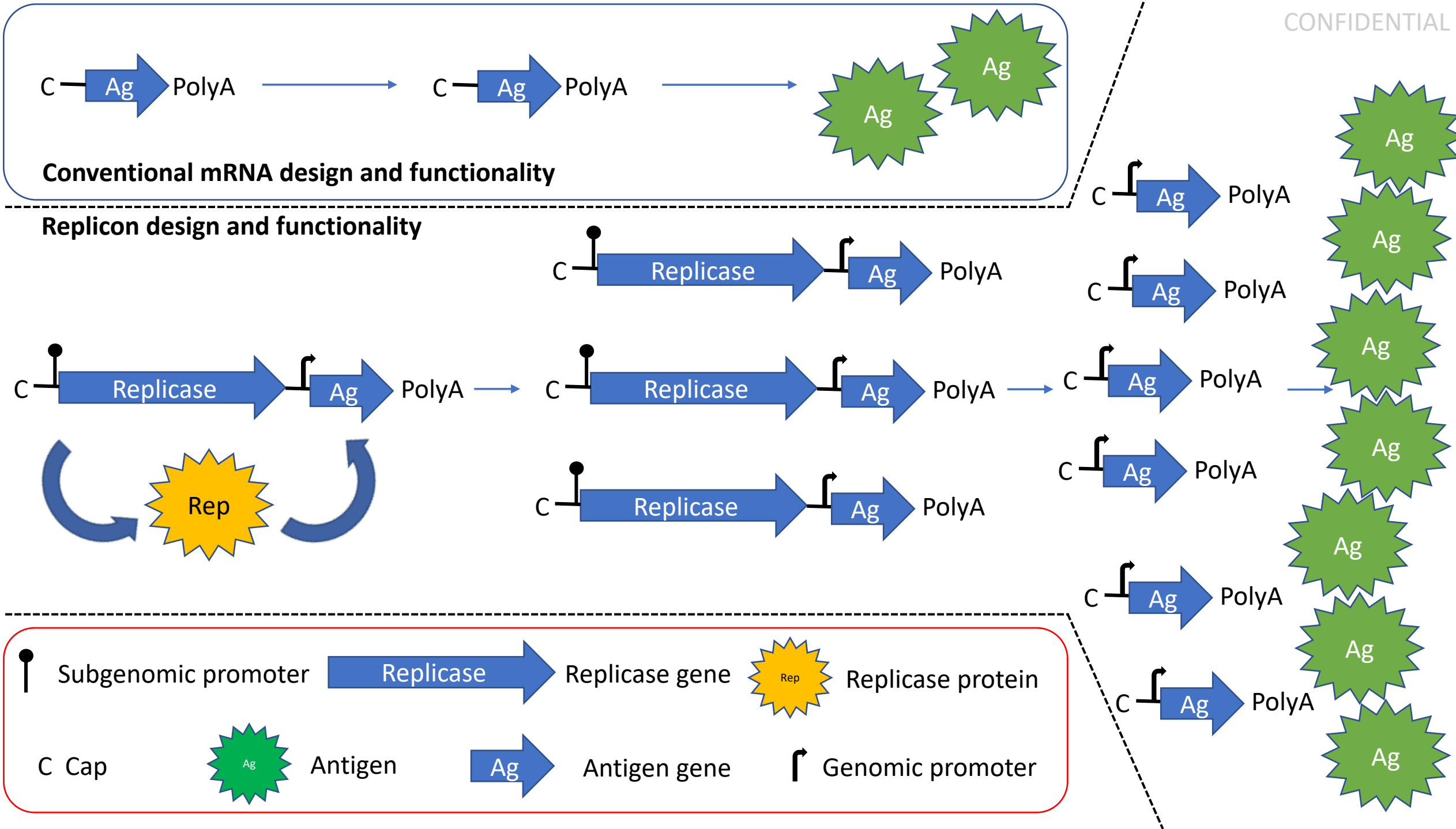
Cytomegalovirus

mRNA-1647 is a vaccine combining six mRNAs in a single vial, which encode for two antigens on the surface of CMV: five mRNAs encoding the subunits that form the membrane-bound pentamer complex and one mRNA encoding the full-length membrane-bound glycoprotein B (gB).

mRNA vs Replicon vaccines

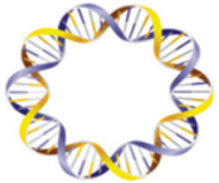


Giulietta Maruggi, Cuiling Zhang, Junwei Li, Jeffrey B. Ulmer and Dong Yu. *Molecular Therapy*. 2019. 27(4): 757-772.



RNA synthesis using IVT

pDNA

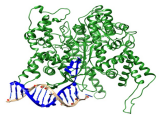


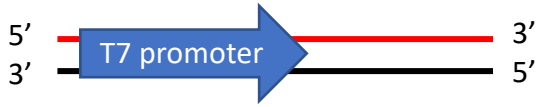
Construct for target antigen

1. Linearize

2. Heat inactivate enzymes

T7 RNA polymerase





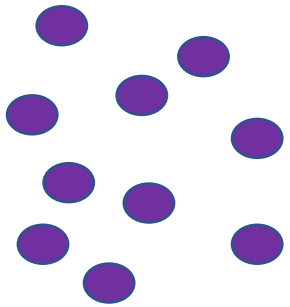
MEGAScript

Uncapped transcripts

ScriptCap

Capped transcripts

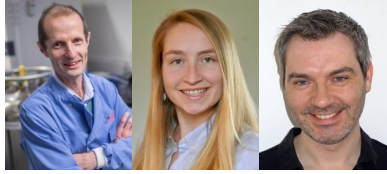
Complexation

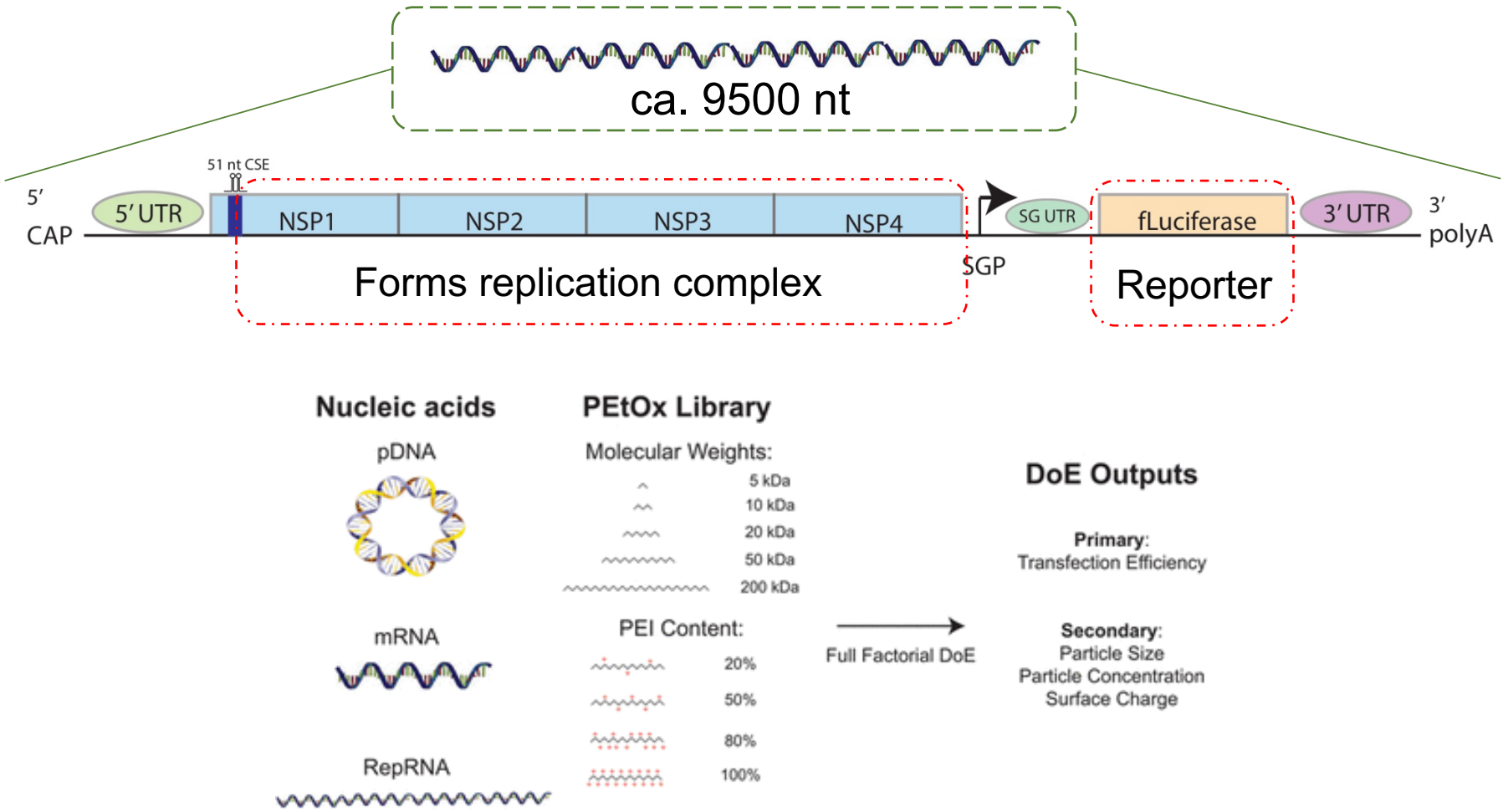


Polyplexed RNA Nanoparticles/LNPs/Liposomes

- ✓ Rapid response potential
- ✓ Cell-free
- ✓ Low cost infrastructure → resource restrained settings
- ✓ In same manufacturing facility
  - ✓ Polyplex synthesis (lower cost, ease of large-scale)
  - ✓ LNPs can be synthesised /stored prior to RNA
  - ✓ RNA designed for various pathogens

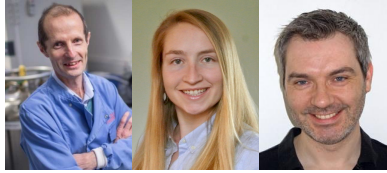
Optimising saRNA delivery





Anna K. Blakney, Gokhan Yilmaz, Paul F. McKay, C. Remzi Becer, and Robin J. Shattock. "One Size Does Not Fit All: The Effect of Chain Length and Charge Density of Poly(ethylene imine) Based Copolymers on Delivery of pDNA, mRNA, and RepRNA Polyplexes." *Biomacromolecules*. 2018. 19(7): 2870-2879.

Optimising saRNA delivery

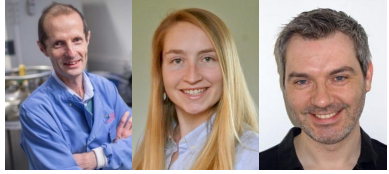


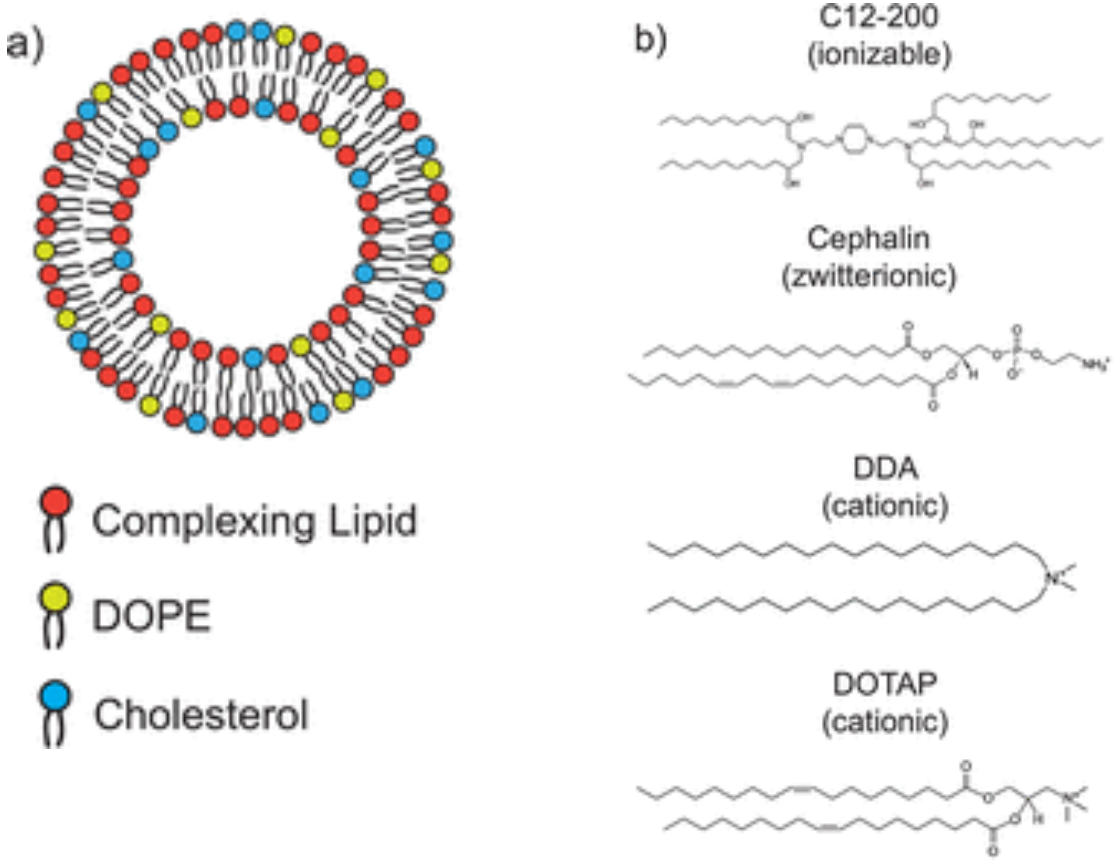
Nucleic acid	optimal % P(EI)	optimal polymer MW
DNA	100	83 kDa
mRNA	80	45 kDa
RepRNA	100	72 kDa

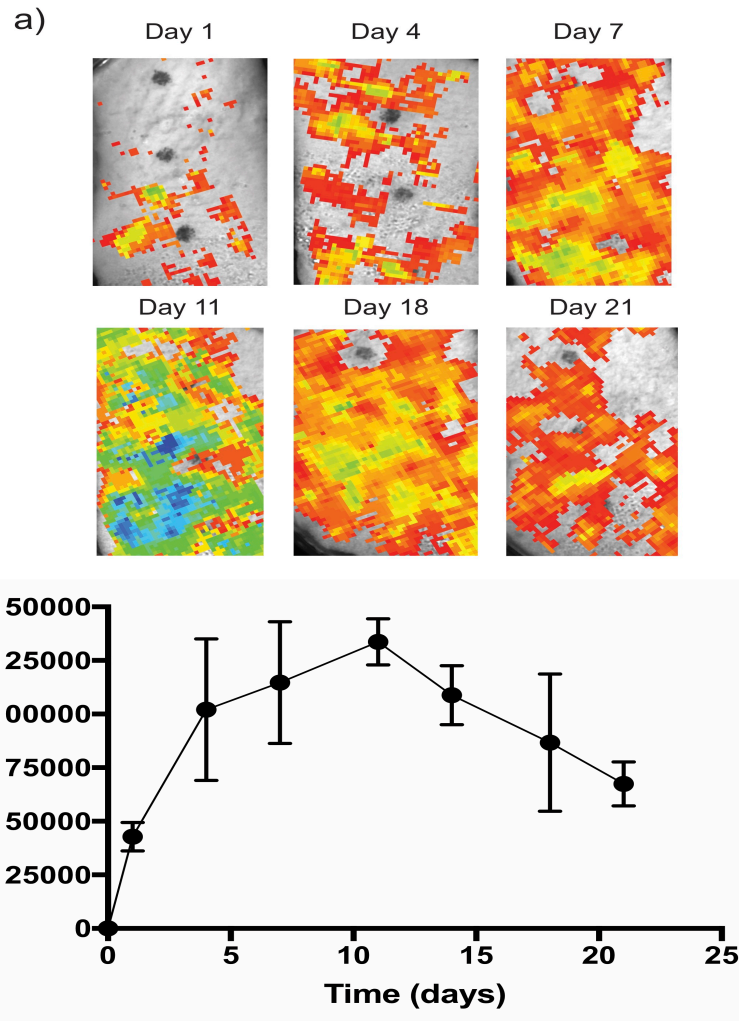
Nucleic Acid	DoE input factor	log worth
DNA	polymer MW	2.960
	% P(EI)	2.393
	polymer MW* % P(EI)	1.028
mRNA	polymer MW	4.687
	% P(EI)	1.887
	polymer MW* % P(EI)	1.477
RepRNA	polymer MW	2.379
	% P(EI)	1.690
	polymer MW* % P(EI)	0.175

Anna K. Blakney, Gokhan Yilmaz, Paul F. McKay, C. Remzi Becer, and Robin J. Shattock. "One Size Does Not Fit All: The Effect of Chain Length and Charge Density of Poly(ethylene imine) Based Copolymers on Delivery of pDNA, mRNA, and RepRNA Polyplexes." *Biomacromolecules*. 2018. 19(7): 2870-2879.

Human skin explants

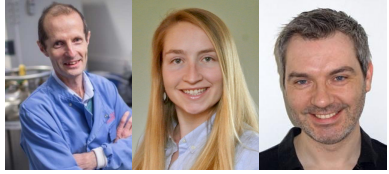


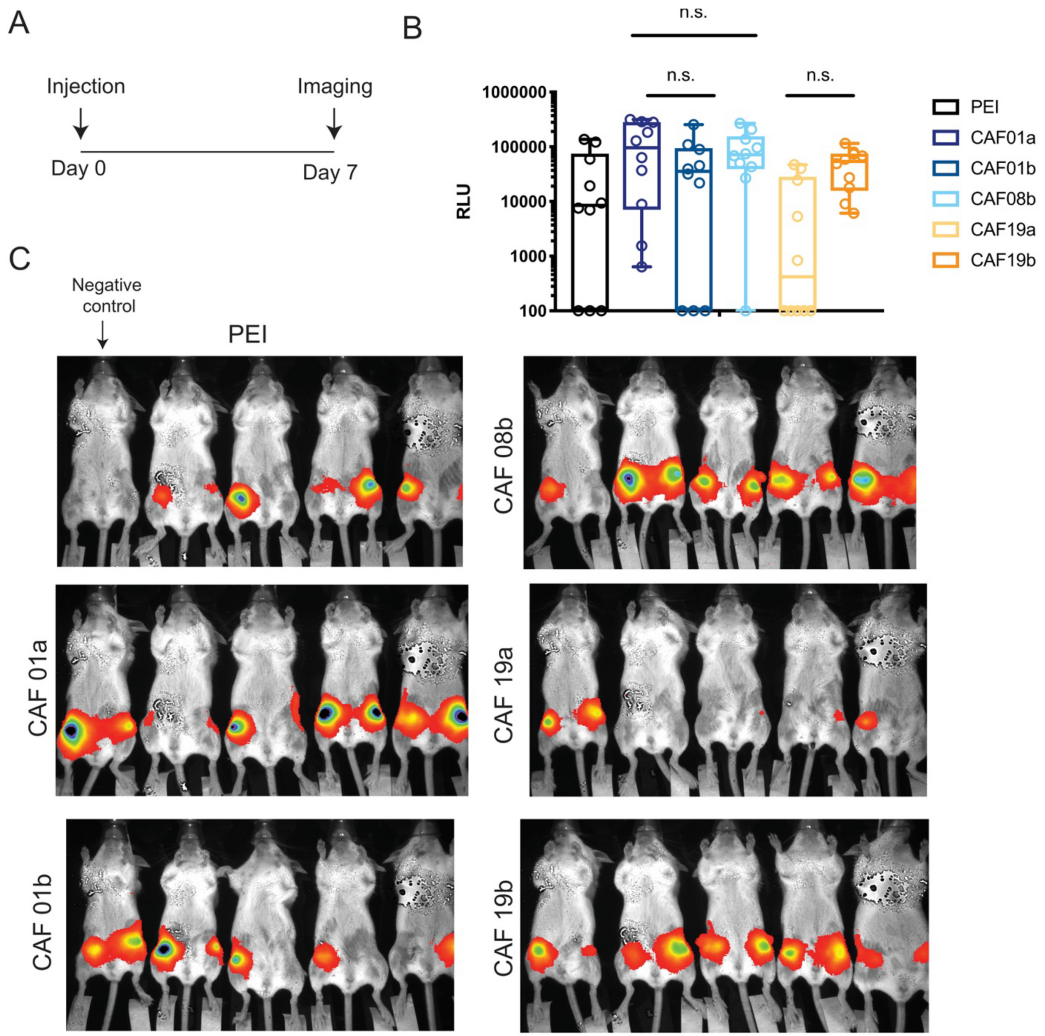




Anna K. Blakney, Paul F. McKay, Bárbara Ibarzo Yus, Judith E. Hunter, Elizabeth A. Dex, and Robin J. Shattock. "The Skin You Are In: Design-of-Experiments Optimization of Lipid Nanoparticle Self-Amplifying RNA Formulations in Human Skin Explants." *ACS Nano*. 2019. 13(5): 5920-5930.

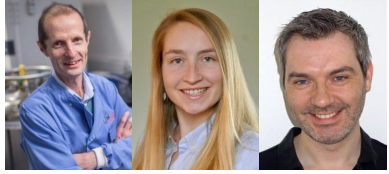
Optimising formulations for saRNA



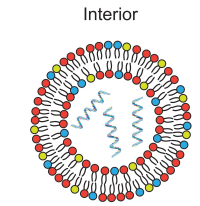


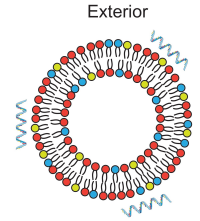
Ongoing: Mice, Ferrets, NHPs, Guinea Pigs, Rabbits

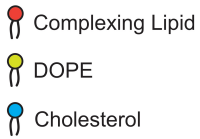
Anna K. Blakney, Paul F. McKay, Dennis Christensen, Bárbara Ibarzo Yus, Yoann Aldon, Frank Follman, and Robin J. Shattock. "Effects of cationic adjuvant formulation particle type, fluidity and immunomodulators on delivery and immunogenicity of saRNA." *Journal of Controlled Release.* 2019. 304: 65-74.



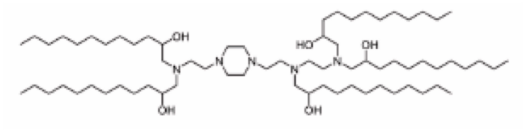
Inside-out?



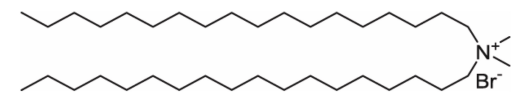




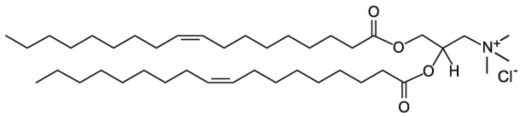
C12-200
(ionizable)

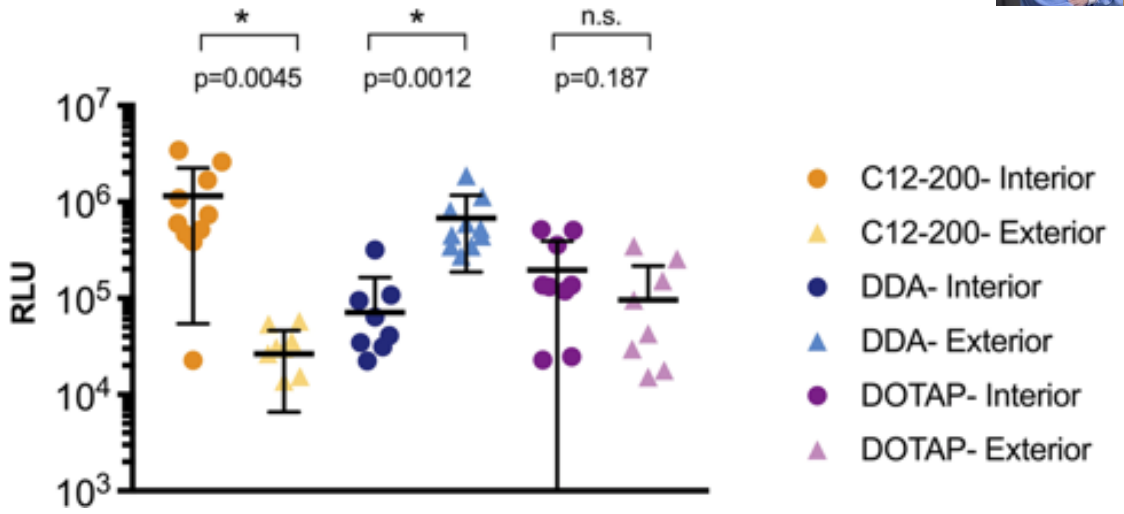


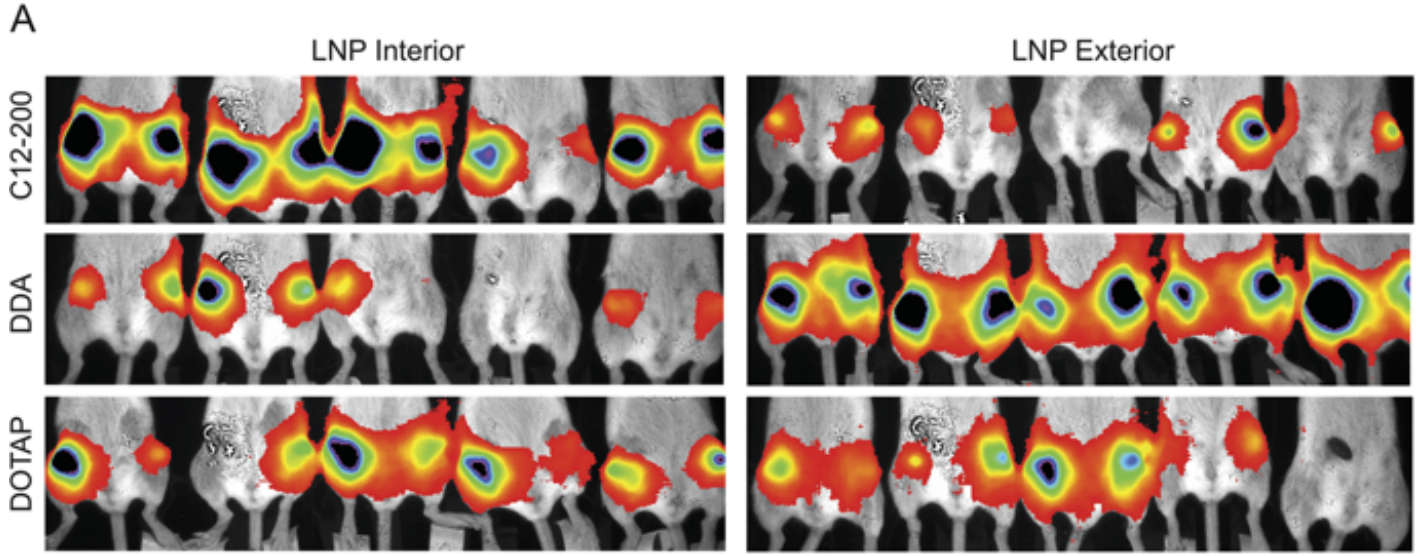
DDA
(cationic)



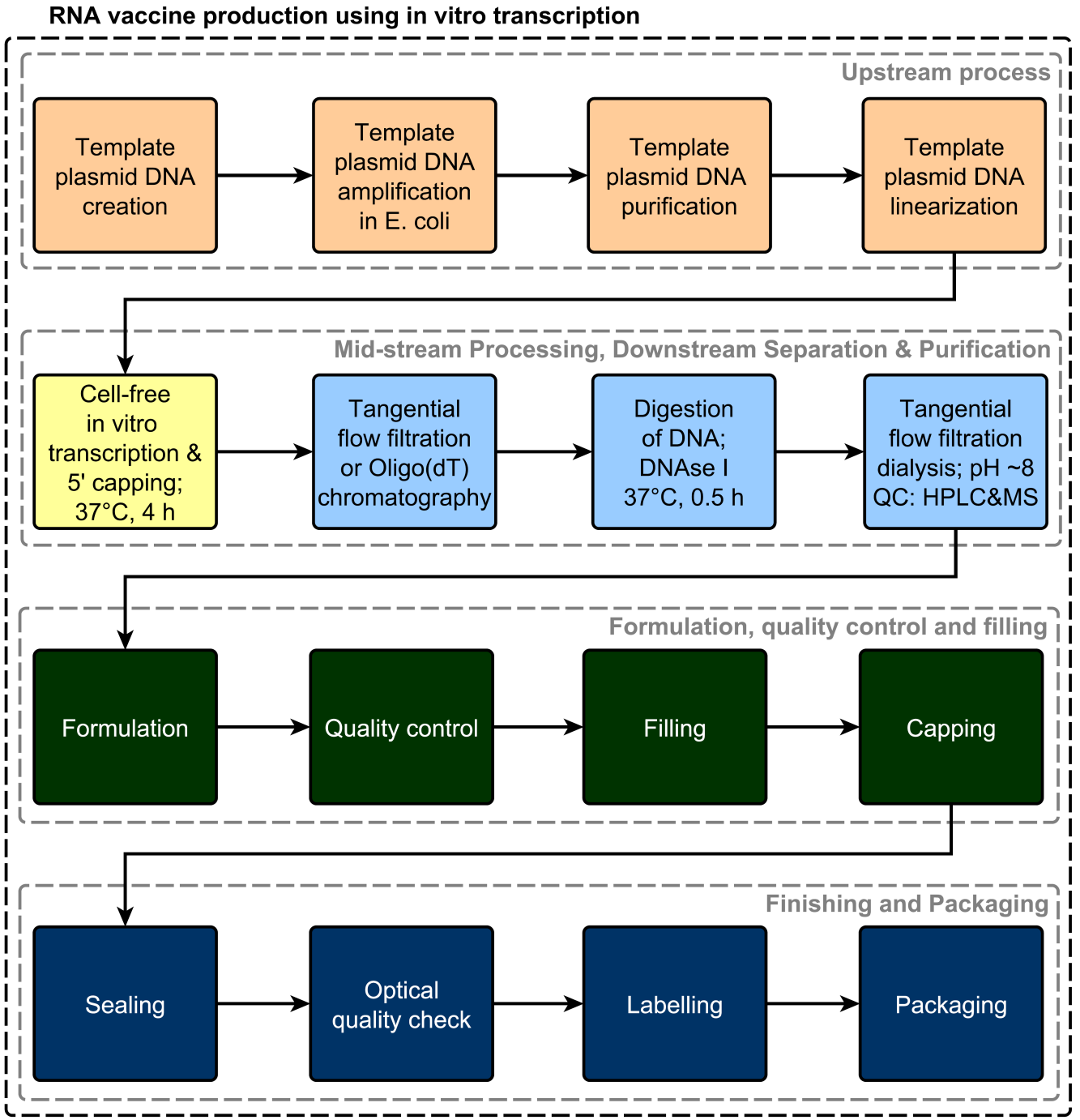
DOTAP
(cationic)







Anna K. Blakney, Paul F. McKay, Bárbara Ibarzo Yus, Yoann Aldon, and Robin Shattock. "Inside out: optimization of lipid nanoparticle formulations for exterior complexation and in vivo delivery of saRNA." *Gene Therapy*. 2019. 26: 363-372.



Zoltán Kis, Robin Shattock, Nilay Shah, and Cleo Kontoravdi."Emerging Technologies for Low-Cost, Rapid Vaccine Manufacture." *Biotechnology Journal*. 2019. 14: 1800376.

Critical steps in saRNA production and processing

NHS Blood and Transplant

Re-engineering of elements on template constructs (Imperial)

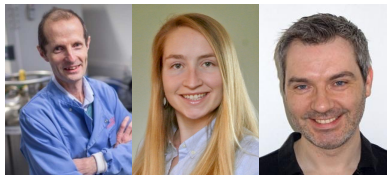
Plasmid engineering/construct design → ***In vitro* transcription/modification** → **Full-length transcript isolation** → **QC analysis** → **Batch release**

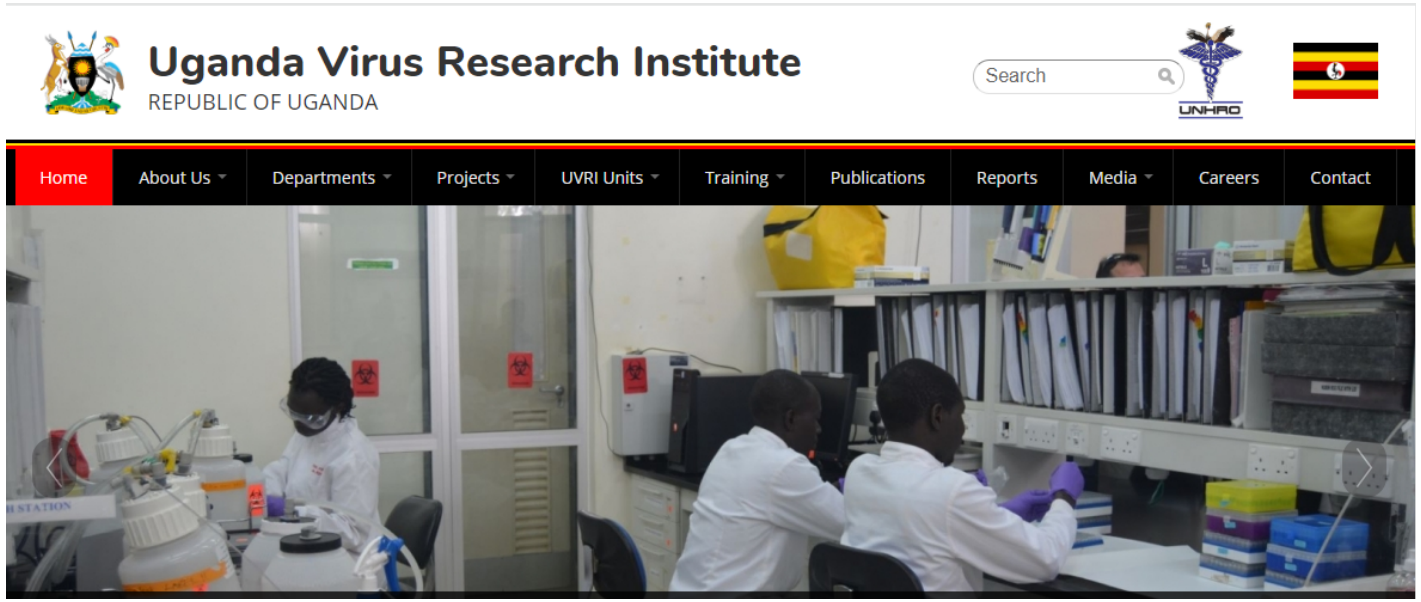
QP at NHSBT

Platform modular GMP process for *in vitro transcription* (IVT) to produce clinical-grade saRNA product

G.M. Cheetham and T.A. Steitz. *Science*. 1999. 286: 2305-9.

RNA – UVRI, Uganda



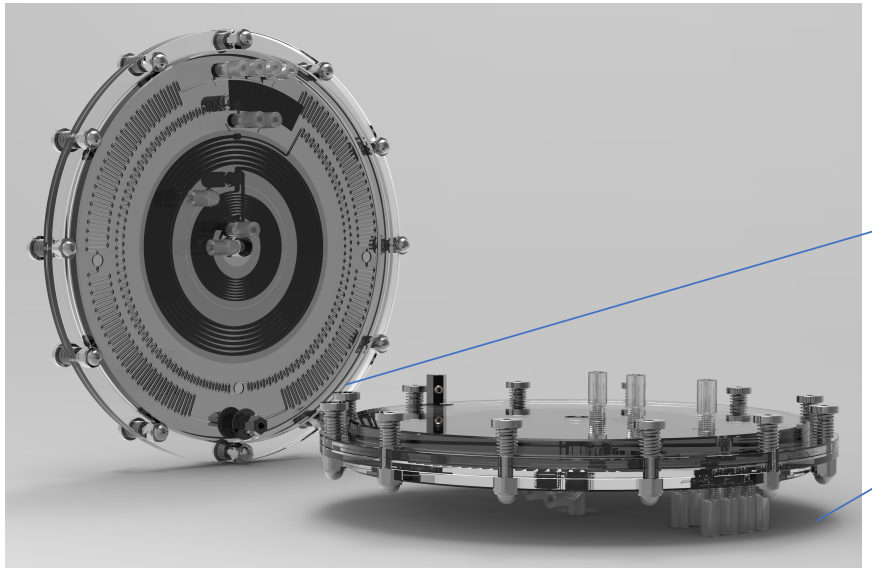


Rift Valley Fever is focus for proof of concept in Uganda

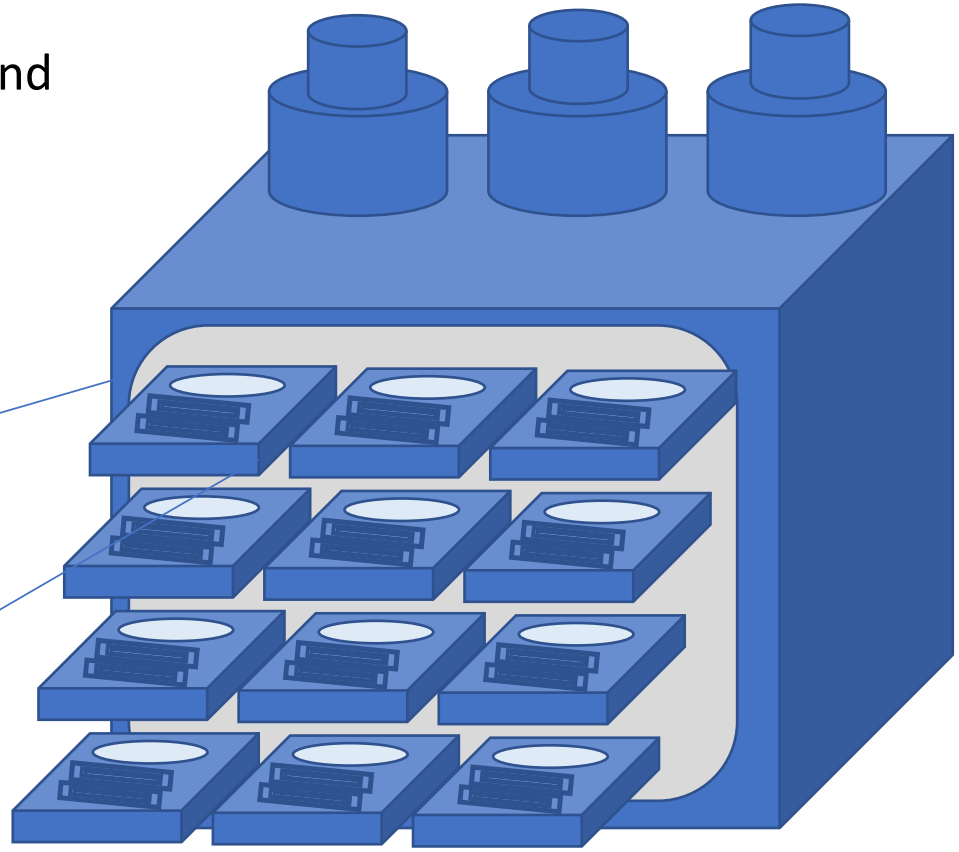
UVRI staff training within Prof Robin Shattock's labs in Imperial College London.

Modular manufacture – Factory-in-a-Box

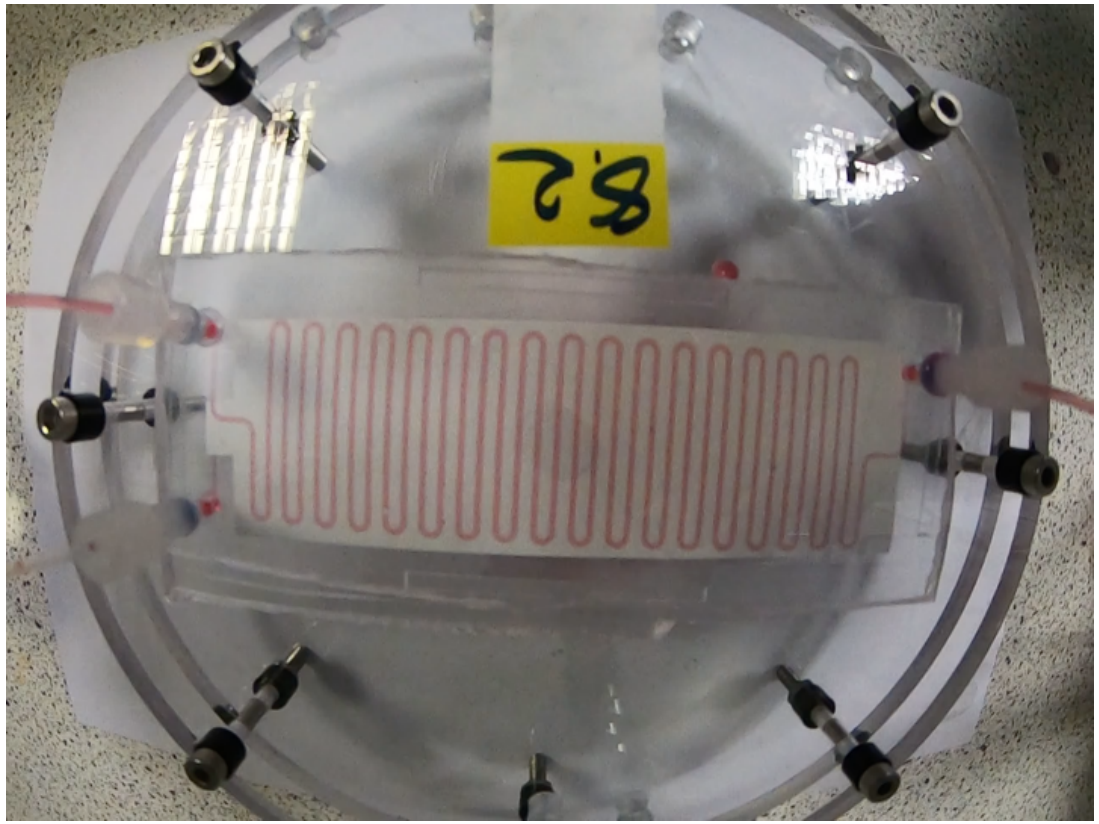
- A modular micro/meso fluidic device to synthesise and cap RNA
- Continuous operation
- On-line and in-situ analytical techniques
- Scale out rather than scale up
- Production of sufficient number of dosages within a few hours of continuous operation
- Potential for significant cost savings and rapid on-demand and in-situ manufacture of synthetic RNA vaccines



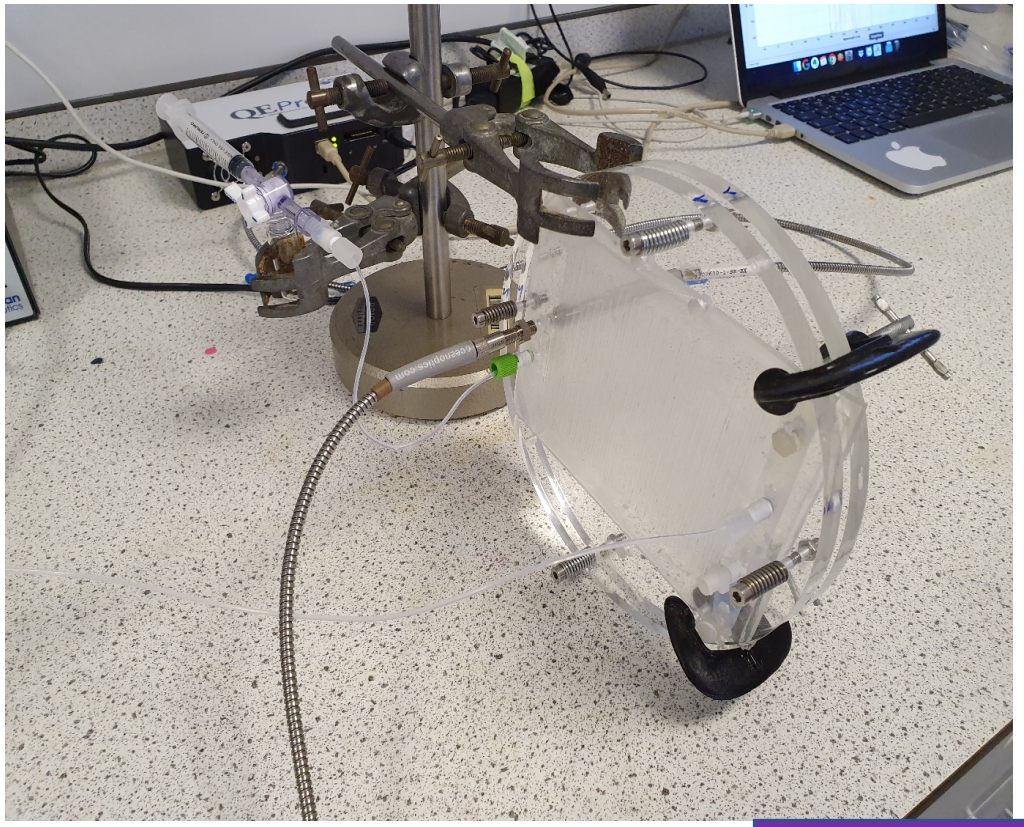
Integrated flow Reaction-Filtration module



Connected System – Experimental set up



Flow Filtration Unit



Reaction Unit with in-situ monitoring

1. Makatsoris, C, UK Patent Application No. 1906422.9, Microfluidic Sensing and Control System
2. Makatsoris, C, UK Patent Application No. 1906421.1, Microfluidic Filtration Apparatus
3. Makatsoris, C,UK Patent Application No. 1906413.8, Microfluidic Processing Apparatus and Method

Future Vaccine Manufacturing Hub












Future Vaccine Manufacturing Hub Outputs

Publications

1. Charles Vragniau, Joshua C. Bufton, Frédéric Garzoni, Emilie Stermann, Fruzsina Rabi, Céline Terrat, Mélanie Guidetti, Véronique Josserand, Matt Williams, Christopher J. Woods, Gerardo Viedma, Phil Bates, Bernard Verrier, Laurence Chaperot, Christiane Schaffitzel, Imre Berger, and Pascal Fender. "Synthetic self-assembling ADDomer platform for highly efficient vaccination by genetically encoded multiepitope display." *Science Advances*. 2019. 5: eaaw2853.
2. Ankur Matreja, Gordon Dougan. "Molecular epidemiology and intercontinental spread of cholera." Vaccine. 2019. https://doi.org/10.1016/j.vaccine.2019.07.038.
3. Anna K. Blakney, Paul F. McKay, Bárbara Ibarzo Yus, Yoann Aldon, and Robin Shattock. "Inside out: optimization of lipid nanoparticle formulations for exterior complexation and in vivo delivery of saRNA." *Gene Therapy*. 2019. https://doi.org/10.1038/s41434-019-0095-2
4. Zoltán Kis, Maria M. Papthanasiou, Raul Calvo-Serrano, Cleo Kontoravdi, and Nilay Shah. "A model-based quantification of the impact of new manufacturing technologies on developing country vaccine supply chain performance: a Kenyan case study." *Journal of Advanced Manufacturing and Processing*. 2019. https://doi.org/10.1002/amp2.10025
5. Anna K. Blakney, Paul F. McKay, Dennis Christensen, Bárbara Ibarzo Yus, Yoann Aldon, Frank Follman, Robin J. Shattock. "Effects of cationic adjuvant formulation particle type, fluidity and immunomodulators on delivery and immunogenicity of saRNA." *Journal of Controlled Release.* 2019. 304: 65-74.
6. Maria M. Papthanasiou, Baris Burnak, Justin Katz, Nilay Shah, Efstratios N. Pistikopoulos. "Assisting continuous biomanufacturing through advanced control in downstream purification." *Computers & Chemical Engineering*. 2019. 125: 232-248.
7. Anna K. Blakney, Paul F. McKay, Bárbara Ibarzo Yus, Judith E. Hunter, Elizabeth A. Dex, and Robin J. Shattock. "The Skin You Are In: Design-of-Experiments Optimization of Lipid Nanoparticle Self-Amplifying RNA Formulations in Human Skin Explants." *ACS Nano*. 2019. 13(5): 5920-5930.

Anna K. Blakney was awarded a Provost's Award for excellence in animal research for the above work.

8. Anna K. Blakney, Gokhan Yilmaz, Paul F. McKay, C. Remzi Becer, Robin J. Shattock. "One Size Does Not Fit All: The Effect of Chain Length and Charge Density of Poly(ethylene imine) Based Copolymers on Delivery of pDNA, mRNA, and RepRNA Polyplexes." *Biomacromolecules*. 2018. 19(7): 2870-2879.
9. Anna K. Blakney, Paul F. McKay, Robin J. Shattock. "Structural Components for Amplification of Positive and Negative Strand VEEV Splitzicons." *Frontiers in Molecular Biosciences*. 2018. 5: 71.
10. Luke Muir, Paul F. McKay, Velislava N. Petrova, Oleksiy V. Klymenko, Sven Kratochvil, Christopher L. Pinder, Paul Kellam, Robin J. Shattock. "Optimisation of ex vivo memory B cell expansion/differentiation for interrogation of rare peripheral memory B cell subset responses." *Wellcome Open Research*. 2018. 2: 97.
11. Zoltán Kis, Robin Shattock, Nilay Shah, Cleo Kontoravdi. "Emerging Technologies for Low-Cost, Rapid Vaccine Manufacture." *Biotechnology Journal*. 2019. 14: 1800376.

Awards

Anna K. Blakney was awarded a Provost's Award for excellence in animal research.
Alex Brogan was awarded the Postdoc and Fellows Development Centre (PFDC) Reps Award 2018.
Yunqing (Frank) Zhu was appointed as Professor at Tongji University in Shanghai.
Alex Brogan was appointed Lecturer at King's College London.

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Thank you for your attention

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