UMRA Forum

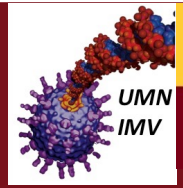
mRNA Technology:

fundamentals and possibilities

(from a virologist's point of view…)

Louis M. Mansky, Ph.D.

February 22, 2022
(2/22/22)



OACA

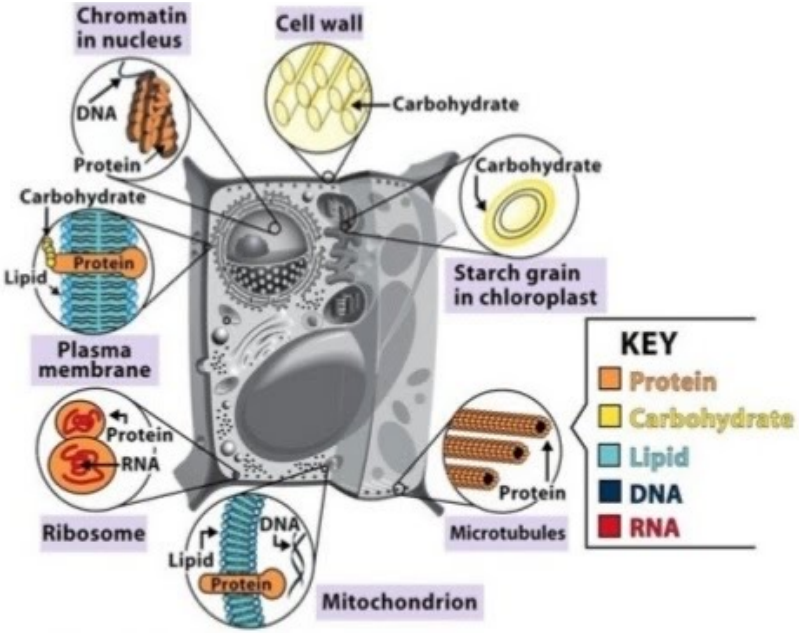
UNIVERSITY OF MINNESOTA
Driven to Discover®

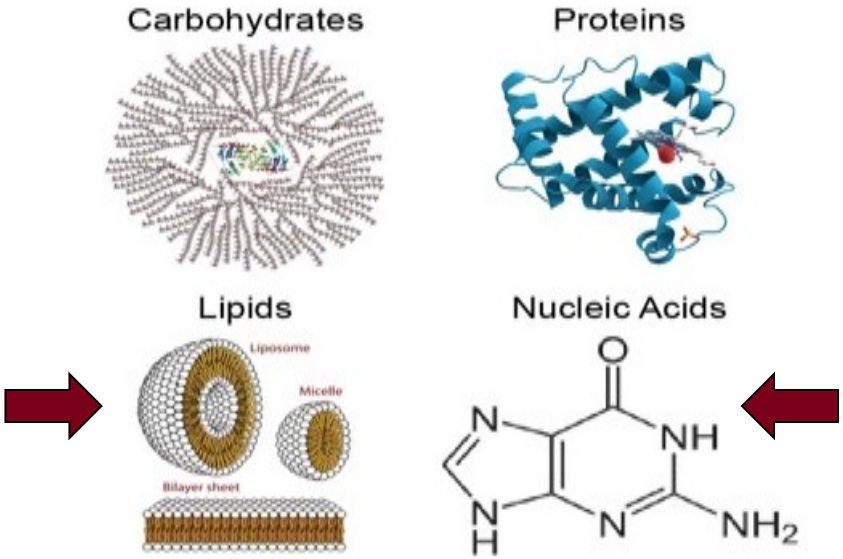
School of Dentistry

Overview of today's discussion

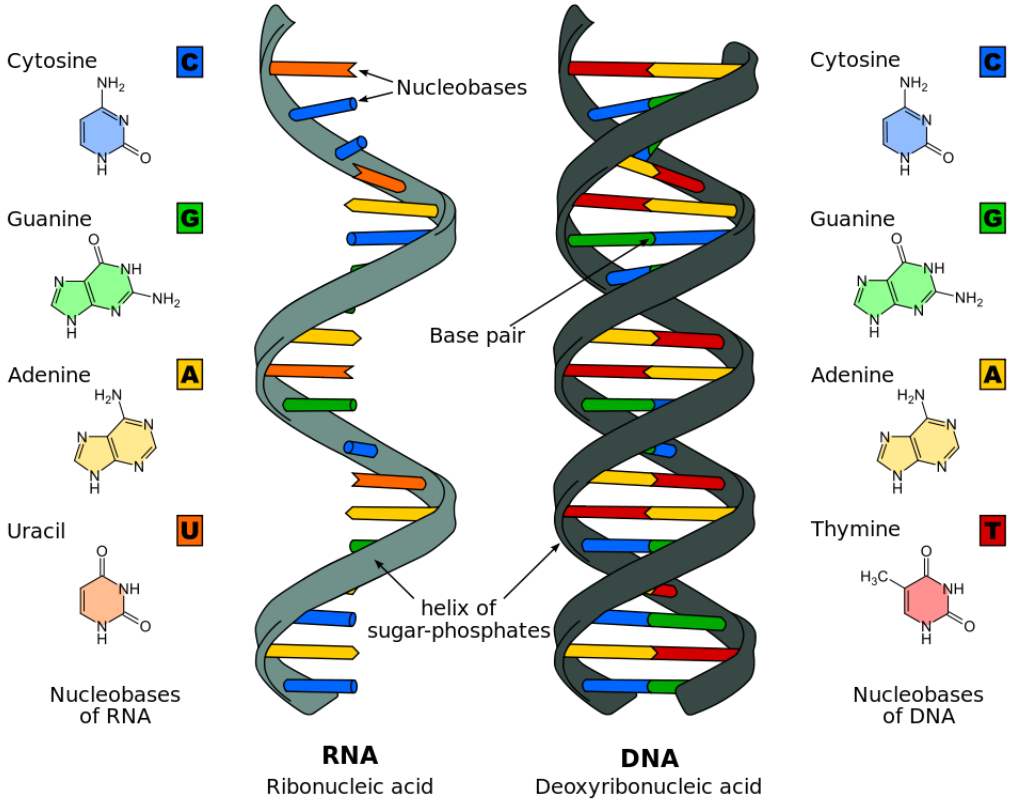
- General background and fundamentals
- Possibilities 1: Use in COVID-19
- Possibilities 2: Other potential applications

Biological molecules in a cell





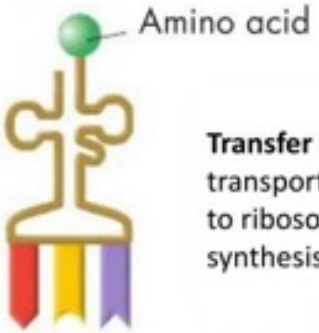
Nucleic acids: DNA and RNA



Main types of RNA in a cell

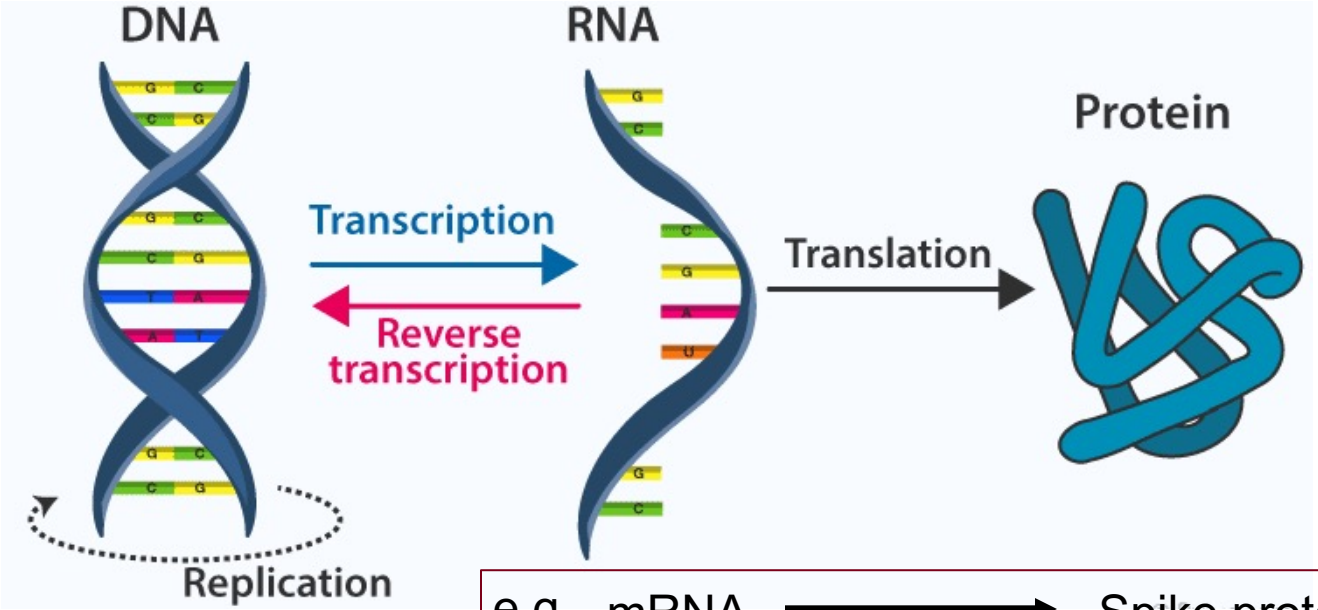
Messenger RNA (mRNA) – carries code from DNA to ribosome for protein synthesis

Ribosomal RNA (rRNA) – assembles amino acids brought by tRNA in a specific order from mRNA to make proteins; made of RNA by the nucleolus



Transfer RNA (tRNA) – transports specific amino acid to ribosome for protein synthesis

Central dogma of molecular biology

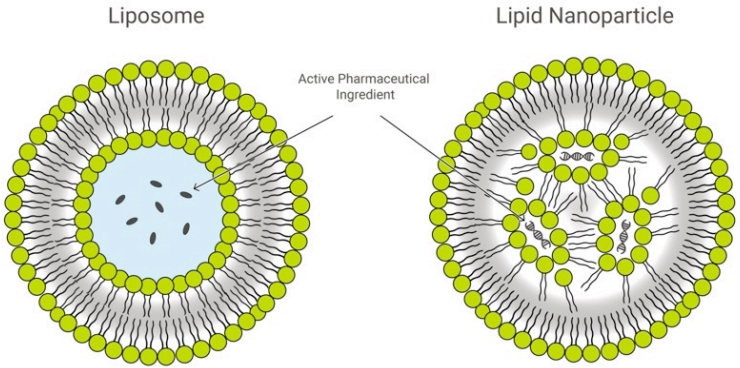


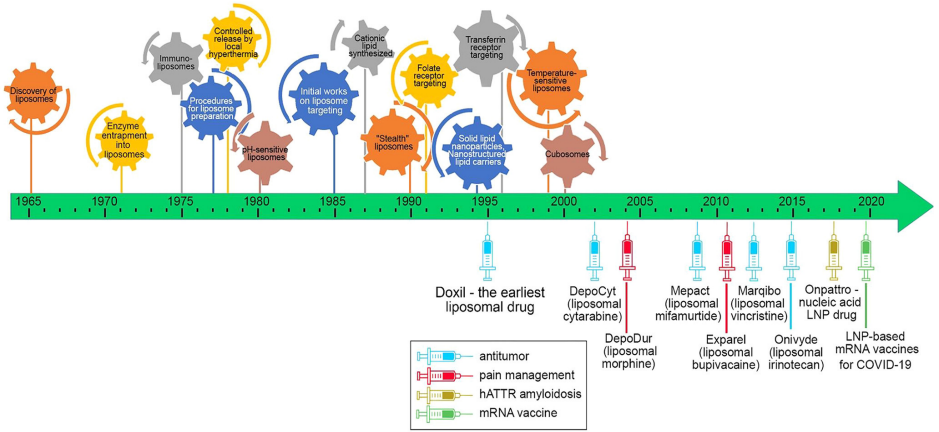
e.g., mRNA → Spike protein

Some milestones in mRNA biology & therapy

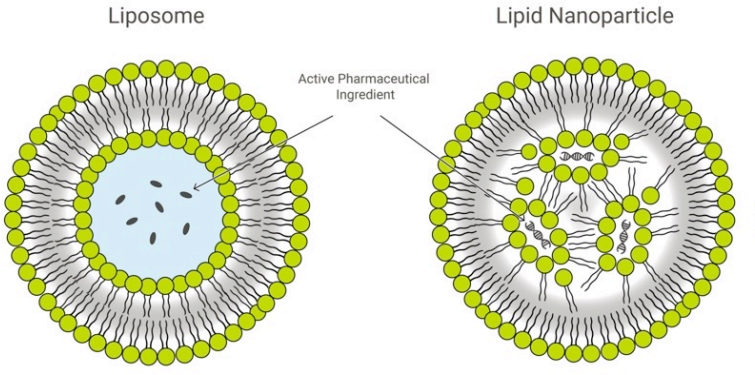
- 1961 - mRNA discovered (Brenner)
- 1969 – 1st evidence of in vitro translation of mRNA (Lingrel)
- 1984 – 1st evidence of in vitro transcription using bacterial virus SP6 RNA polymerase (Krieg)
- 1990 – 1st demonstration of translation of mRNA injected into mice (Wolff)
- 1992 – mRNA injection into rat brains for therapeutic treatment (Jirikowski)
- 1995 – 1st mRNA designed vaccine encoding cancer antigens (Conry)
- 2005 – nucleoside modified, non immunogenic mRNA transcript designed (Kariko)
- 2009 – 1st trial of cancer immunotherapy using mRNA–based vaccines (Weide)

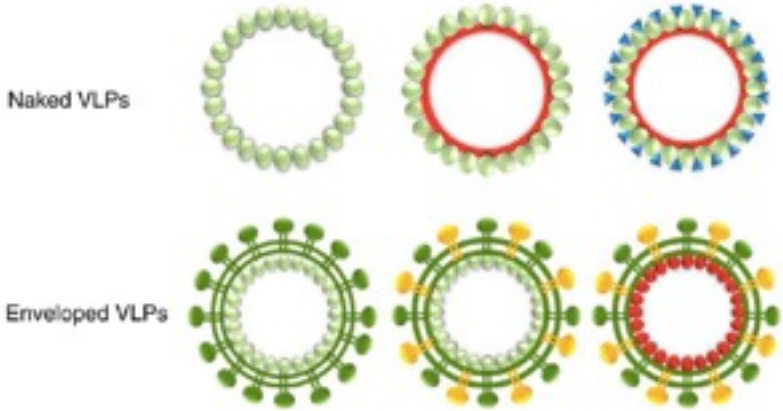
Liposomes, lipid nanoparticles, and technological advances in therapy





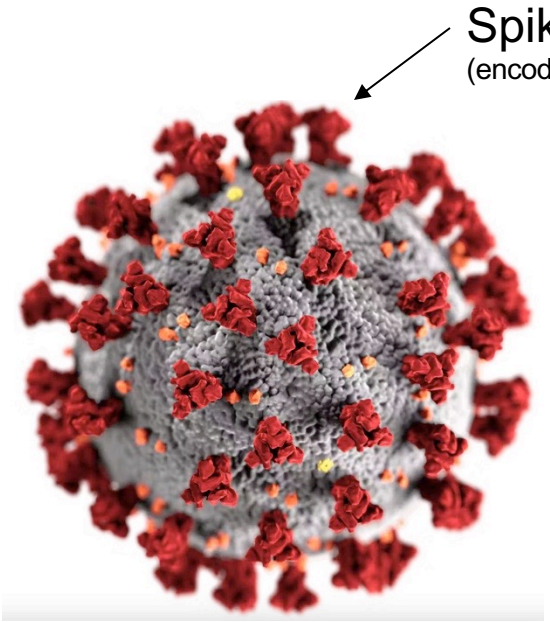
Similarities between liposomes & LNPs with virus-like particles (VLPs)





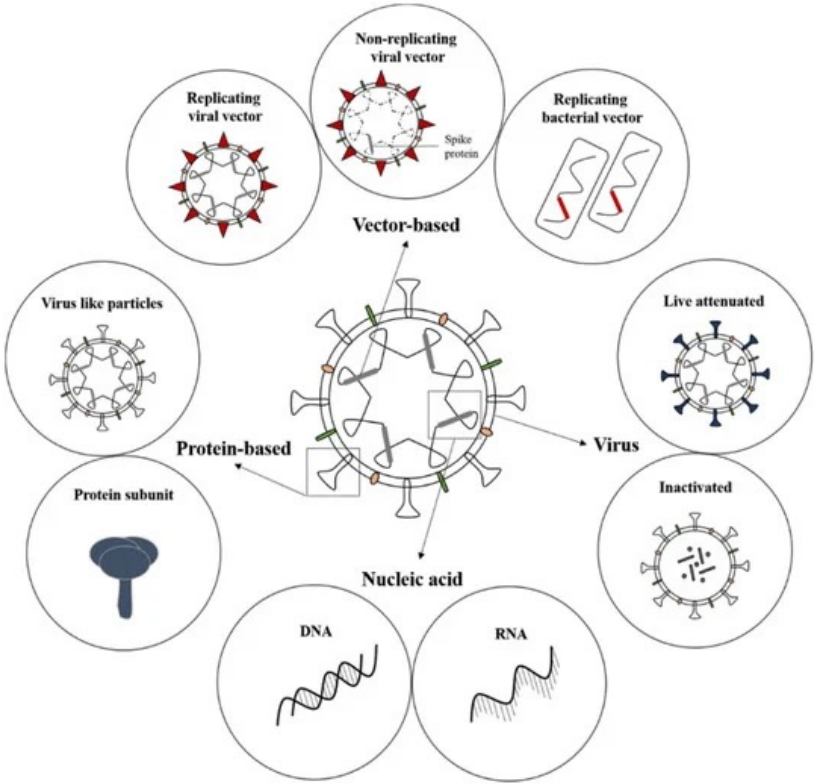
Example of an approved VLP vaccine:

COVID 19 mRNA vaccines



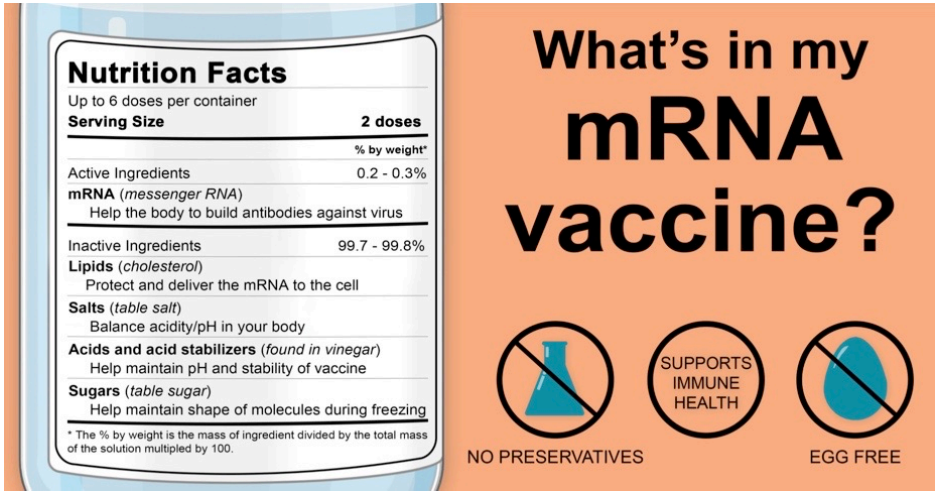
SARS-CoV-2
(cause of COVID-19)

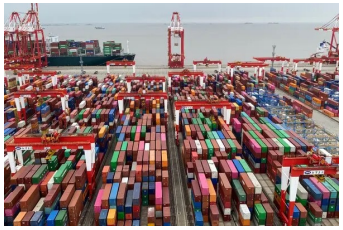
Other types of COVID-19 vaccines investigated



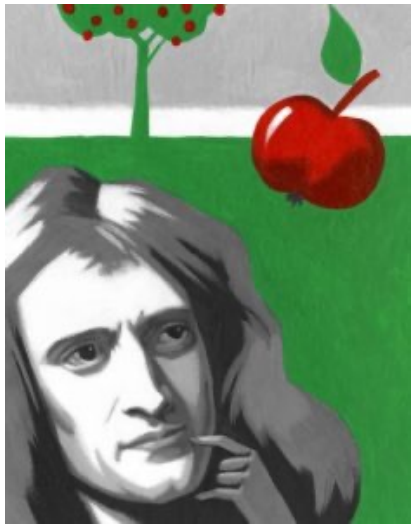
Mass production of mRNA vaccines presents many challenges…

- Kilogram amounts (and beyond) of the ingredients are needed!



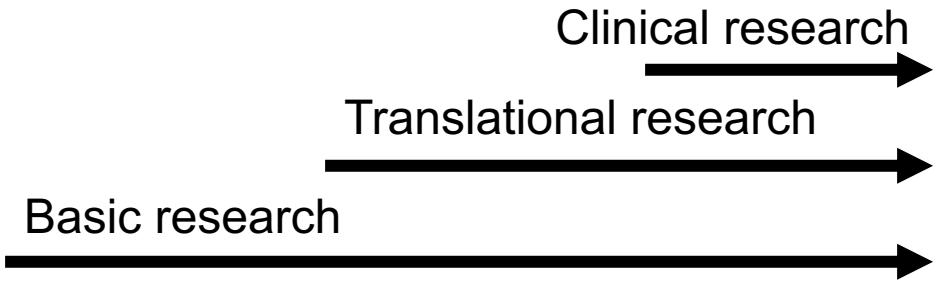


Previous research/knowledge not associated with COVID-19 was required for mRNA success



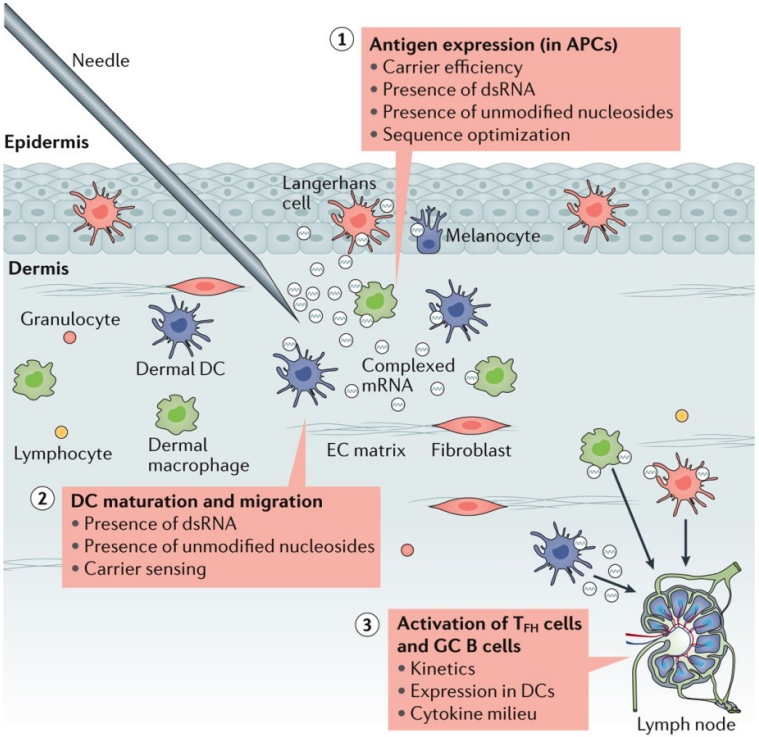
Serendipity in scientific discovery – or subsequent application of past discoveries (particularly in a crisis) – should not be underestimated

Creation of therapeutics is a continuum that always requires troubleshooting and experimentation to achieve success



Research continuum – basic research is needed all along the path…

mRNA vaccine delivery and response



mRNA vaccine applications under study

(as of 2018)

Delivery system type	Route of delivery	Species	Target
Commercial transfection reagent	i.n.	Mouse	OVA[145]
Protamine	i.d.	Mouse, ferret, pig and human	Influenza virus[18,52], melanoma[150], non-small-cell lung cancer[200], prostate cancer[36,52,151], rabies virus[56], OVA[36,52,155] and Lewis lung cancer[155]
Protamine liposome	i.v.	Mouse	Lung cancer[201]
Polysaccharide particle	s.c.	Mouse and rabbit	Influenza virus[98]
Cationic nanoemulsion	i.m.	Mouse, rabbit, ferret and rhesus macaque	Influenza virus[96], RSV[50], HIV-1 (REFS 50,97), HCMV[50], *Streptococcus* spp.[100], HCV and rabies virus[87]
Cationic polymer	s.c. and i.n.	Mouse	Influenza virus[99], andHIV-1 (REFS 110,111)
Cationic polymer liposome	i.v.	Mouse	Melanoma[202,203], pancreatic cancer[204]
Cationic lipid nanoparticle	i.d., i.v. and s.c.	Mouse	HIV-1 (REF. 109) and OVA[152]
Cationic lipid, cholesterol nanoparticle	i.v., s.c. and i.s.	Mouse	Influenza virus[59,108], melanoma[59,141], Moloney murine leukaemia virus, OVA, HPV andc olon cancer[59]
Cationic lipid, cholesterol, PEG nanoparticle	i.d., i.m. and s.c.	Mouse, cotton rat and rhesus macaque	Zika virus[20,85,112], influenza virus[22,94,95,205], RSV[19], HCMV, rabies virus[87] and melanoma[153]
Dendrimer nanoparticle	i.m.	Mouse	Influenza virus, Ebola virus, *Toxoplasma gondii*[89] and Zika virus[88]

HCMV, human cytomegalovirus; HCV, hepatitis C virus; HPV, human papillomavirus; i.d., intradermal; i.m., intramuscular; i.n., intranasal; i.s., intrasplenic; i.v., intravenous; OVA, ovalbumin-expressing cancer models; PEG, polyethylene glycol; RSV, respiratory syncytial virus; s.c., subcutaneous.

Clinical trials with mRNA vaccines against viral diseases

(as of 2018)

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Argos Therapeutics	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	• NCT00672191 (II) • NCT01069809 (II) • NCT02042248 (I)	• Completed[105] • Completed; results NA • Completed; results NA
CureVac AG	RNActive viral Ag mRNA (i.m., i.d.)	Rabies virus	NCT02241135 (I)	Active[56,91]
Erasmus Medical Center	DC loaded with viral Ag mRNA with TriMix (i.nod.)	HIV-1	NCT02888756 (II)	Recruiting
Fundació Clínic per la Recerca Biomèdica	Viral Ag mRNA with TriMix (NA)	HIV-1	NCT02413645 (I)	Active
Massachusetts General Hospital	DC loaded with viral Ag mRNA (i.d.)	HIV-1	NCT00833781 (II)	Completed[104]
McGill University Health Centre	DC EP with autologous viral Ag and CD40L mRNAs (i.d.)	HIV-1	NCT00381212 (I/II)	Completed[102]
Moderna Therapeutics	Nucleoside-modified viral Ag mRNA (i.m.)	Zika virus	NCT03014089 (I/II)	Recruiting[85]
		Influenza virus	NCT03076385 (I)	Ongoing[22]

The table summarizes the clinical trials registered at ClinicalTrials.gov as of 5 May 2017. Ag, antigen; CD40L, CD40 ligand; DC, dendritic cell; EP, electroporated; i.d., intradermal; i.m., intramuscular; i.nod., intranodal; NA, not available.

Clinical trials with mRNA vaccines against cancer (as of 2018)

Sponsoring institution	Vaccine type (route of administration)	Targets	Trial numbers (phase)	Status
Antwerp University Hospital	DC EP with TAA mRNA (i.d. or NA)	AML	• NCT00834002 (I) • NCT01686334 (II)	• Completed[206,207] • Recruiting
		AML, CML, multiple myeloma	NCT00965224 (II)	Unknown
		Multiple solid tumours	NCT01291420 (I/II)	Unknown[208]
		Mesothelioma	NCT02649829 (I/II)	Recruiting
		Glioblastoma	NCT02649582 (I/II)	Recruiting
Argos Therapeutics	DC EP with autologous tumour mRNA with or without CD40L mRNA (i.d. or NA)	Renal cell carcinoma	• NCT01482949 (II) • NCT00678119 (II) • NCT00272649 (I/II) • NCT01582672 (III) • NCT00087984 (I/II)	• Ongoing • Completed[209] • Completed; results NA • Ongoing • Completed; results NA
		Pancreatic cancer	NCT00664482 (NA)	Completed; results NA
Asterias Biotherapeutics	DC loaded with TAA mRNA (NA)	AML	NCT00510133 (II)	Completed[210]
BioNTech RNA Pharmaceuticals GmbH	Naked TAA or neo-Ag mRNA (i.nod.)	Melanoma	• NCT01684241 (I) • NCT02035956 (I)	• Completed; results NA • Ongoing
	Liposome-complexed TAA mRNA (i.v.)	Melanoma	NCT02410733 (I)	Recruiting[59]
	Liposome-formulated TAA and neo-Ag mRNA (i.v.)	Breast cancer	NCT02316457 (I)	Recruiting
CureVac AG	RNActive TAA mRNA (i.d.)	Non-small-cell lung cancer	• NCT00923312 (I/II) • NCT01915524 (I)	• Completed[211] • Terminated[200]
		Prostate cancer	• NCT02140138 (II) • NCT00831467 (I/II) • NCT01817738 (I/II)	• Terminated • Completed[151] • Terminated[212]
Duke University	DC loaded with CMV Ag mRNA (i.d. or ing.)	Glioblastoma, malignant glioma	• NCT00626483 (I) • NCT00639639 (I) • NCT02529072 (I) • NCT02366728 (II)	• Ongoing[213] • Ongoing[138,139] • Recruiting • Recruiting
	DC loaded with autologous tumour mRNA (i.d.)	Glioblastoma	NCT00890032 (I)	Completed; results NA
	DC, matured, loaded with TAA mRNA (i.nod.)	Melanoma	NCT01216436 (I)	Terminated
Guangdong 999 Brain Hospital	DC loaded with TAA mRNA (NA)	Glioblastoma	• NCT02808364 (I/II) • NCT02709616 (I/II)	• Recruiting • Recruiting
		Brain metastases	NCT02808416 (I/II)	Recruiting
Herlev Hospital	DC loaded with TAA mRNA (i.d.)	Breast cancer, melanoma	NCT00978913 (I)	Completed[214]
		Prostate cancer	NCT01446731 (II)	Completed[215]
Life Research Technologies GmbH	DC, matured, loaded with TAA mRNA (NA)	Ovarian cancer	NCT01456065 (I)	Unknown
Ludwig-Maximilian-University of Munich	DC loaded with TAA and CMV Ag mRNA (i.d.)	AML	NCT01734304 (I/II)	Recruiting
MD Anderson Cancer Center	DC loaded with AML lysate and mRNA (NA)	AML	NCT00514189 (I)	Terminated
Memorial Sloan Kettering Cancer Center	DC (Langerhans) EP with TAA mRNA (i.d.)	Melanoma	NCT01456104 (I)	Ongoing
		Multiple myeloma	NCT01995708 (I)	Recruiting
Oslo University Hospital	DC loaded with autologous tumour or TAA mRNA (i.d. or NA)	Melanoma	• NCT00961844 (I/II) • NCT01278940 (I/II)	• Terminated • Completed[216]
		Prostate cancer	• NCT01197625 (I/II) • NCT01278914 (I/II)	• Recruiting • Completed; results NA
		Glioblastoma	NCT00846456 (I/II)	Completed[217]
		Ovarian cancer	NCT01334047 (I/II)	Terminated
Radboud University	DC EP with TAA mRNA (i.d. and i.v. or i.nod)	Colorectal cancer	NCT00228189 (I/II)	Completed[218]
		Melanoma	• NCT00929019 (I/II) • NCT00243529 (I/II) • NCT00940004 (I/II) • NCT01530698 (I/II) • NCT02285413 (II)	• Terminated • Completed[219,220] • Completed[220,221] • Completed[144,220,221] • Completed; results NA
Universitair Ziekenhuis Brussel	DC EP with TAA and TriMix mRNA (i.d. and i.v.)	Melanoma	• NCT01066390 (I) • NCT01302496 (II) • NCT01676779 (II)	• Completed[137] • Completed[140] • Completed; results NA
University Hospital Erlangen	DC, matured, loaded with autologous tumour RNA (i.v.)	Melanoma	NCT01983748 (III)	Recruiting
University Hospital Tübingen	Autologous tumour mRNA with GM-CSF protein (i.d. and s.c.)	Melanoma	NCT00204516 (I/II)	Completed[222]
	Protamine-complexed TAA mRNA with GM-CSF protein (i.d. and s.c.)	Melanoma	NCT00204607 (I/II)	Completed[150]
University of Campinas, Brazil	DC loaded with TAA mRNA (NA)	AML, myelodysplastic syndromes	NCT03083054 (I/II)	Recruiting
University of Florida	RNActive* TAA mRNA (i.d.)	Prostate cancer	NCT00906243 (I/II)	Terminated
	DC loaded with CMV Ag mRNA with GM-CSF protein (i.d.)	Glioblastoma, malignant glioma	NCT02465268 (II)	Recruiting

The table summarizes the clinical trials registered at ClinicalTrials.gov as of 5 May 2017. Ag, antigen; AML, acute myeloid leukaemia; CD40L, CD40 ligand; CML, chronic myeloid leukaemia; CMV, cytomegalovirus; DC, dendritic cell; EP, electroporated; GM-CSF, granulocyte–macrophage colony-stimulating factor; i.d., intradermal; ing., inguinal injection; i.nod., intranodal injection; i.v., intravenous; NA, not available; neo-Ag, personalized neoantigen; s.c., subcutaneous; TAA, tumour-associated antigen. *Developed by CureVac AG.

Leading mRNA vaccine developers

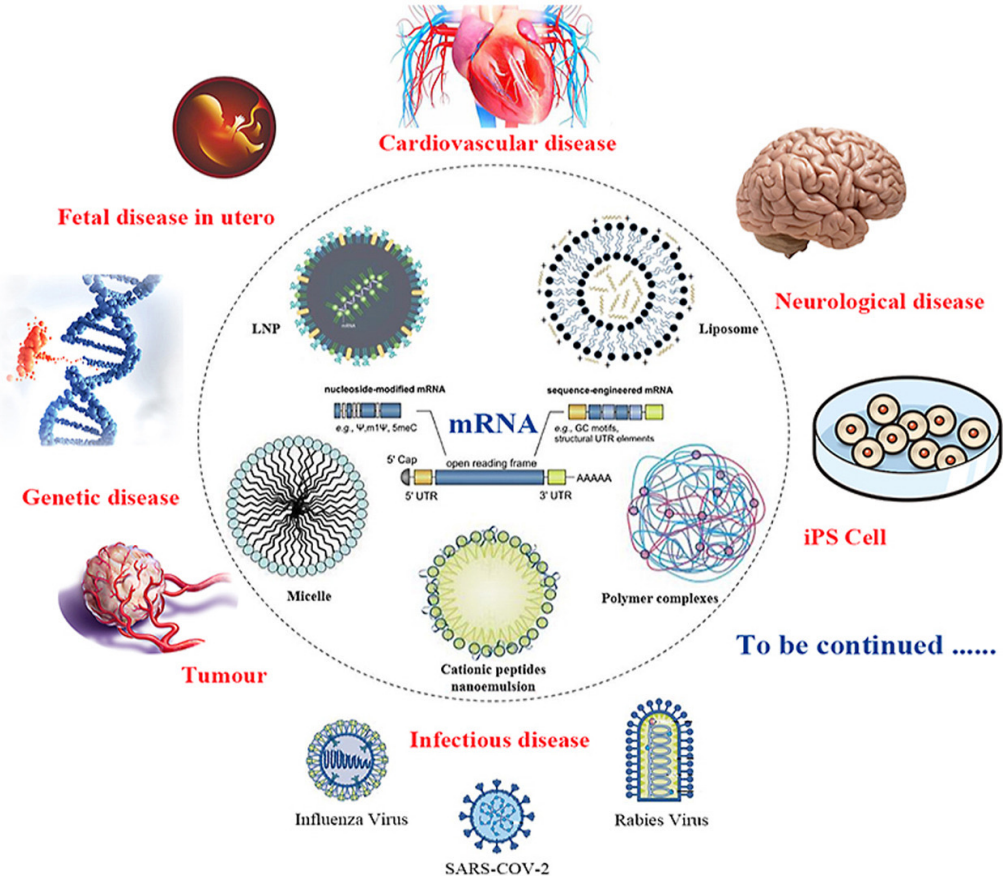
(as of 2018)

Institution	mRNA technology	Partners	Indication (disease target)
Argos Biotechnology	mRNA neoantigens (Arcelis platform)	NA	Individualized cancer vaccines, HIV-1
BioNTech RNA Pharmaceuticals GmbH	Nucleoside-modified mRNA (IVAC Mutanome, FixVAC)	Genentech/Roche	Individualized cancer vaccines
		Bayer AG	Veterinary vaccines
CureVac AG	Sequence-optimized, purified mRNA (RNActive, RNArt, RNAdjuvant)	Boehringer Ingelheim GmbH	Cancer vaccines (lung cancer)
		Johnson & Johnson	Viral vaccines
		Sanofi Pasteur	Infectious disease vaccines
		BMGF	Infectious disease vaccines
		IAVI	HIV vaccines
eTheRNA Immunotherapies	Purified mRNA (TriMix)	NA	Cancer (melanoma, breast), viral vaccines (HBV and/or HPV)
GlaxoSmithKline/ Novartis	Self-amplifying mRNA (SAM) (alphavirus replicon)	NA	Infectious disease vaccines
Moderna Therapeutics	Nucleoside-modified mRNA	Merck & Co.	Individualized cancer vaccines, viral vaccines
		BMGF, DARPA, BARDA	Viral vaccines (influenza virus, CMV, HMPV, PIV, chikungunya virus, Zika virus)
University of Pennsylvania	Nucleoside-modified, purified mRNA	NA	Infectious disease vaccines

BARDA, Biomedical Advanced Research and Development Authority; BMGF, Bill & Melinda Gates Foundation; CMV, cytomegalovirus; DARPA, Defense Advanced Research Projects Agency; HBV, hepatitis B virus; HMPV, human metapneumovirus; HPV, human papillomavirus; IAVI, International AIDS Vaccine Initiative; NA, not available; PIV, parainfluenza virus.

Suspicion of vaccination has an old history

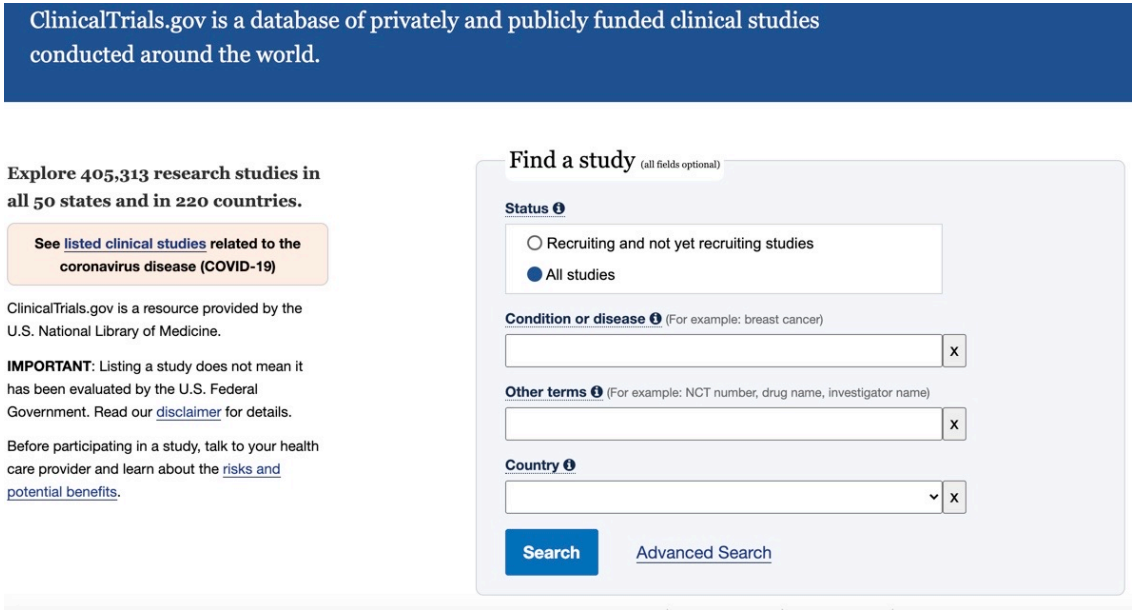
- In 1798, Physician Edward Jenner demonstrated that cowpox (a virus related to smallpox) could provide protection from smallpox (deduced by recognizing that dairymaids infected with cowpox were immune to smallpox)
- In this 1802 cartoon, the British satirist James Gillray implied that vaccination caused people to become part cow



There is a seemingly endless potential for mRNA technology applications to treat various ailments and diseases of humans & animals

Suggested resource for finding ongoing mRNA vaccine clinical trials

https://clinicaltrials.gov/



Questions,
questions,
& more questions…

University of Minnesota

Driven to Discover®

Crookston Duluth Morris Rochester Twin Cities