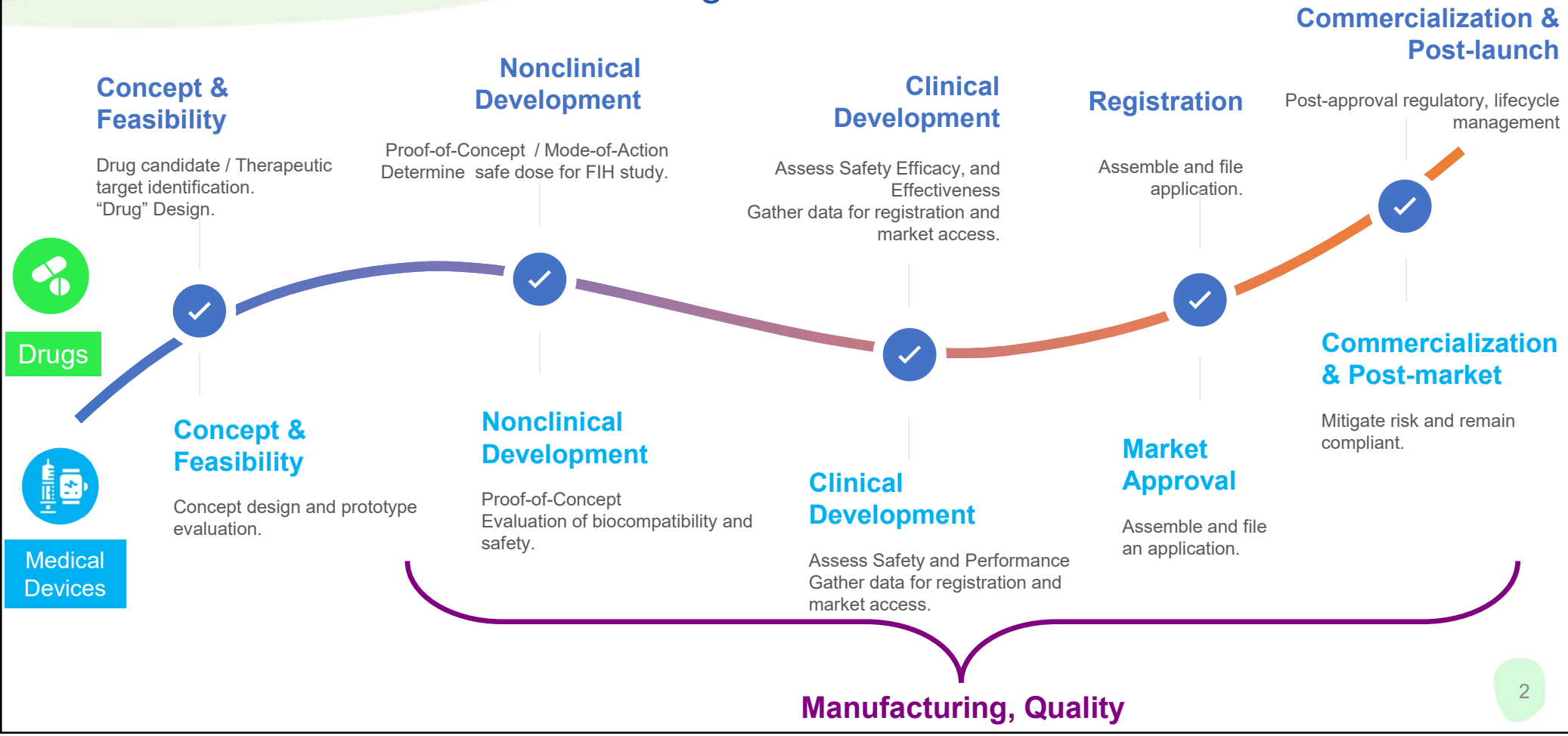
Réunion du 19 Janvier 2023
Cercle des Pharmaciens et Dirigeants des Industries de Santé (CPDIS)

Voisin Consulting Life Sciences (VCLS)

Emmanuelle Voisin
Anne Schlegel

WHAT WE DO
Customized Solutions for both Drugs and Medical Devices



WHAT WE DO

Expert extension of client product development team

The Products We Deal With

Cell, gene & tissue therapies

Biologics

Vaccines

Biosimilars

Small molecules

Microbiome products

Medical devices

Digital health technologies

In Vitro diagnostics

Companion diagnostics

Combination products

Foods & nutraceuticals

THÉRAPIES INNOVANTES À BASE D´ARNm: DE MULTIPLES OPPORTUNITÉS

ANNE SCHLEGEL, PhD

Présentation au Cercle des Pharmaciens et Dirigeants des Industries de Santé (CPDIS)

19 Janvier 2023

ANNE SCHLEGEL, PhD

- 2007: Diplôme d´ingénieur de l´Ecole supérieure de Biotechnologie de Strasbourg (ESBS)
- 2007-2009: Ingénieur de recherche chez PX Therapeutics (Grenoble)
- 2009-2012: Doctorat, bourse CIFRE

  UPCGI (Faculté de Pharmacie Paris 5, Prof. D. Scherman) & DNA Therapeutics
- Depuis 2013: **BioNTech** (Mayence, Allemagne)
  - 2013-2016: Scientist « RNA Formulation and Drug Delivery »
  - 2016-2019: Head of « Formulation Discovery and Testing » Unit
  - Depuis mai 2019: congé parental
- Septembre 2021: Master 2 DEIM « Développement et enregistrement international des médicaments », Université Paris-Saclay
- Depuis 2022: Senior Regulatory Scientist chez **Voisin Consulting Life Sciences**

AGENDA

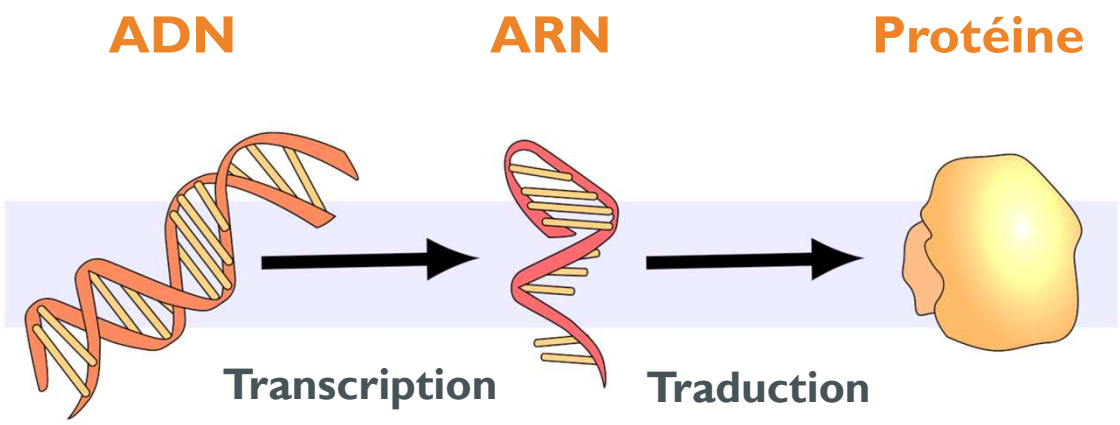
- Introduction
- La plateforme ARNm
- Les applications thérapeutiques de l´ARNm
- Conclusion

Disclaimer:

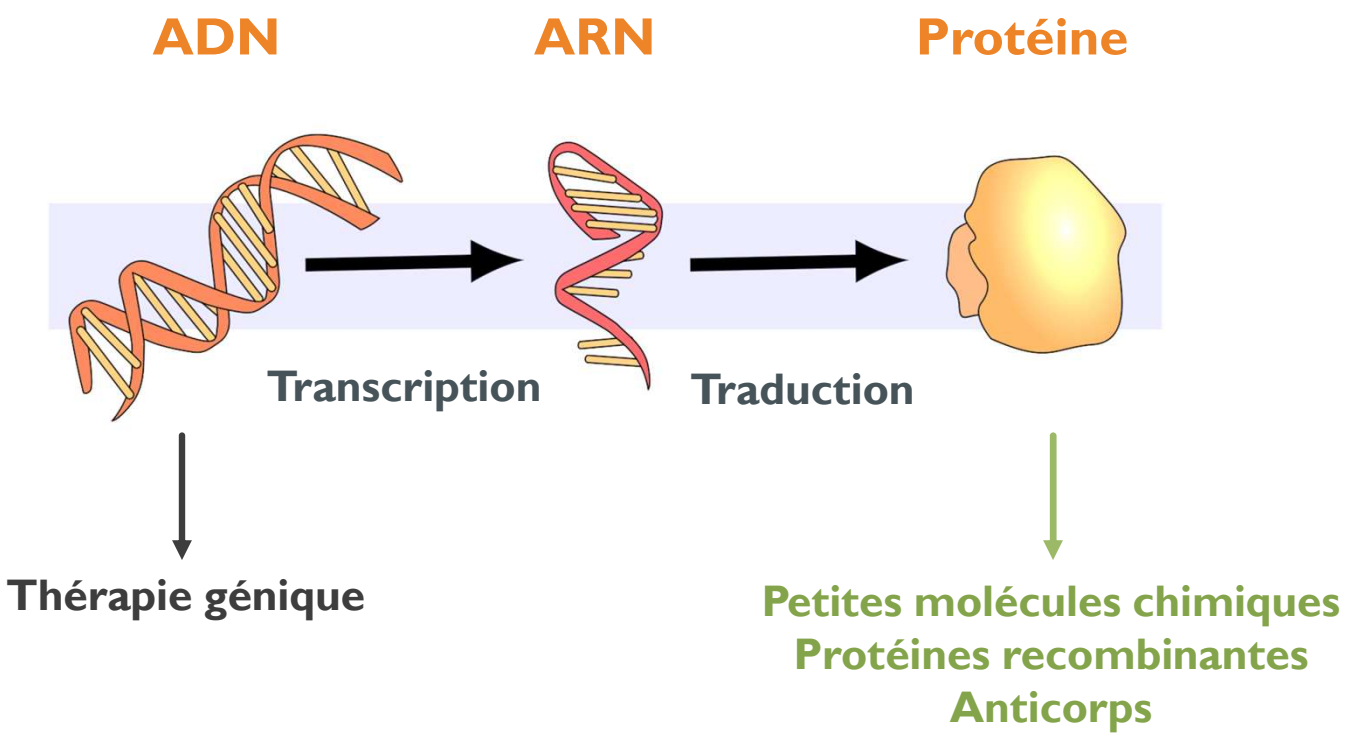
The views and statements expressed on this document and made during the presentation are those of the presenter, and do not represent those of the BioNTech Group.

INTRODUCTION

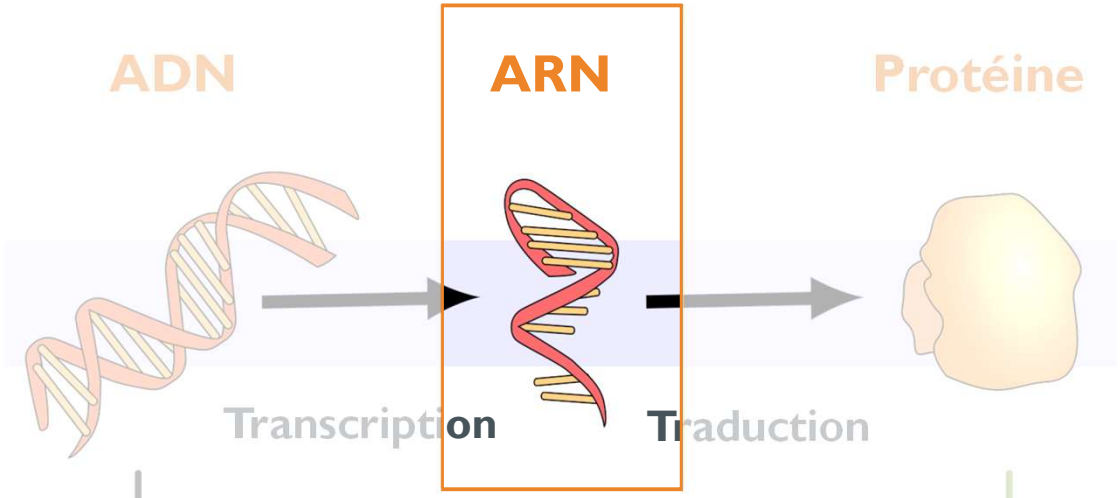
LE DOGME CENTRAL DE LA BIOLOGIE MOLÉCULAIRE



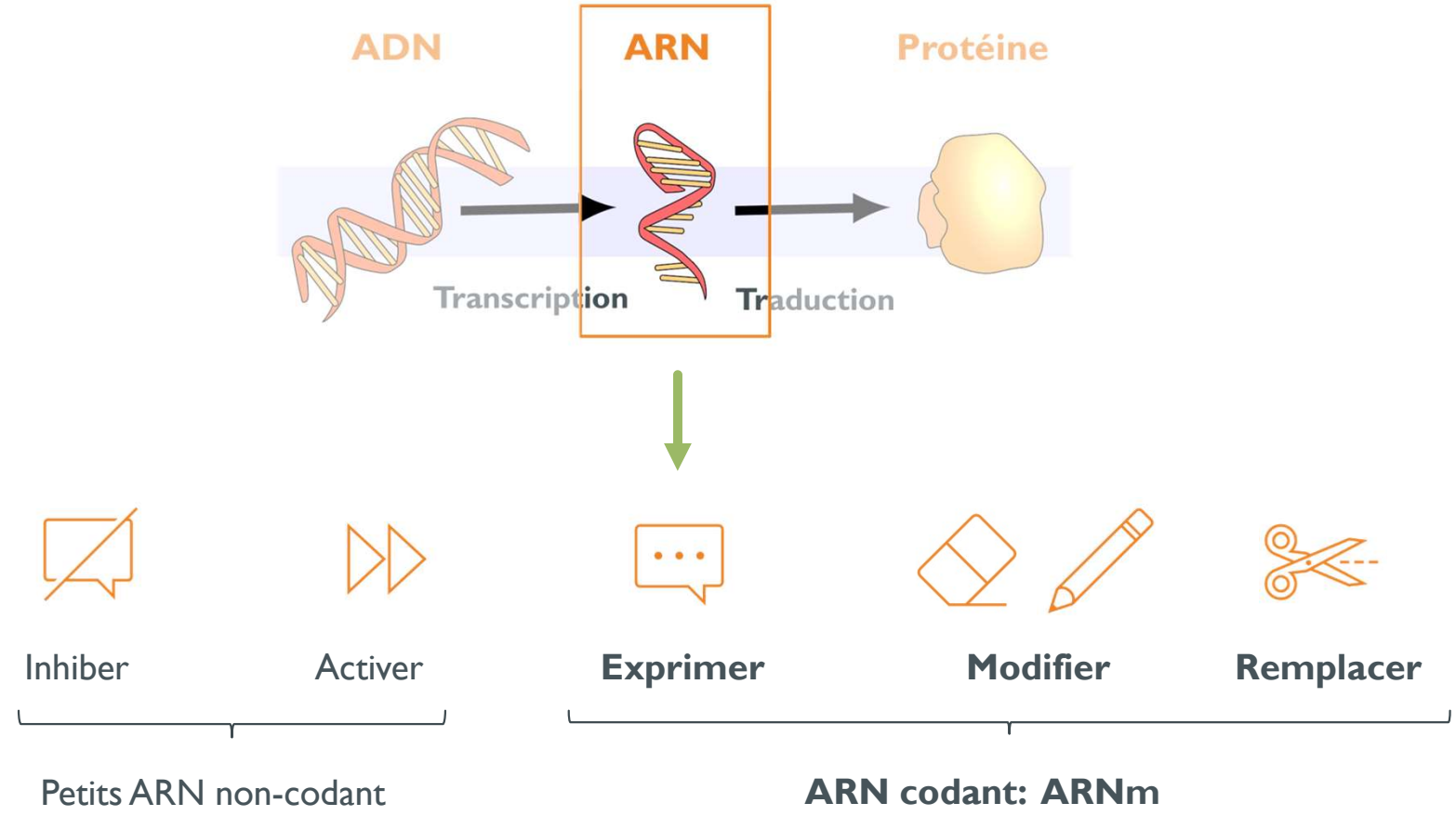
LES APPROCHES THÉRAPEUTIQUES



LES APPROCHES THÉRAPEUTIQUES

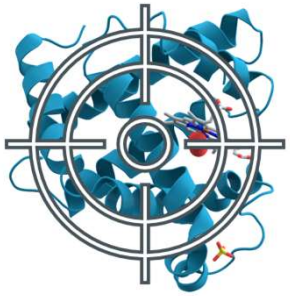


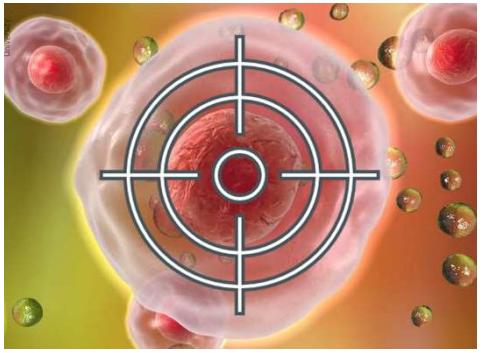
LES APPROCHES THÉRAPEUTIQUES BASÉES SUR L´ARN



LE BUT…

Une médecine de précision…





Et une médecine personnalisée !

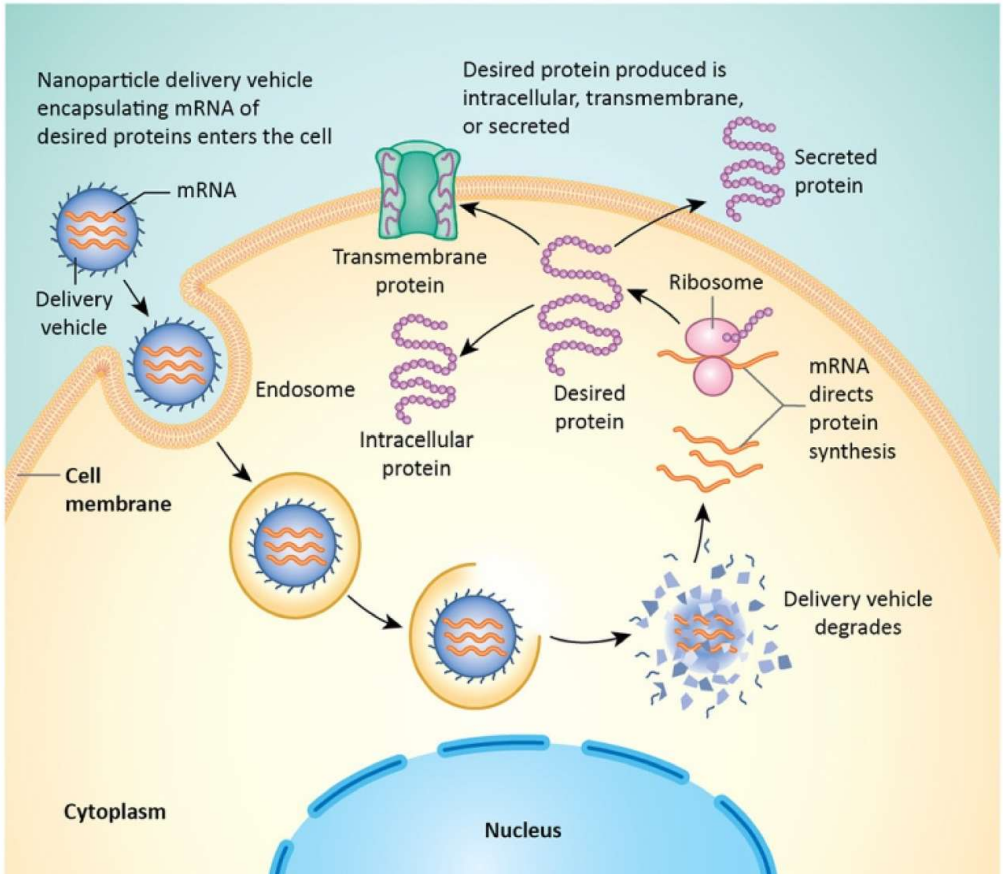
LA PLATEFORME ARNm

L´ARNm COMME MOLÉCULE THÉRAPEUTIQUE: MÉCANISME D´ACTION

- Traduction de l´information génétique contenue dans l´ARNm en une protéine thérapeutiquement active:

  ADN → ARNm → protéine

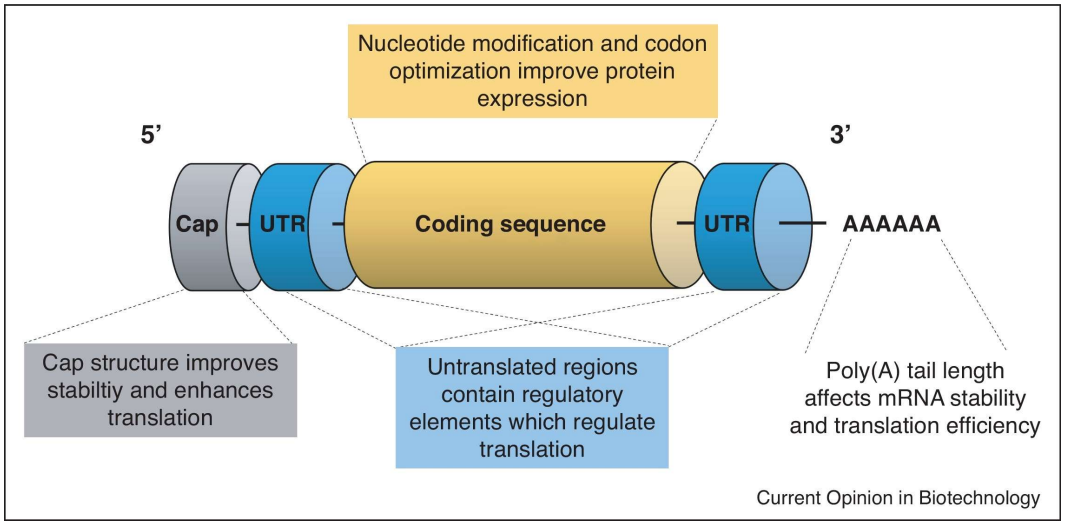
- La séquence nucléotidique de l'ARNm détermine la séquence d'acides aminés de la protéine
- Protéine exprimée: thérapeutique ou antigénique
- Différentes localisations:
  - Intracellulaire
  - Secrétée
  - Transmembranaire
  - Peptide-CMH I /II



Bhat et al., 2021

COMPOSITION D´UN ARNm SYNTHETIQUE

- **Similaire à un ARNm naturel**
- Contient différents éléments:
  - La coiffe: 5′ cap 7-methylguanosine (m^7G)
  - Des régions non codantes: 5´UTR et 3´UTR
  - La séquence codante pour le gène d´intérêt
  - La queue poly(A)
- Séquence et structure optimisées afin de:
  - ↗ Efficacité de traduction de l´ARNm
  - ↗ Stabilité (Demi-vie)
  - Immunogénicité +/-



Kon et al., 2021

Bloom et al., 2021

DIFFÉRENTS FORMATS D´ARNm THERAPEUTIQUES

- ARNm non modifié

Immunogène → Immunothérapie

- ARNm modifié (nucléosides)

Non-immunogène → Production de protéines thérapeutiques

- Self-amplifying mRNA (saRNA)
  (self-replicating mRNA, replicon)

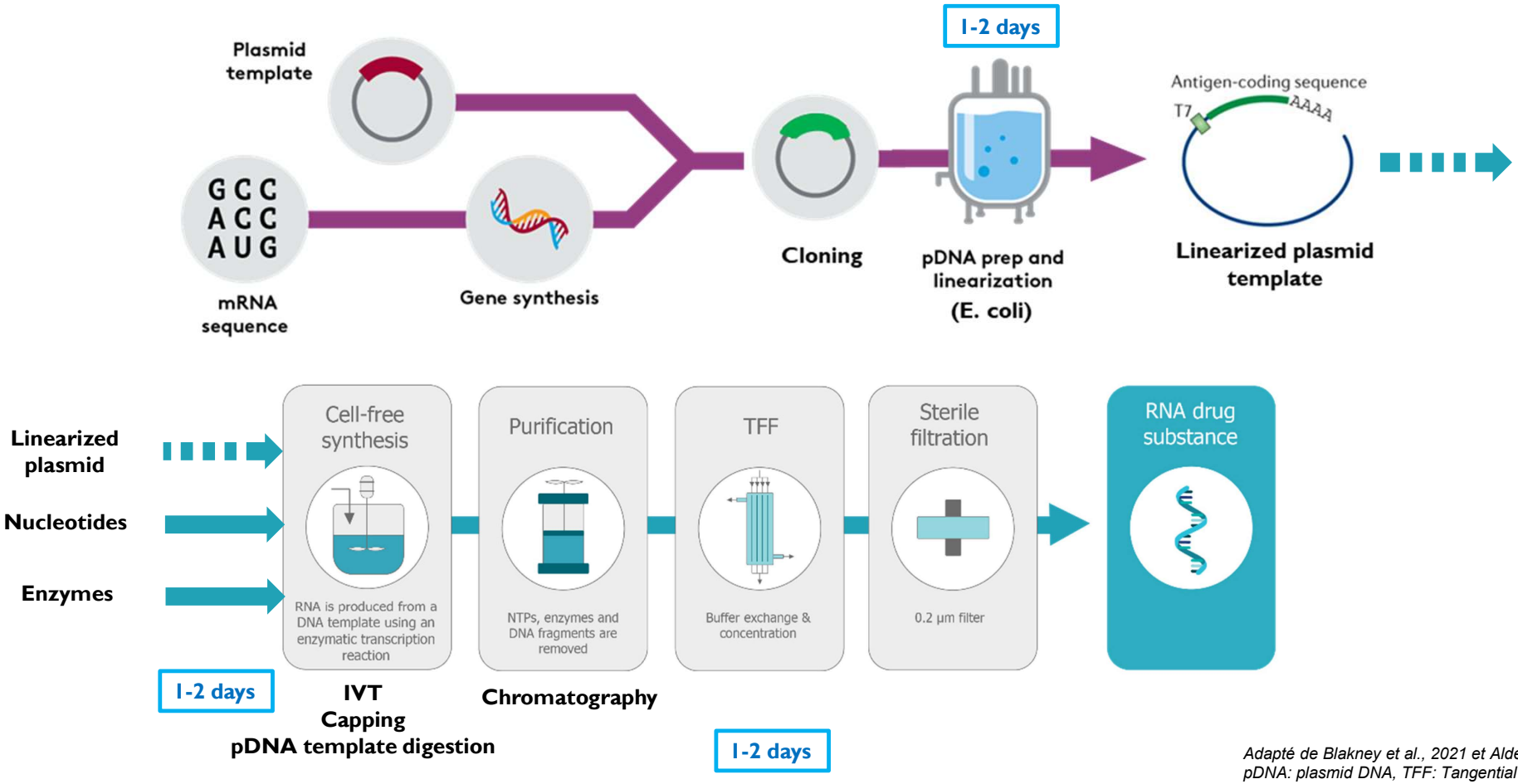
Forte expression à dose réduite + Immunogène → Immunothérapie & Vaccination

Adapted from BioNTech website; GOI: gene of Interest; SGP: Subgenomic Promoter

FABRICATION D´UN ARNm THERAPEUTIQUE

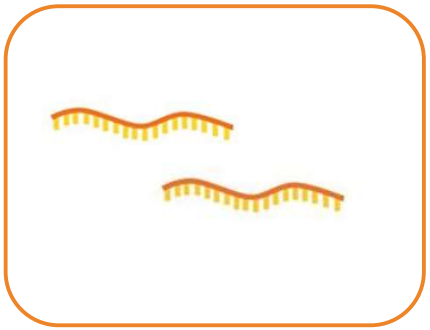
- Synthèse d´ARNm par transcription enzymatique (in vitro transcribed RNA, IVT) établie dans les années 90s
- Procédé de fabrication acellulaire:
  - évite les problèmes de complexité de fabrication et de sécurité associés à la production en culture cellulaire de protéines recombinantes ou de vecteurs viraux.
  - fabrication facile et rapide: avantage de la plateforme ARNm notamment pour les vaccins et l´immunothérapie personnalisée
- Des kits commerciaux de transcription in vitro existent depuis plusieurs années (~mg)
- Plusieurs CDMO (Contract Development and Manufacturing Organization) proposent de l´ARNm de grade pharmaceutique (GMP): TriLink, Aldevron, Eurogentec, Lonza, ThermoFisher…

FABRICATION D´UN ARNm THERAPEUTIQUE

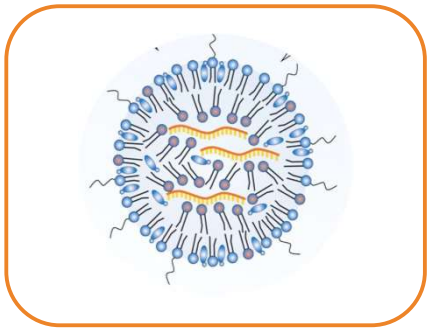


Adapté de Blakney et al., 2021 et Aldevron website
pDNA: plasmid DNA, TFF: Tangential Flow FIltration

L´ARNm THERAPEUTIQUE

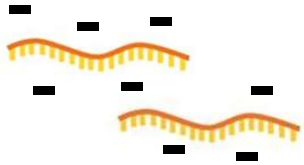


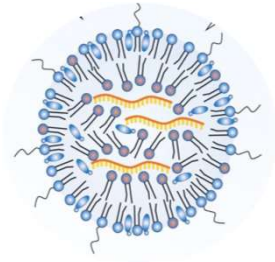
ARNm
=
Substance active (DS)



ARNm formulé
=
Produit fini (DP)

POURQUOI L´ARNm DOIT-IL ETRE FORMULÉ ?





ARNm nu

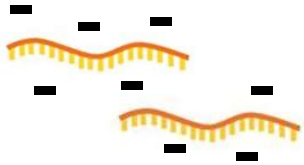
- Macromolécule
- Chargée négativement et hydrophile
- Facilement dégradée par les ribonucléases (RNAse) dans le milieu extracellulaire
- Ne pénètre pas librement la membrane cellulaire

ARNm formulé

- ARN protégé de la dégradation
- Meilleure interaction et internalisation dans les cellules
- Ciblage sélectif de l´organe/cellule d'intérêt
- Facilite la sortie de l´endosome
- Empêche les interactions non spécifiques

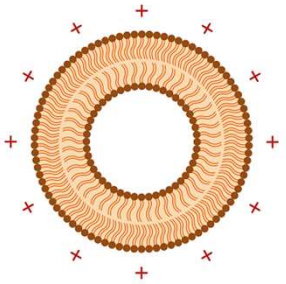
PRINCIPE GÉNÉRAL DE LA VECTORISATION DE L´ARNm

ARN
chargé négativement

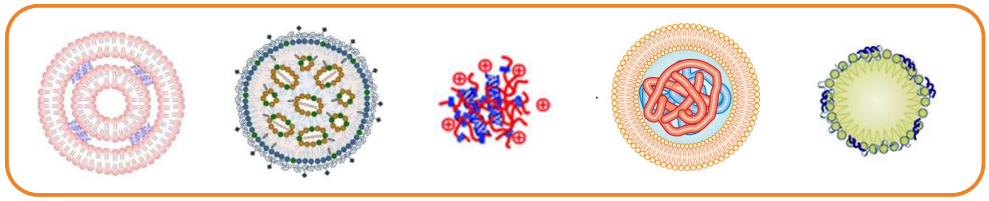


Interactions électrostatiques

Vecteur/véhicule
chargé positivement



- Systèmes lipidiques
- Systèmes polymériques
- Systèmes hydrides



Nanoparticules → Taille: 30-500 nm (moyenne 100 nm)
Surface chargée: positive, négative ou neutre

LES MÉDICAMENTS À BASE DE LNP APPROUVÉS

Onpattro®
2018

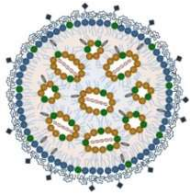
siRNA dirigé contre la transthyrétine (TTR) (patisiran) pour le traitement de l´amyloïdose hATTR

Comirnaty®
Dec 2020

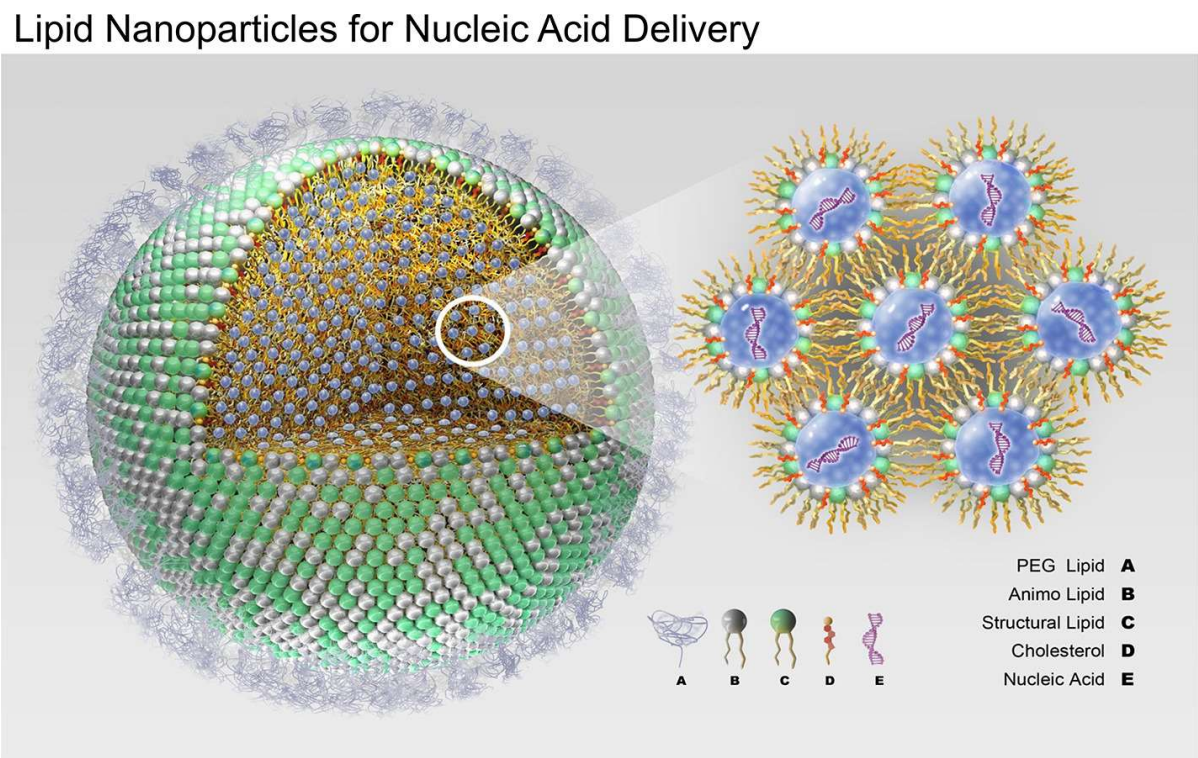
Spikevax®
Jan 2021

ARNm codant pour la protéine spike du SARS-CoV2

Formulation sous forme de LNP (Lipid NanoParticle)



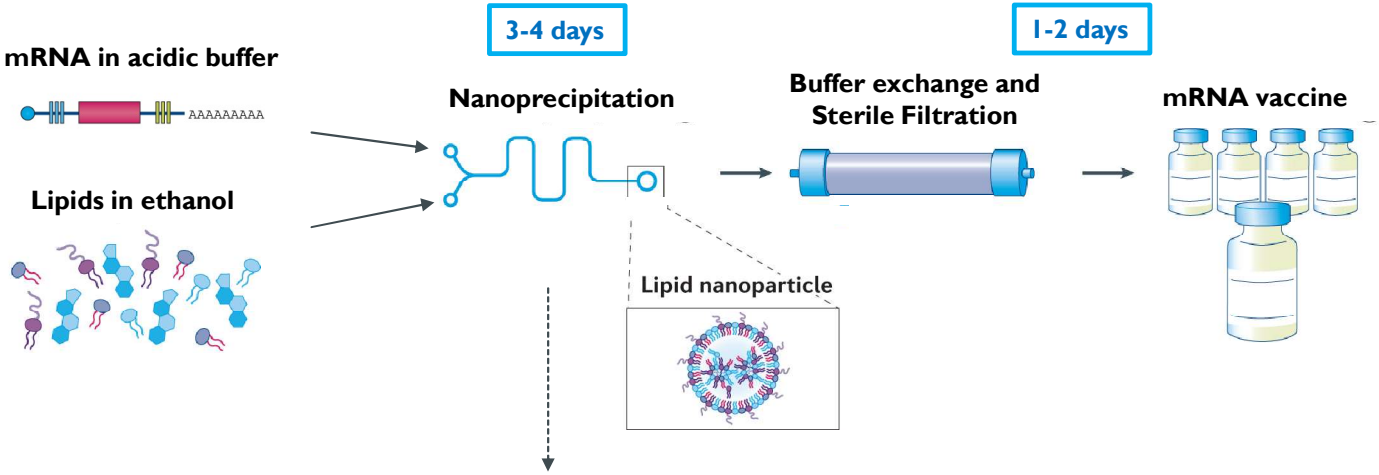
LES NANO-VECTEURS „LNP“



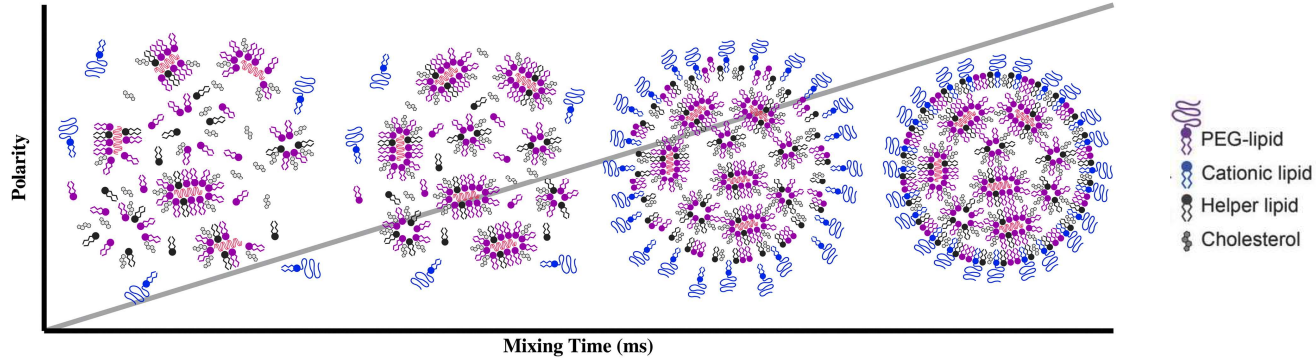
Samaridou et al., 2020

- **Lipide cationique ionisable:** complexation avec l´acide nucléique, fusion membranaire
- **Lipide structurel « helper »:** formation de bicouche
- **Cholestérol:** intégrité et stabilité des membranes, sortie de l´endosome
- **Lipide PEGylé:** surface hydrophile, encombrement stérique

FABRICATION DES LNP



Adapté de Chaudhary et al., 2021



Kulkarny et al., 2018

LES APPLICATIONS THÉRAPEUTIQUES DE L´ARNm

2 MÉDICAMENTS À BASE D´ARNm APPROUVÉS

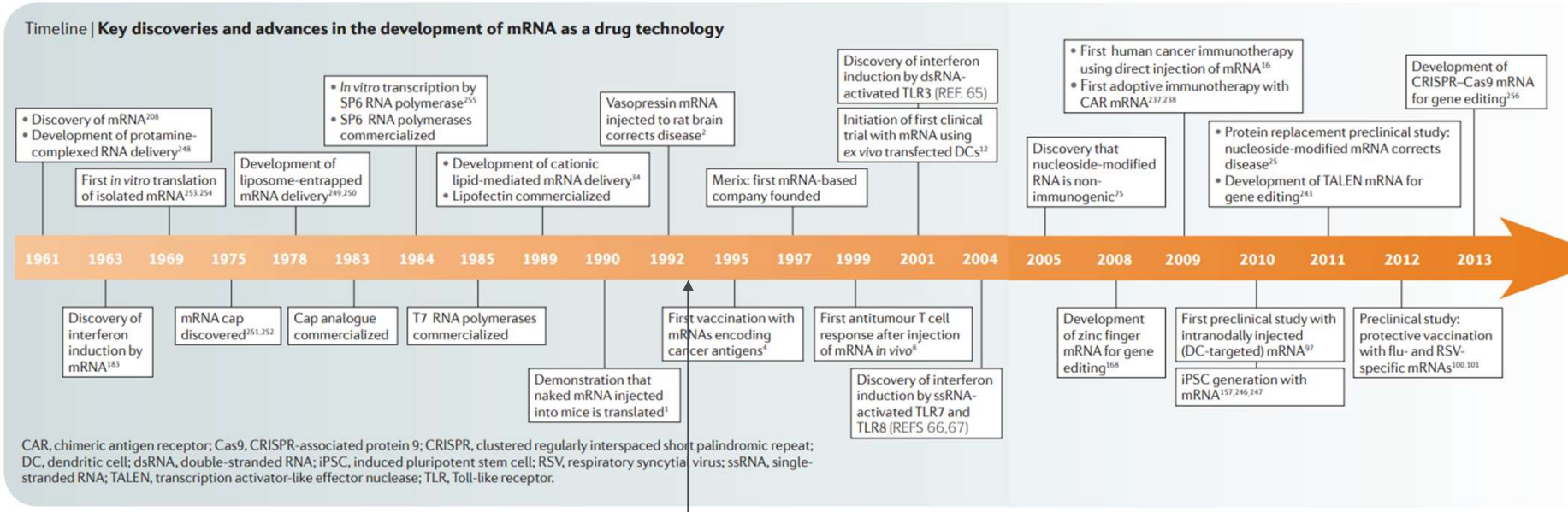
Comirnaty®
Dec 2020

Spikevax®
Jan 2021

ARNm codant pour la protéine spike du SARS-CoV2
Vaccins – Intramusculaire

+ versions adaptées aux variants Omicrons

HISTORIQUE DU DEVELOPEMENT DE L´ARNm THERAPEUTIQUE

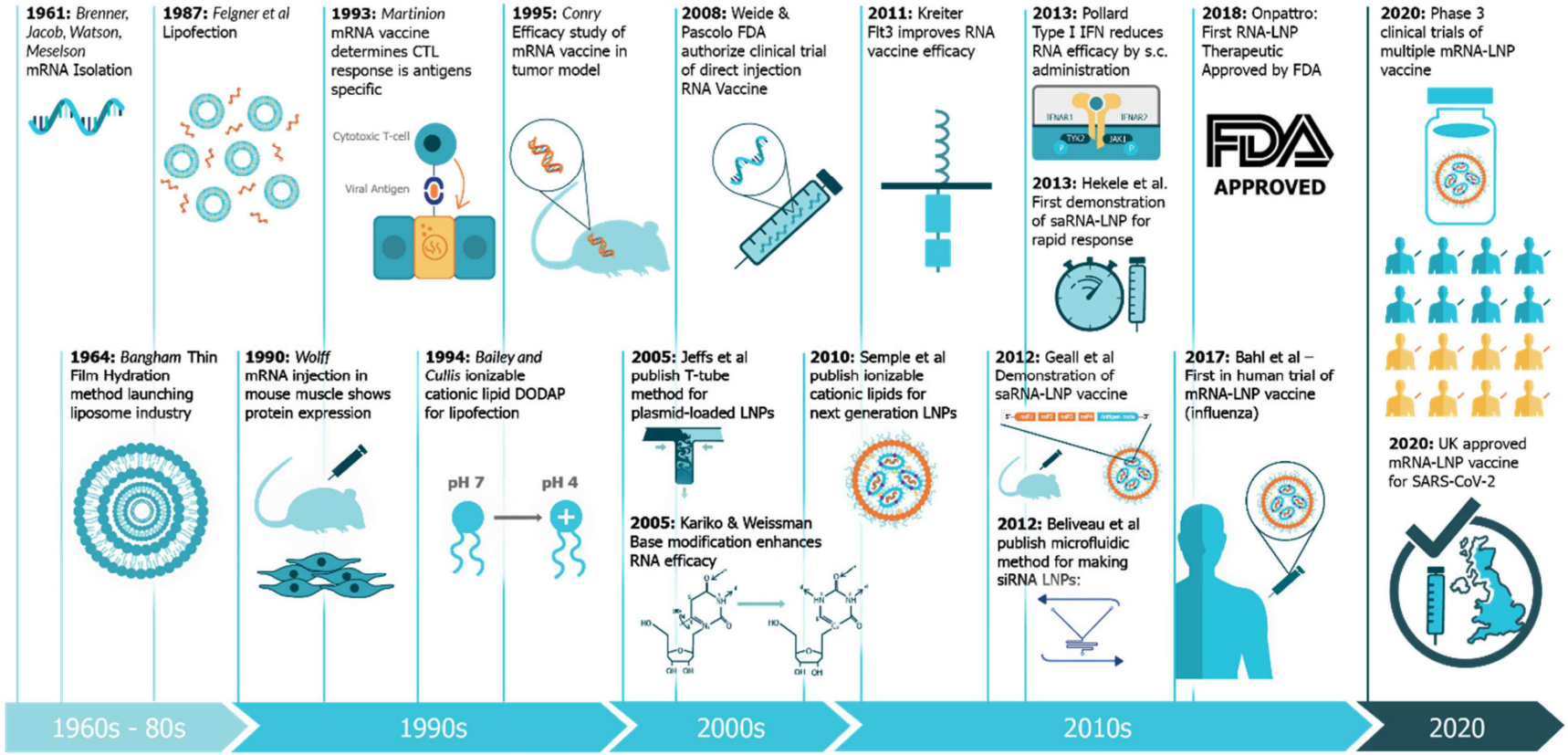


Sahin et al., 2014

1993
Martinon et al. (INSERM)
First mRNA vaccine
against a virus (influenza)

+ see Dolgin E., 2021 for the complete history

HISTORIQUE DES INNOVATIONS AYANT CONTRIBUÉES AU DÉVELOPPEMENT DES VACCINS ARNm

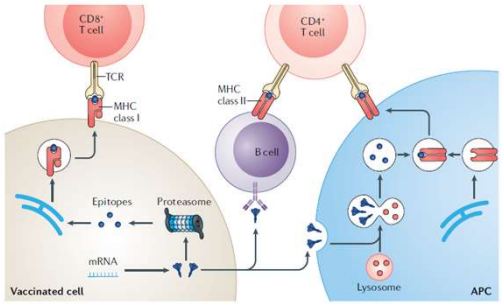


Blakney et al., 2021

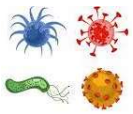
APERÇU DES APPLICATIONS THÉRAPEUTIQUES DE L´ARNm

mRNA vaccines

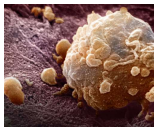
Stimulation of the immune system



- mRNA design: immunogenic
- In Vivo Applications (i.v., i.m.)
- Therapeutic areas:



Infectious diseases

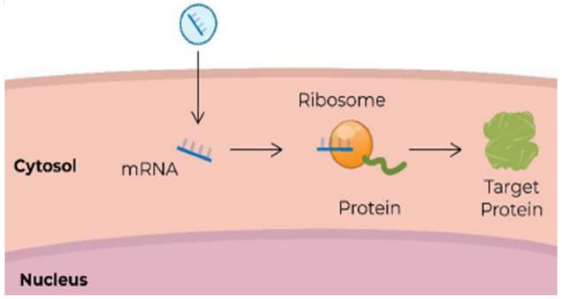


Oncology

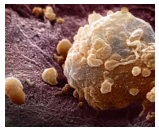
Allergy

mRNA therapeutics

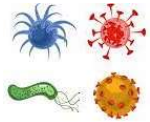
in situ production of proteins, antibodies, nucleases,…



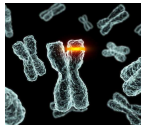
- mRNA design: non-immunogenic
- In Vivo or Ex Vivo Applications
- Therapeutic areas:



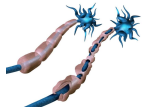
Oncology



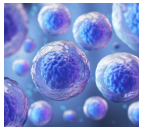
Infectious diseases



Genetic disorders



Autoimmunity



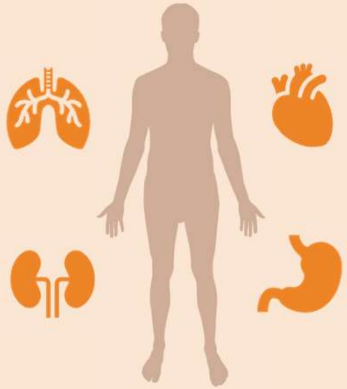
Regenerative medicine

APERÇU DES APPLICATIONS THÉRAPEUTIQUES DE L´ARNm

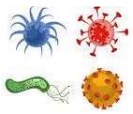
mRNA vaccines

- **RNA Formulation**

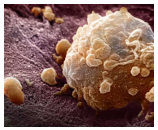
Delivery vehicle properties
Targeting
Route of administration



- Therapeutic areas:



Infectious diseases

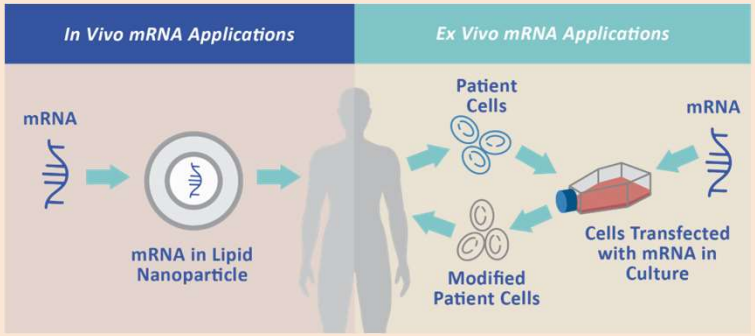


Oncology

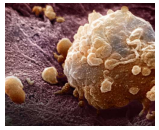
Allergy

mRNA therapeutics

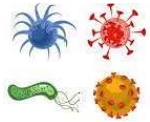
- **In Vivo or Ex Vivo Applications**



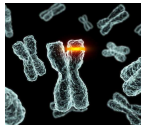
- Therapeutic areas:



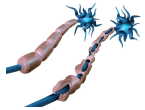
Oncology



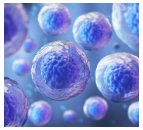
Infectious diseases



Genetic disorders

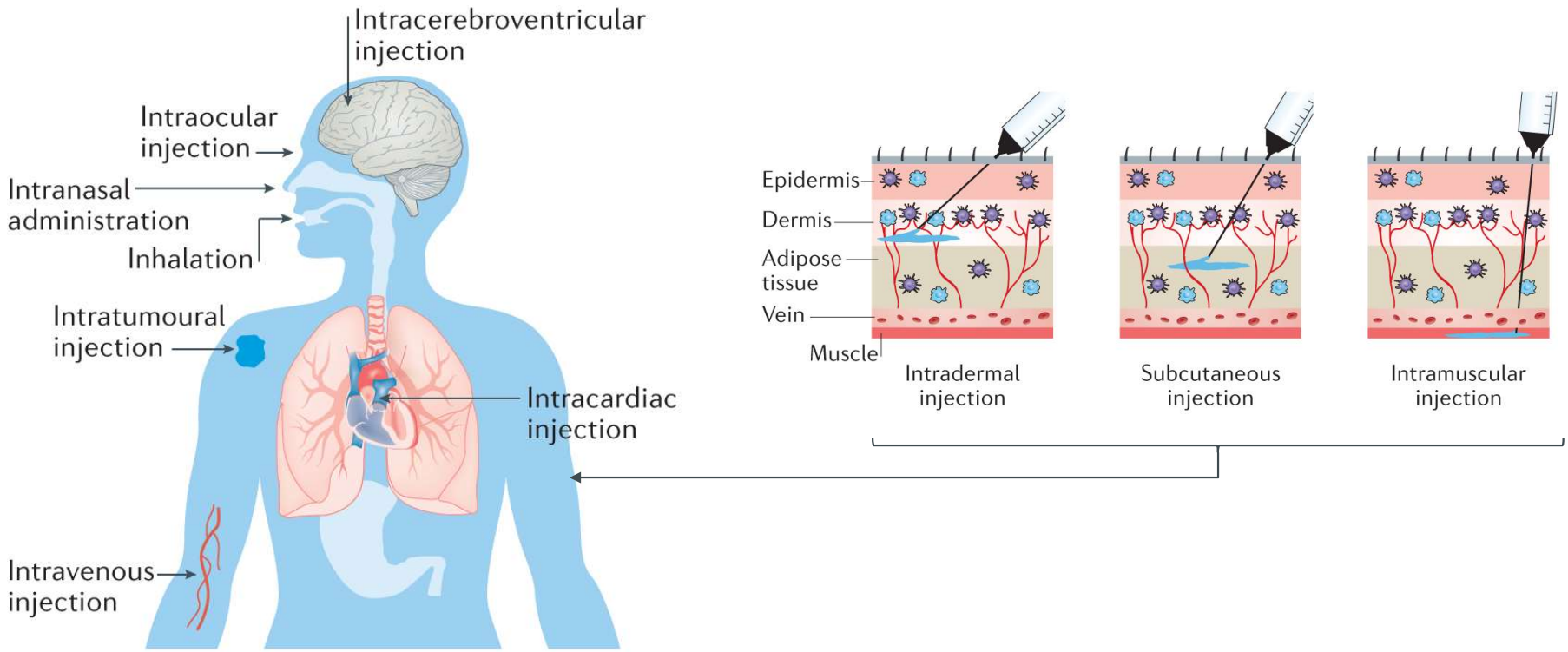


Autoimmunity



Regenerative medicine

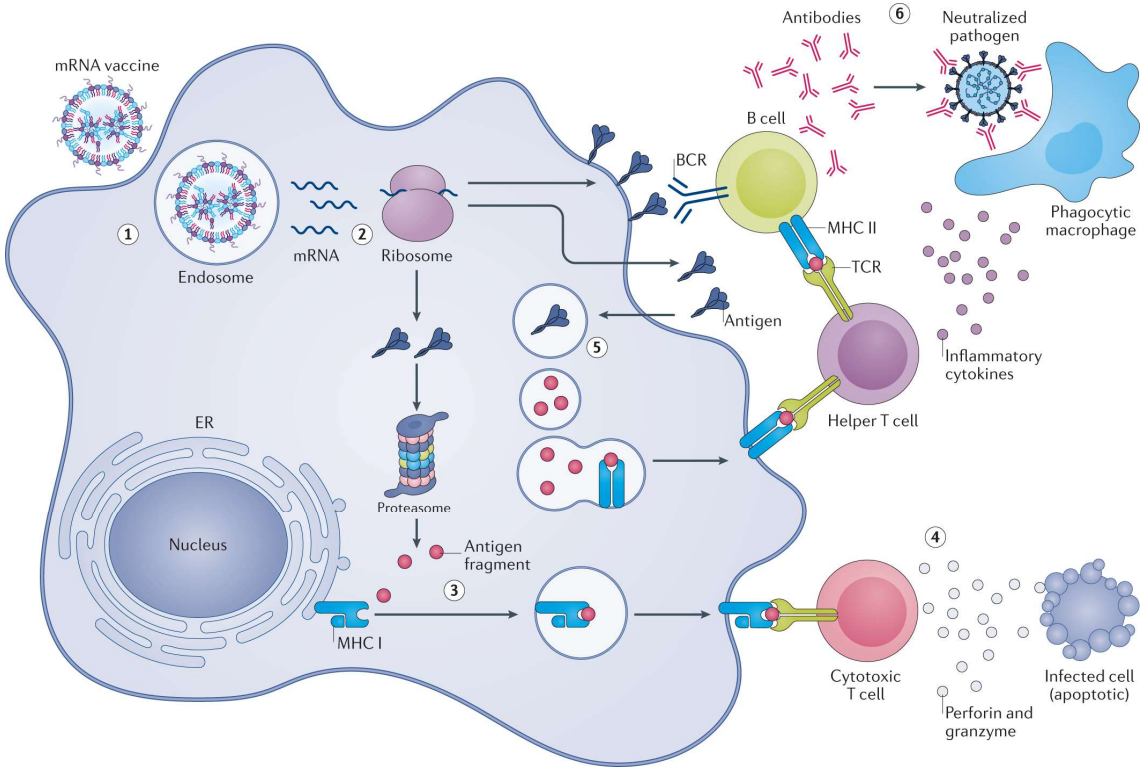
DIFFÉRENTES VOIES D´ADMINISTRATION



Adapté de Hou et al., 2021

PREVENTION ET TRAITEMENT DES MALADIES INFECTIEUSES

- **Immunisation active**: vaccins à ARNm
  - Le vaccin ARNm-LNP induit une réponse inflammatoire transitoire recrutant monocytes, neutrophiles et cellules dendritiques au site d´injection (i.m.).
  - Induit une réponse immunitaire humorale et cellulaire.
  - Expression d´un ou de plusieurs antigènes (ex. vaccin Moderna mRNA-1647 contre CMV contient 6 ARNm, en Phase 3)
- **Immunisation passive**: anticorps neutralisant
  - Injection i.v., synthèse et sécrétion par le foie
  - Par ex. sécrétion d´anticorps contre Chikungunya (Moderna mRNA-1944, Phase 1)



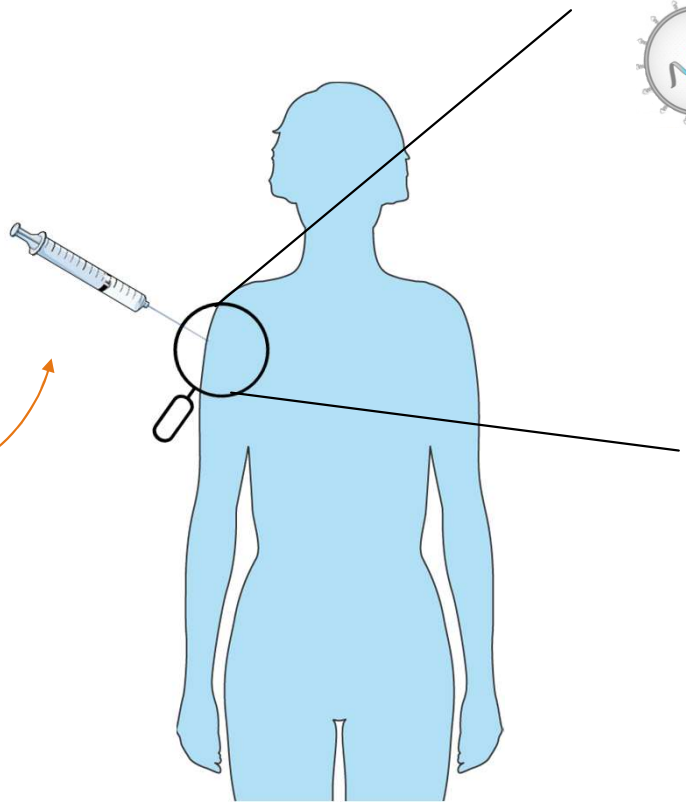
Chaudhary et al., 2021

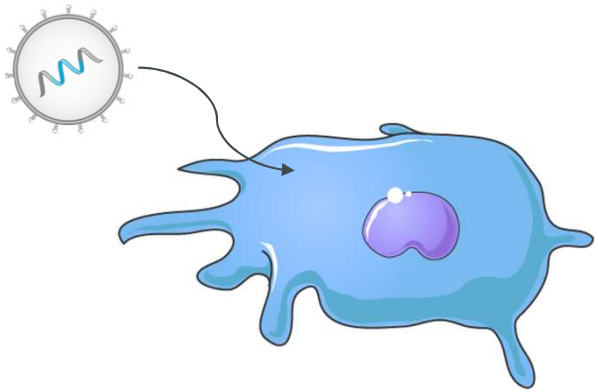
MÉCANISME D´ACTION DES VACCINS ARNm PROPHYLACTIQUES

ARNm codant pour un antigène

Particule lipidique contenant l'ARNm

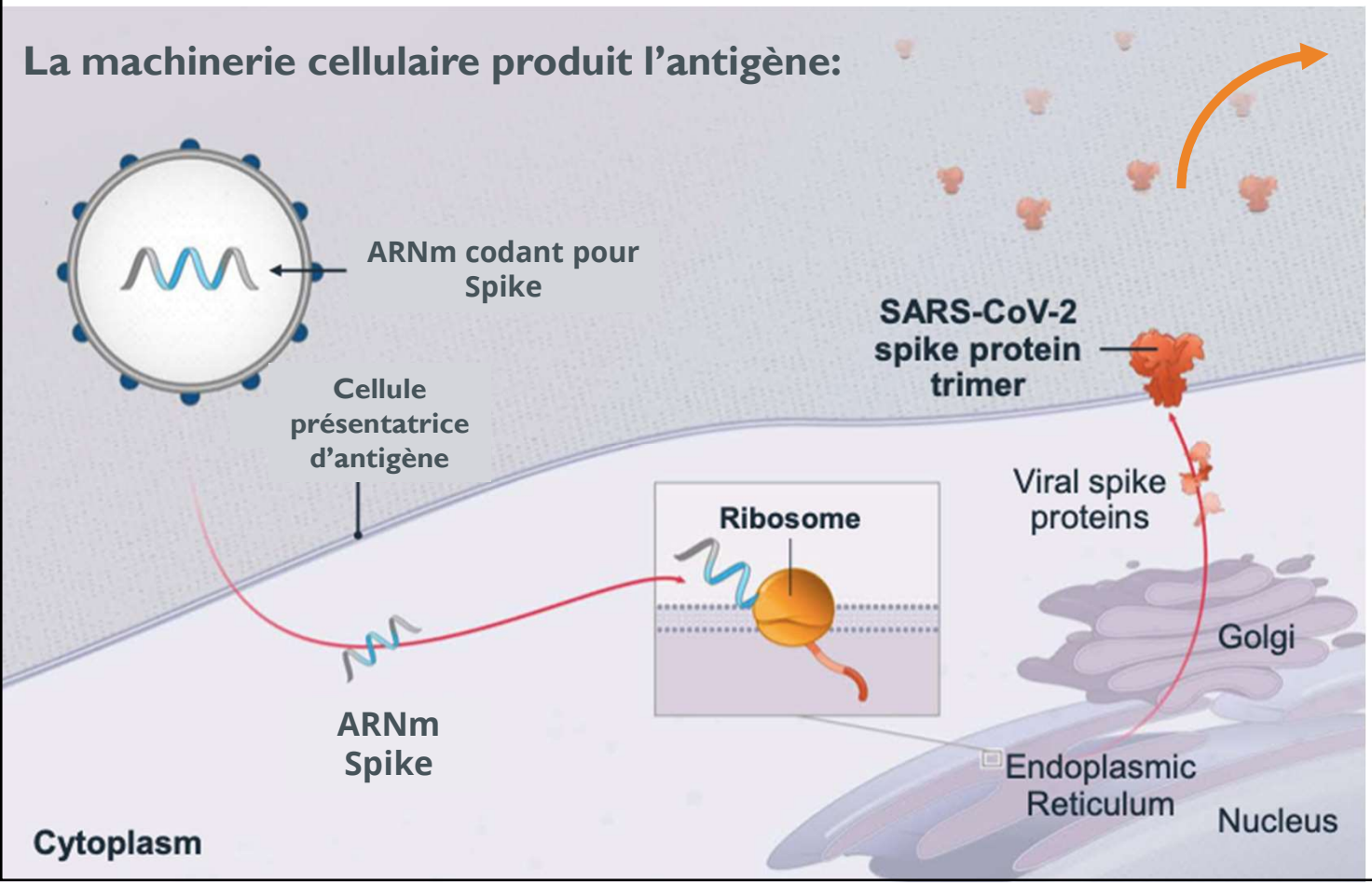
VACCIN mRNA





Cellule présentatrice d'antigène

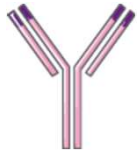
MÉCANISME D´ACTION DES VACCINS ARNm PROPHYLACTIQUES



Réponse immunitaire

Humorale

Anticorps neutralisant le virus



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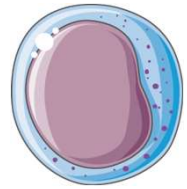
Cellulaire

Lymphocyte T8 détruisant les cellules infectées



Adapté de Moderna, Advisory Commitee presentation

MALADIES INFECTIEUSES, QUELQUES EXEMPLES…

Table 1. Clinical Trials of mRNA vaccines against infectious diseases.

Infectious Disease	Biological Active/Encoding Sequence	Strategy/Delivery System	Administration Route	NCT Number/Phase
Rabies	CV7201 mRNA/Rabies virus glycoprotein (RABV-G)	In vivo/Polypeptide system	i.d. or i.m.	NCT02241135/Phase I
	CV7202 mRNA/Rabies virus glycoprotein (RABV-G)	In vivo/Lipid nanosystem	i.m.	NCT03713086/Phase I
Zika Virus	mRNA-1893/Structural proteins of Zika virus	In vivo/Lipid nanosystem		NCT04064905/Phase I
	mRNA-1325/Zika virus antigen	In vivo/Lipid nanosystem	i.d.	NCT03014089/Phase I
Cytomegalovirus (CMV)	mRNA-1647 and mRNA-1443/Pentamer complex and full-length membrane-bound glycoprotein B (gB) and pp65 T cell antigen of CMV	In vivo/Lipid nanosystem	i.d.	NCT03382405/Phase I
	AVX601/Alphavirus replicon vaccine expressing gB, pp65 and IE1 proteins of CMV	In vivo/Viral vector	i.m. or s.c.	NCT00439803/Phase I
hMPV and PIV3	mRNA-1653: Fusion proteins of hMPV and PIV3	In vivo/Lipid nanosystems	i.d.	NCT03392389/Phase I
Tuberculosis	GSK 692,342/Immunogenic fusion protein (M72) derived from Mycobacterium tuberculosis	In vivo/Lipid nanosytems	i.m.	NCT01669096/Phase II
HIV-1	HIV-1 Gag and Nef	Ex vivo/mRNA transfected autologous DCs	i.d.	NCT00833781/Phase I
	iHIVARNA: TriMix and HTI/APC activation molecules (CD40L+CD70+caTLR4) and HIV immunogen sequences (Gag, Pol, Vif and Nef)	In vivo/Naked mRNA	Inguinal intranodal	NCT02413645 and NCT02888756/Phase I and Phase II
	AVX101/Alphavirus replicon vaccine expressing HIV Gag antigen	In vivo/Viral vector	s.c.	NCT00097838 and NCT00063778/Phase I
Influenza	VAL-506440/H10N8 antigen	In vivo/Lipid nanosystems	i.d. or i.m.	NCT03076385/Phase I
	VAL-339851/H7N9 antigen	In vivo/Lipid nanosystems	i.d. or i.m.	NCT03345043/Phase I
	AVX502/Alphavirus replicon vaccine expressing Influenza A/Wyoming/03/2003 Hemagglutinin	In vivo/Viral vector	i.m. or s.c.	NCT00440362 and NCT00706732/Phase I/II

DCs, dendritic cells; i.d., intradermal; i.m., intramuscular; s.c., subcutaneous; i.v., intravenous; hMPV, human metapneumovirus; PIV3, human parainfluenza virus type 3; HIV-1, human immunodeficiency virus type 1; APC, antigen-presenting cells.

Gómez-Aguado et al., 2020

Table 1 | **Representative clinical trials of lipid nanoparticle–mRNA vaccines against infections and cancer**

Name	Disease	Encoded antigen	Administration route	ClinicalTrials.gov identifier	Phase
Infections					
mRNA-1273	SARS-CoV-2	Spike	i.m.	NCT04470427	III (EUA and CMA)
BNT162b2	SARS-CoV-2	Spike	i.m.	NCT04368728	III (EUA and CMA)
CVnCoV	SARS-CoV-2	Spike	i.m.	NCT04652102	III
LNP-nCoVsaRNA	SARS-CoV-2	Spike	i.m.	ISRCTN17072692	I
ARCT-021	SARS-CoV-2	Spike	i.m.	NCT04728347	II
ARCoV	SARS-CoV-2	Receptor-binding domain	i.m.	ChiCTR2000034112	I
mRNA-1440	Influenza H10N8	Haemagglutinin	i.m.	NCT03076385	I
mRNA-1851	Influenza H7N9	Haemagglutinin	i.m.	NCT03345043	I
mRNA-1893	Zika virus	Pre-membrane and envelope glycoproteins	i.m.	NCT04064905	I
mRNA-1345	Respiratory syncytial virus	F glycoprotein	i.m.	NCT04528719	I
mRNA-1653	Metapneumovirus and parainfluenza virus type 3 (MPV/PIV3)	MPV and PIV3 F glycoproteins	i.m.	NCT03392389	I
mRNA-1647	Cytomegalovirus	Pentameric complex and B glycoprotein	i.m.	NCT04232280	II
mRNA-1388	Chikungunya virus	Chikungunya virus antigens	i.m.	NCT03325075	I
CV7202	Rabies virus	G glycoprotein	i.m.	NCT03713086	I

Table 2 | **Representative clinical trials of lipid nanoparticle–mRNA therapeutics against infections, cancer and genetic disorders**

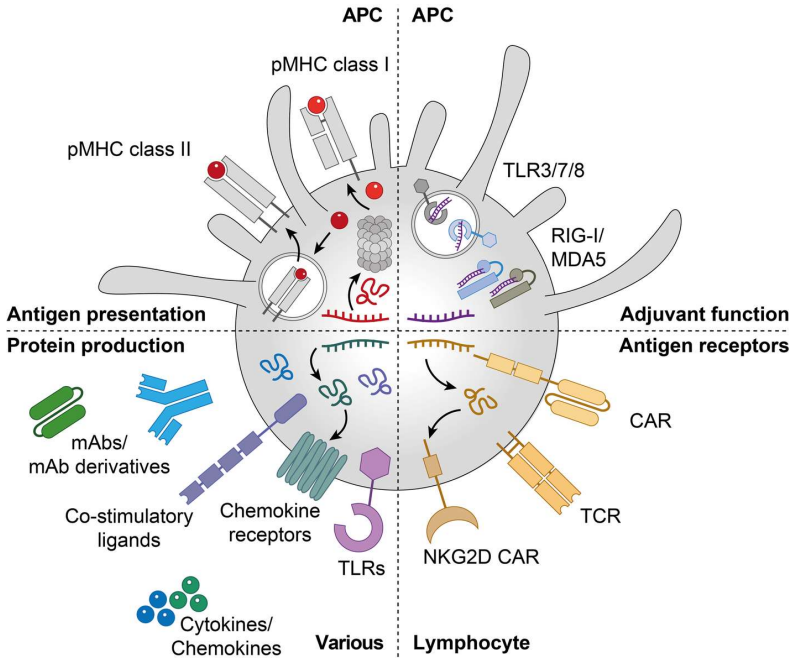
Name	Disease	Encoded protein	Administration route	ClinicalTrials.gov identifier	Phase
Infections					
mRNA-1944	Chikungunya virus	Antibody against chikungunya virus	i.v.	NCT03829384	I

Hou et al., 2020

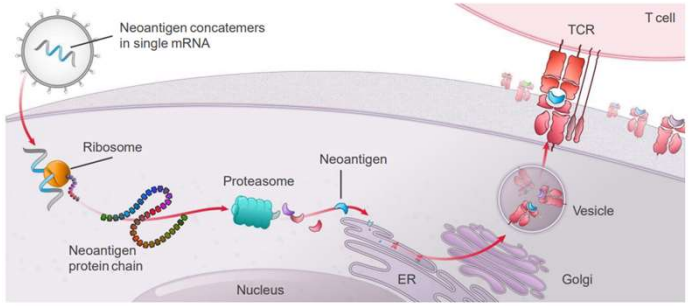
ONCOLOGIE

Approches immuno-oncologiques :

- **Immunisation active**: vaccin à ARNm
  - Expression d'un ou de plusieurs antigènes tumoraux (34 pour Moderna, 20 pour BioNTech).
  - Effets adjuvant intrinsèque de l'ARNm.
  - Ex-vivo ou in-vivo (injection i.v., cible les cellules dendritiques de la rate).
  - Médecine personnalisée
- **Immunisation passive**:
  - Expression d'anticorps, mono ou bispécifiques (Van Hoecke, 2019)
  - Injection i.v., synthèse et sécrétion par le foie
- **Immunomodulation** du microenvironnement tumoral:
  - injection intratumorale d´ARNm codant pour des cytokines
- **Thérapie immuno-cellulaire CAR-T cell**:
  - ARNm codant pour un CAR (Chimeric Antigen Receptor)
  - Ex vivo ou in vivo

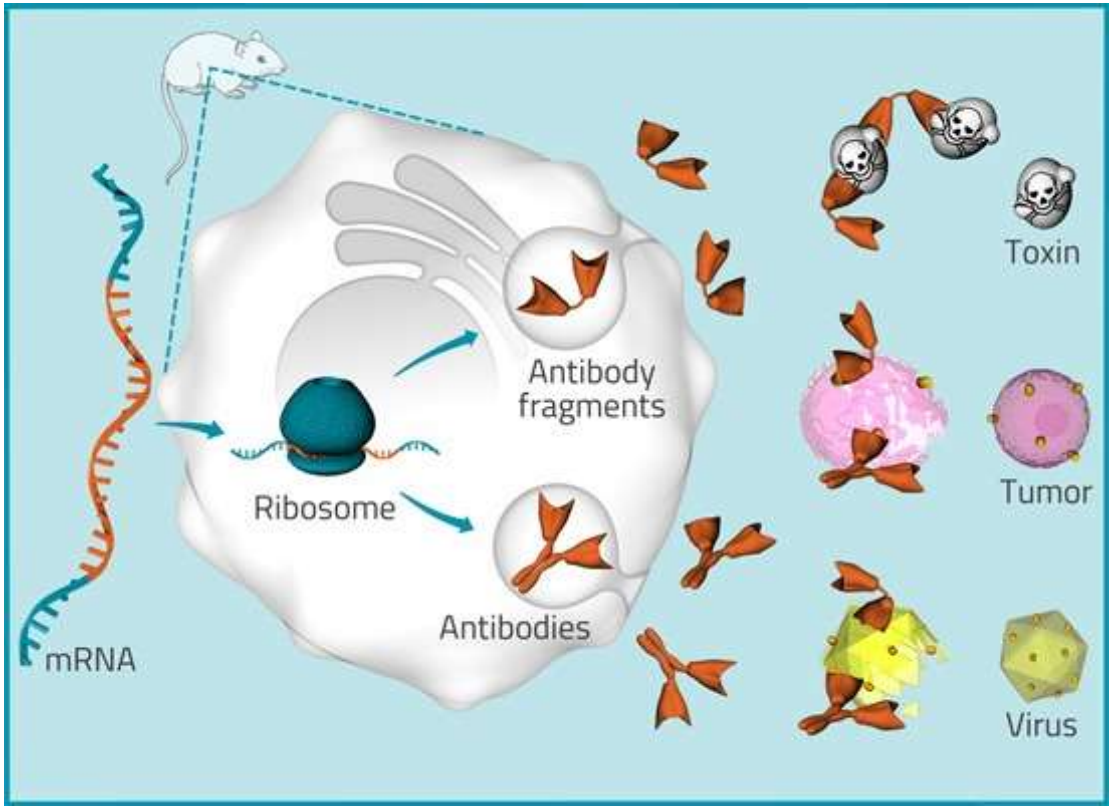


Beck et al., 2021



Moderna Corporate presentation 2021

EXEMPLE D' IMMUNISATION PASSIVE



Thran et al, mRNA mediates passive vaccination against infectious agents, toxins, and tumors; EMBO Mol Med (2017)9:1434-1447

ONCOLOGIE, QUELQUES EXEMPLES…

Table 2. Clinical trials of mRNA for cancer immunotherapy.

Type of Cancer	Biological Active/Encoding Sequence	Strategy/Delivery System	Administration Route	NCT Number/Phase
Non-small-cell lung carcinoma (NSCLC)	CV9201/five mRNAs encoding antigens which are overexpressed or exclusively expressed in NSCLC cells	In vivo/Polypeptide system	i.d.	NCT00923312/Phase I/II
	CV9202/six mRNAs encoding antigens which are overexpressed in NSCLC compared to healthy tissue	In vivo/Polypeptide system	i.d.	NCT01915524/Phase I
Metastatic NSCLC	BI 1,361,849/NSCLC-associated antigens (NY-ESO-1, MAGE-C1, MAGE-C2, 5T4, and MUC-1)	In vivo/Polypeptide system	i.d.	NCT03164772/Phase I and II
Esophageal Cancer and NSCLC	Personalized mRNA Tumor Vaccine/Neoantigen (tumor associated specific antigens)	In vivo/-	s.c.	NCT03908671/NotA
Malignant Melanoma	mRNA coding for melanoma associated antigens	In vivo/Naked mRNA	s.c.	NCT00204516/Phase I/II
	mRNA coding melanoma associated antigens (Melan-A, Mage-A1, Mage-A3, Survivin, GP100 and Tyrosinase)	In vivo/Polypeptide system	i.d.	NCT00204607/Phase I/II
	mRNA coding the unique spectrum of tumor antigens in each patient	Ex vivo/mRNA transfected DCs	i.d. or intranodal	NCT01278940/Phase I/II
Malignant Melanoma III and IV	TriMix-DC encoding melamona tumor-associated antigens (MAGE-A3, MAGE-C2, tyrosinase and gp100)	Ex vivo/autologous TriMix-DC	i.v.	NCT01302496/Phase II
Melanoma	mRNA-4157/personalized cancer vaccine targeting twenty tumor-associated antigens	In vivo/Lipid nanosystems	i.d.	NCT03897881/Phase II
	(RBL001; RBL002)/malignant melanoma associated antigens	In vivo/Naked mRNA	intranodal	NCT01684241/Phase I
	IVAC MUTANOME/poly-neo-epitopic personalized cancer vaccine targeting tumor-associated antigens (with or without initial treatment with RBL001/RBL002)	In vivo/Naked mRNA	intranodal	NCT02035956/Phase I
	RBL001.1; RBL002.2; RBL003.1; RBL004.1/malignant melanoma-associated antigens	In vivo/Lipid nanosystems	i.v.	NCT02410733/Phase I
	mRNA encoding TriMix	Ex vivo/mRNA-transfected autologous DCs	i.d. and i.v.	NCT01066390/Phase I
	mRNA encoding melanoma-associated tumor antigens (gp100 and tyrosinase) and TriMix	Ex vivo/mRNA-transfected autologous DCs	intranodal	NCT01530698/Phase I/II
Melanoma Stage III or IV	mRNA encoding melanoma associated antigens (gp100 and tyrosinase)	Ex vivo/mRNA-transfected DCs	i.v., i.d., intranodal	NCT00243529/Phase I/II
Metastatic Malignant Melanoma	hTERT-, Survivin- and tumor cell derived mRNA + ex vivo T cell expansion	Ex vivo/mRNA-transfected DCs	i.d. and i.v.	NCT00961844/Phase I/II
Uveal Melanoma	mRNA coding tumor associated antigens	Ex vivo/mRNA-transfected DCs	i.d./i.v.	NCT00929019/Phase I/II
Acute Myeloid Leukemia (AML)	mRNA coding for the Wilms' tumor protein (WT1)	Ex vivo/mRNA transfected autologous DCs	i.d.	NCT00834002/Phase I
	AML-specific mRNA	Ex vivo/mRNA transfected autologous DCs	i.d.	NCT00514189/Phase I
	mRNA encoding WT1, PRAME, and CMVpp65	Ex vivo/mRNA transfected autologous DCs	i.d.	NCT01734304/Phase I/II

Gómez-Aguado et al., 2020

ONCOLOGIE, QUELQUES EXEMPLES…

Table 2. *Cont.*

Type of Cancer	Biological Active/Encoding Sequence	Strategy/Delivery System	Administration Route	NCT Number/Phase
Relapsed or Refractory AML	Autologous Anti-CD 123 CAR TCR/4-1BB-expressing T-lymphocytes/anti-CD123 chimeric antigen receptors expressing tandem TCR and 4-1BB (TCR/4-1BB) costimulatory domains	Ex vivo/mRNA transfected autologous CAR T cells	iv	NCT02623582/Early Phase I
Multiple Myeloma	mRNA encoding CT7, MAGE-A3, and WT1	Ex vivo/mRNA-transfected autologous Langerhans-type DCs	i.d.	NCT01995708/Phase I
Prostate Cancer	CV9104/mRNAs encoding PSA, PSCA, PSMA, STEAP1, PAP and Mucin 1 antigens	In vivo/Polypeptide system	i.d.	NCT01817738 and NCT02140138/Phase I/II and Phase II
	mRNA coding tumor associated antigens	Ex vivo/mRNA-transfected DCs	i.d.	NCT01278914/Phase I/II
	mRNA extracted from Primary Prostate Cancer Tissue, combined with mRNA encoding hTERT and Survivin	Ex vivo/mRNA-transfected DCs	i.d.	NCT01197625/Phase I/II
Metastatic Prostate Cancer	mRNA derived from the patient's own tumor	Ex vivo/mRNA-transfected autologous DCs	i.d.	NCT01153113/Phase I/II (withdrawn)
Hormonal Refractory Prostate Cancer	CV9103/mRNAs encoding PSA, PSCA, PSMA and STEAP1 antigens	In vivo/Polypeptide system	i.d.	NCT00831467 (eudract 2008-003967-37) and NCT00906243/Phase I/II
Glioblastoma	mRNA encoding Survivin, hTERT or autologous tumor stem cells derived from tumorspheres	Ex vivo/mRNA-transfected autologous DCs	id	NCT03548571/Phase II/III
Ovarian Cancer	W_ova1 vaccine: Three ovarian cancer tumor associated antigens mRNAs	In vivo/Lipid nanosystems	i.v.	NCT04163094/Phase I
Recurrent Epithelial Ovarian Cancer	mRNA encoding hTERT and Survivin in addition to amplified cancer stem cell mRNA	Ex vivo/mRNA-transfected DCs	i.d.	NCT01334047/Phase I/II
Breast Cancer	cMet RNA CAR T cells	Ex vivo/mRNA transfected autologous CAR T cells	intratumoral	NCT01837602/Phase I
Early Breast Cancer	mRNA encoding TriMix	In vivo/naked mRNA	intratumoral	NCT03788083/Phase I
Triple-negative breast cancer	IVAC_WAREHOUSE_bre1_uID; IVAC MUTANOME _uID/personalized cancer vaccine targeting tumor-associated antigens	In vivo/Lipid nanosystems	i.v.	NCT02316457/Phase I
Solid tumors	mRNA-4157/personalized cancer vaccine targeting twenty tumor-associated antigens	In vivo/Lipid nanosystem	i.m.	NCT03313778/Phase I
Hodgkin Lymphoma	RNA anti-CD19 CAR T cells/CD19 chimeric antigen receptors expressing tandem TCR/4-1BB costimulatory domains	Ex vivo/mRNA transfected autologous CAR T cells	i.v.	NCT02277522 and NCT02624258/Early Phase I
Metastatic Pancreatic Ductal Adenocarcinoma	RNA mesothelin re-directed autologous T cell/chimeric anti-mesothelin immunoreceptor SS1	Ex vivo/mRNA transfected autologous CAR T cells	i.v.	NCT01897415/Phase I
Malignant Pleural Mesothelioma	Autologous anti-mesothelin CAR T cells/chimeric anti-mesothelin immunoreceptor	Ex vivo/mRNA transfected autologous CAR T cells	i.v.	NCT01355965/Phase I
Malignant Melanoma, Breast Cancer	RNA CART-cMET/MET chimeric antigen receptors with tandem TCRζ and 4-1BB (TCRζ/4-1BB) co-stimulatory domains	Ex vivo/mRNA transfected autologous CAR T cells	i.v.	NCT03060356/Early Phase I

Gómez-Aguado et al., 2020

ONCOLOGIE, QUELQUES EXEMPLES…

Table 2. *Cont.*

Type of Cancer	Biological Active/Encoding Sequence	Strategy/Delivery System	Administration Route	NCT Number/Phase
Brain Cancer, Neoplasm Metastases	Personalized cellular vaccine/tumor associated antigen mRNA	Ex vivo/mRNA transfected autologous DCs	NA	NCT02808416/Phase I
Advanced Esophageal Squamous Carcinoma, Gastric Adenocarcinoma, Pancreatic Adenocarcinoma, Colorectal Adenocarcinoma	Personalized mRNA Tumor Vaccine/Neoantigen (tumor associated specific antigens)	In vivo/-	s.c.	NCT03468244/NotA
Melanoma, Colon cancer, Gastrointestinal cancer, Genitourinary cancer, hepatocellular cancer	NCI-4650/mRNA-based, Personalized Cancer Vaccine	In vivo/Lipid nanosystems	i.m.	NCT03480152/Phase I/II
Melanoma, NSCLC, Bladder Cancer, Colorectal Cancer, Triple Negative Breast Cancer, Renal Cancer, Head and Neck Cancer, Other Solid Cancers	RO7198457/personalized cancer vaccine targeting tumor-associated antigens	In vivo/Lipid nanosystem	i.v.	NCT03289962/Phase I
Relapsed/Refractory Solid Tumor Malignancies or Lymphoma, Ovarian Cancer	mRNA-2416/OX40L	In vivo/Lipid nanosystems	Intratumoral	NCT03323398/Phase I and II
Squamous Cell Carcinoma, Head and Neck Neoplasm, Cervical Neoplasm, Penile Neoplasms Malignant	human papillomavirus (HPV16) mRNA vaccine/HPV16-derived E6, E7 tumor antigens	In vivo/Naked mRNA	i.d.	NCT03418480/Phase I and II
Advanced or Metastatic Malignancies Expressing CEA (Colorectal Cancer, Breast Cancer, Lung Cancer, Pancreatic Cancer) or Stage III Colon Cancer	AVX701/Alphaviral replicon particle vaccine expressing Carcinoembryonic Antigen Gene (CEA(6D)).	In vivo/Viral vector	i.m.	NCT00529984, NCT01890213/Phase I and II, Phase I
Glioblastoma, Renal Cell Carcinoma, Sarcomas, Breast Cancers, Malignant Mesothelioma, Colorectal Tumor	mRNA encoding WT1	Ex vivo/mRNA-transfected autologous DCs	i.d.	NCT01291420/Phase I/I,

NA, not available; NotA, not applicable; DCs, dendritic cells; i.d., intradermal; s.c., subcutaneous; i.v., intravenous; i.m., intramuscular.

Gómez-Aguado et al., 2020

ONCOLOGIE, QUELQUES EXEMPLES…

Table 1 | **Representative clinical trials of lipid nanoparticle–mRNA vaccines against infections and cancer**

Name	Disease	Encoded antigen	Administration route	ClinicalTrials.gov identifier	Phase
mRNA-5671/V941	Non-small-cell lung cancer, colorectal cancer, pancreatic adenocarcinoma	KRAS antigens	i.m.	NCT03948763	I
mRNA-4157	Melanoma	Personalized neoantigens	i.m.	NCT03897881	II
mRNA-4650	Gastrointestinal cancer	Personalized neoantigens	i.m.	NCT03480152	I/II
FixVac	Melanoma	NY-ESO-1, tyrosinase, MAGE-A3, TPTE	i.v.	NCT02410733	I
TNBC-MERIT	Triple-negative breast cancer	Personalized neoantigens	i.v.	NCT02316457	I
HARE-40	HPV-positive cancers	HPV oncoproteins E6 and E7	i.d.	NCT03418480	I/II
RO7198457	Melanoma	Personalized neoantigens	i.v.	NCT03815058	II
W_ova1	Ovarian cancer	Ovarian cancer antigens	i.v.	NCT04163094	I

CMA, conditional marketing authorization; EUA, Emergency Use Authorization; HPV, human papillomavirus; i.d., intradermal; i.m., intramuscular; i.v., intravenous; KRAS, Kirsten rat sarcoma 2 viral oncogene homologue; MAGE-A3, melanoma antigen family A; NY-ESO-1, New York esophageal squamous cell carcinoma 1; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TPTE, putative tyrosine-protein phosphatase.

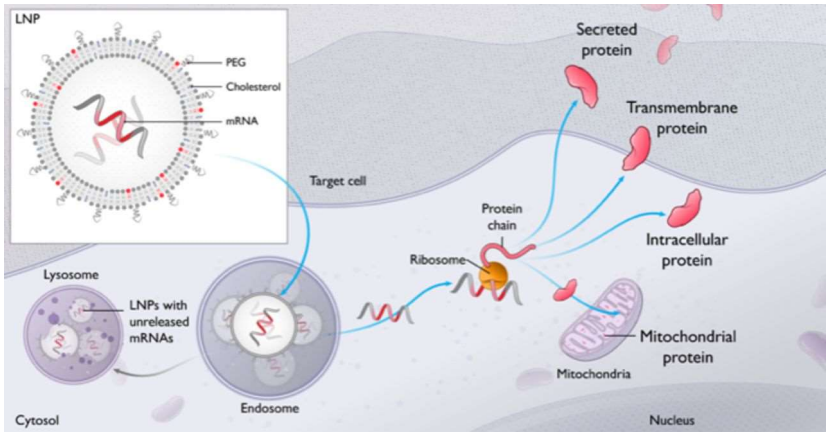
Table 2 | **Representative clinical trials of lipid nanoparticle–mRNA therapeutics against infections, cancer and genetic disorders**

Name	Disease	Encoded protein	Administration route	ClinicalTrials.gov identifier	Phase
mRNA 2416	Solid tumours	OX40L	Intratumour	NCT03323398	II
mRNA-2752	Solid tumours	OX40L, IL-23 and IL-36γ	Intratumour	NCT03739931	I
MEDI1191	Solid tumours	IL-12	Intratumour	NCT03946800	I
SAR441000	Solid tumours	IL-12sc, IL-15sushi, IFNα and GM-CSF	Intratumour	NCT03871348	I

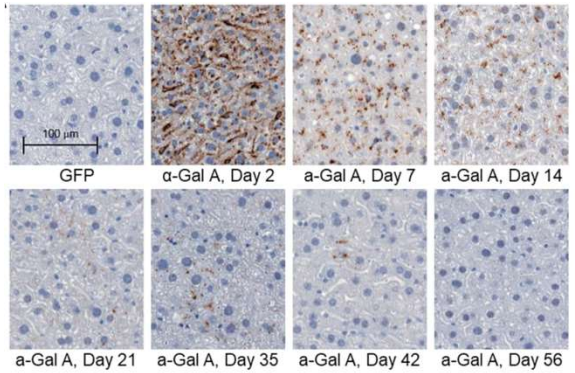
Hou et al., 2020

THÉRAPIE DE REMPLACEMENT DE PROTÉINE

- « Protein replacement therapy »: l´ARNm est administré au patient afin de compenser une protéine dysfonctionnelle, ou pour fournir des protéines thérapeutiques (souvent pour maladies génétiques).
- Expression de protéine intracellulaire (cytosolique, mitochondriale, nucléaire,…), transmembranaire ou secrétée
- Design: ARNm non-immunogène, administrations répétées, vecteur ciblant la cellule à traiter
- Limité pour l´instant aux maladies hépatiques (i.v.), pulmonaires (inhalation) et cardiaques (injection locale) + quelques essais sur l´œil et la peau.
- Quelques exemples:
  - Déficit en alpha-1-antitrypsine (Connolly et al. ,2018) (Alexion)
  - Déficit en citrine (Cao et al., 2019)
  - Déficit en ornithine transcarbamylase (OTC): Arcturus ARCT-810, Phase 2
  - Déficit en méthylmalonyl-CoA mutase (mitochondriale): Moderna mRNA-3705, Phase 1/2
  - Maladie de Fabry: ARNm codant pour l´alpha galactosidase A en administration i.v., efficace pendant 6 semaines (Zhu et al., 2019).
  - Mucoviscidose: ARNm codant pour CFTR délivré aux cellules épithéliales pulmonaires par nébulisation (TranslateBio, MRT5005, Phase 1/2)
  - Réduction de l'ischémie myocardique: A-VEGF en injection épicardique (Moderna/AstraZeneca AZD8601, Phase 2)



Berraondo et al., 2019



Liver

Duration of Action of h-α-Gal A in Fabry mice injected i.v. with either 0.5 mg/kg h-α-Gal A mRNA or 0.5 mg/kg eGFP mRNA encapsulated in LNPs

Zhu et al., 2019

PROTEIN REPLACEMENT, QUELQUES EXEMPLES…

Table 3. Clinical trials of mRNA for protein-replacement therapies.

Disease	Biological Active/Encoding Sequence	Strategy/Delivery System	Administration Route	NCT Number/Phase
Heart Failure	AZD8601/Vascular endothelial growth factor-A (VEGF-A)	Naked mRNA	Epicardial injection	NCT03370887/Phase II
Ulcers associated with type II diabetes	AZD8601/Vascular endothelial growth factor-A (VEGF-A)	Naked mRNA	Intradermal	NCT02935712/Phase I
Propionic Acidemia	mRNA-3927/alpha and beta subunits of the mitochondrial enzyme propionyl-CoA carboxylase	In vivo/Lipid nanosytems	Intravenous	NCT04159103/Phase I and II
Isolated Methylmalonic Acidemia	mRNA-3704/methylmalonyl-coenzyme A mutase (MUT)	In vivo/Lipid nanosytems	Intravenous	NCT03810690/Phase I and II
Ornithine Transcarbamylase Deficiency	MRT5201/Ornithine transcarbamylase	In vivo/Lipid nanosystems	Intravenous	NCT03767270/Phase I and II
Cystic Fibrosis	MRT5005/Human Cystic Fibrosis Transmembrane Regulator protein (CFTR)	In vivo/Lipid nanosystems	Nebulization	NCT03375047/Phase I and II

Gómez-Aguado et al., 2020

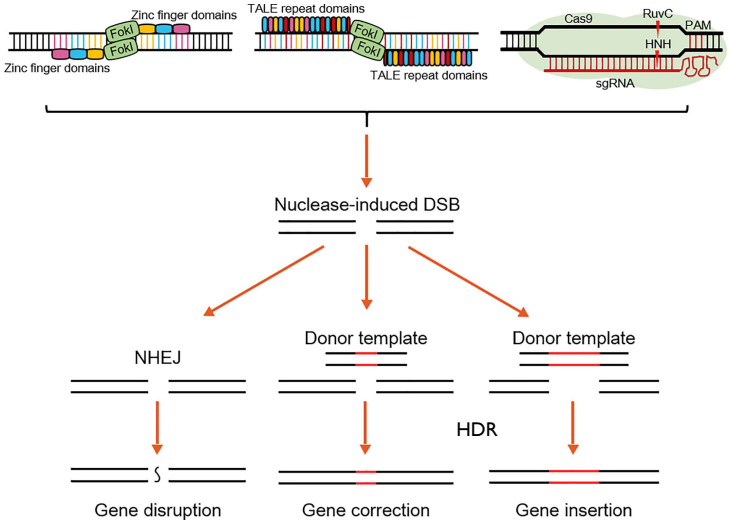
Table 2 | **Representative clinical trials of lipid nanoparticle–mRNA therapeutics against infections, cancer and genetic disorders**

Name	Disease	Encoded protein	Administration route	ClinicalTrials.gov identifier	Phase
mRNA-3704	Methylmalonic acidaemia	Methylmalonyl-CoA mutase	i.v.	NCT03810690	I/II
mRNA-3927	Propionic acidaemia	Propionyl-CoA carboxylase	i.v.	NCT04159103	I/II
MRT5201	Ornithine transcarbamylase deficiency	Ornithine transcarbamylase	i.v.	NCT03767270	I/II
MRT5005	Cystic fibrosis	Cystic fibrosis transmembrane conductance regulator	Inhalation	NCT03375047	I/II

Hou et al., 2020

ÉDITION GÉNOMIQUE (GENE EDITING)

- L´édition génomique apparait comme une alternative à la thérapie génique: modification spécifique du génome grâce à de nombreux outils (CRISPR-Cas9, TALEN, ZFN, base editing, transposase…)
- ARNm codant pour des nucléases: par ex. co-délivrance d´ARNm Cas9 + ARN guide
- Particularité: expression transitoire de la nucléase permettant de minimiser les effets off-target (vs. vecteur ADN)
- Administration in vivo ou ex vivo
- Domaines d´application variés:
  - Maladie génétiques:
    - Amyloïdose à transthyrétine, knock-out du gène TTR dans le foie (Intellia TX, Phase 3 prévue);
    - Angiœdème bradykinique, knock-out du gène KLKB1 dans le foie (Intellia TX, Phase 1);
    - Hémophilie B, knock-in du gène FIX dans le foie (Intellia TX, préclinique);
    - Glycogénose de type 1a , correction E342K du gène G6PC (Beam TX, préclinique);…
  - Maladies chroniques:
    - Hypercholestérolémie familiale, knock-out du gène PCSK9 (Verve TX, Phase 1)
  - Cancer:
    - Apoptose de cellules tumorales (knock-out de PLK1, Rosenblum et al., 2021)



Zhang et al., 2019

HDR: Homologous-dependent repair
NHEJ: non-homologous end joining

ÉDITION GÉNOMIQUE, QUELQUES EXEMPLES…

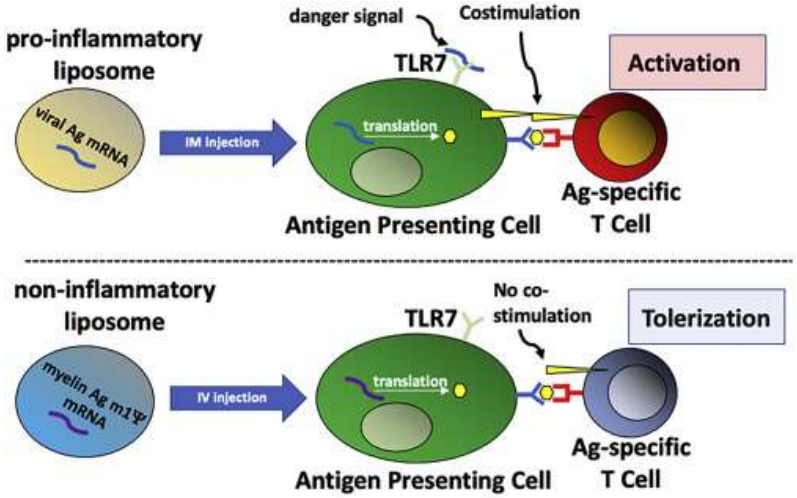
Table 4. Clinical trials of mRNA for gene editing therapy.

Disease	Biological Active	Therapeutic mRNA	Target Protein	Strategy/Delivery System	Administration Route	NCT Number/Phase
HIV	SB-728mR	ZFN mRNA	CCR5	Ex vivo/Autologous CD4+ T Cells	Intravenous	NCT02388594/Phase I
	SB-728mR	ZFN mRNA	CCR5	Ex vivo/Autologous CD4 CAR+ T cells	Intravenous	NCT03617198/Phase I
	SB-728mR-T	ZFN mRNA	CCR5	Ex vivo/Autologous T cells	Intravenous	NCT02225665, NCT04201782,/Phase I, Phase I/II
	SB-728mR-HSPC	ZFN mRNA	CCR5	Ex vivo/Autologous CD34+ hHSPCs	Intravenous	NCT02500849/Phase I
Sickle Cell Disease	BIVV003	ZFN mRNA	B-cell lymphoma/leukemia 11A (BCL11A)	Ex vivo/Autologous CD34 + hematopoietic stem cells (HSPC)	Intravenous	NCT03653247/Phase I/II
B acute lymphoblastic leukemia	UCART19	TALEN mRNA	TCR and CD52	Ex vivo/Allogenic T cells	Intravenous	NCT02808442, NCT02746952, NCT02735083/Phase I
B cell leukemia and B cell lymphoma	UCART019	CRISPR/Cas9 mRNA	TCR, B2M	Ex vivo/Allogenic T cells	Intravenous	NCT03166878/Phase I/II

Gómez-Aguado et al., 2020

MALADIES AUTO-IMMUNES ET INFLAMMATOIRES

- **Maladies auto-immunes:**
  - Expression d´IL-2 pour restaurer l´homéostasie immunitaire (Moderna, mRNA-6231, Phase 1)
  - Utilisation de vaccin à ARNm non-immunogène pour administrer l´auto-antigène aux cellules dendritiques → Développement un type distinct de cellule T régulatrice effectrice (Treg) spécifique de l'antigène qui supprime l'autoréactivité contre les auto-antigènes ciblés. Ex: Sclérose en plaque (Krienke et al., 2020)
  - Prévention du diabète de type 1 chez les souris, par l'administration de cellules T modifiées redirigées contre les cellules T CD8+ diabétogènes (Fishman et al., 2017).



Furlan R., 2021

- **Allergie:**
  - Les vaccins à ARNm prophylactiques contre les allergies antagonisent les mécanismes d'allergie en induisant des réponses immunitaires de type TH1 qui suppriment la production d'IgE spécifiques aux allergènes. Ex: Roesler et al., 2009; Hattinger et al., 2015

THÉRAPIE CELLULAIRE, MÉDECINE RÉGÉNÉRATIVE

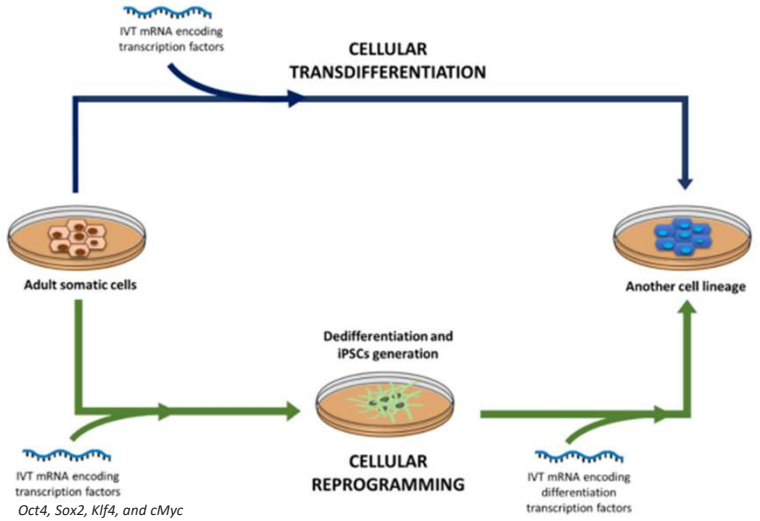
- Médecine régénérative: réparation, le remplacement ou la régénération de cellules, de tissus ou d'organes pour restaurer une fonction altérée

  → Thérapie cellulaire

- Reprogrammation et différenciation des cellules avec l'ARNm codant pour des facteurs de transcription (Warren et al., 2010):
  - Cellules souches: l´ARNm peut être utilisé pour différentier une cellule souche en une cellule somatique.
  - Cellules souches pluripotentes induites (iPSCs): l´ARNm peut être utilisé pour reprogrammer une cellule somatique en cellule pluripotente (Kogut et al., 2018)

  → Particularité: grande efficacité de transfection in vitro, expression transitoire sans intégration génomique et capacité de transférer des mélanges complexes.

- Utilisation de l´ARN ex-vivo
- Statut: préclinique
- Exemples d´applications:
  - cellules sécrétant de l'insuline pour les patients atteints de diabète de type I (Corritore et al., 2016; Koblas et al., 2016);
  - cardiomyocytes pour régénérer le tissu cardiaque après un infarctus du myocarde (Lee et al., 2015) ;
  - régénération osseuse (Elangovan et al., 2015)…



Gómez-Aguado et al., 2020

CONCLUSION

CARACTÉRISTIQUES DE LA TECHNOLOGIE ARNm…

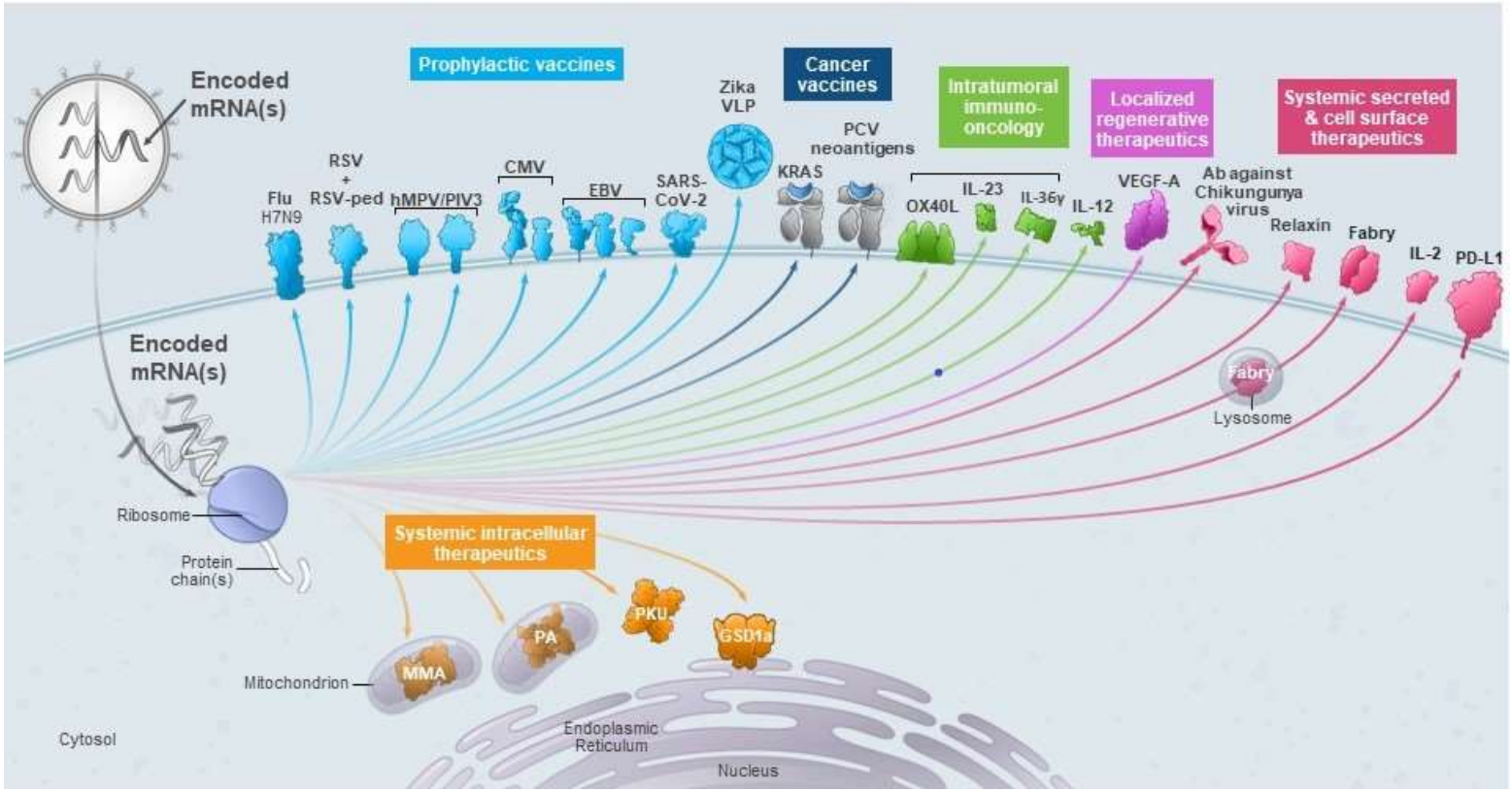
- Molécule naturelle, avec des propriétés bien caractérisées.
- Technologie polyvalente, largement applicable dans le traitement ou la prévention de nombreuses maladies : convient pour coder des anticorps, des antigènes, des cytokines et tout autre type de protéine qu´elle soit intracellulaire, transmembranaire ou sécrétée. → Offre la possibilité de produire des thérapies pour des maladies incurables jusqu´à présent.
- Expression transitoire, avec une activité et une demi-vie adaptables. Possibilité d´expression simultanée de plusieurs protéines.
- Pas de risque d'intégration génomique (potentiellement meilleur profil de sécurité que la thérapie génique).
- Protéine exprimée avec modifications post-transcriptionnelles endogènes → diminuent le risque d'immunogénicité.
- Validation clinique de cette approche grâce aux 2 vaccins à base d'ARNm contre le SARS-CoV2.
- Développement rapide et flexible, de la sélection du gène cible au candidat clinique: adaptation rapide de la séquence codante (émergence de nouveaux variants, médecine personnalisée)
- Modifications minimales du procédé de fabrication pour des cibles multiples.
- Fabrication rapide et peu coûteuse.

DÉFIS ET LIMITES…

- Vectorisation :
  - Les nano-vecteurs doivent être spécifiques aux tissus et cellules ciblées et biodégradables.
  - Limité pour l´instant principalement au foie en i.v. (+ rate), poumon (inhalation) et injection locale (intratumoral, intramusculaire).
- Efficacité:
  - Non prouvée encore pour les ARNm thérapeutiques en i.v. (ex. haut titre en protéine/anticorps atteignable ?)
  - Limitation en terme de doses (stabilité des nanoparticules)
- Toxicité / Immunogénicité :
  - Toxicité des matériaux utilisés (lipides et nucléotides non naturel) à forte dose?
- Stabilité / accessibilité:
  - L'ARNm est susceptible de se dégrader (un seule cassure suffit à le rendre inactif)
  - Problématique du stockage à 4°C ou température ambiante pour que ces thérapies puisent être utilisées dans tout environnement de soin de santé
- Fabrication :
  - Difficulté d'obtenir un ARNm de haute qualité et très pur.
  - Pas totalement « Plug and Play »: procédé devant être légèrement optimisé pour chaque nouvelle séquence

UNE TECHNOLOGIE POLYVALENTE, APPLICABLE À DE NOMBREUX DOMAINES THÉRAPEUTIQUES

- Exemple du pipeline de Moderna (2021):



RSV: respiratory syncytial virus ; CMV: cytomegalovirus ; PCV: personalized cancer vaccine ; Moderna Corporate presentation 2021

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