Universität Zürich UZH

UniversityHospital Zurich

URPP Translational Cancer Research

Basic research

Clinical research

[UZH - URPP Translational Cancer Research - Messenger RNA Platform]

INFIERI, Madrid

August 2021, 28th

RNA nanotechnologies applied to new vaccines (and therapies)

PD Dr. Steve Pascolo; University Hospital of Zurich, Switzerland

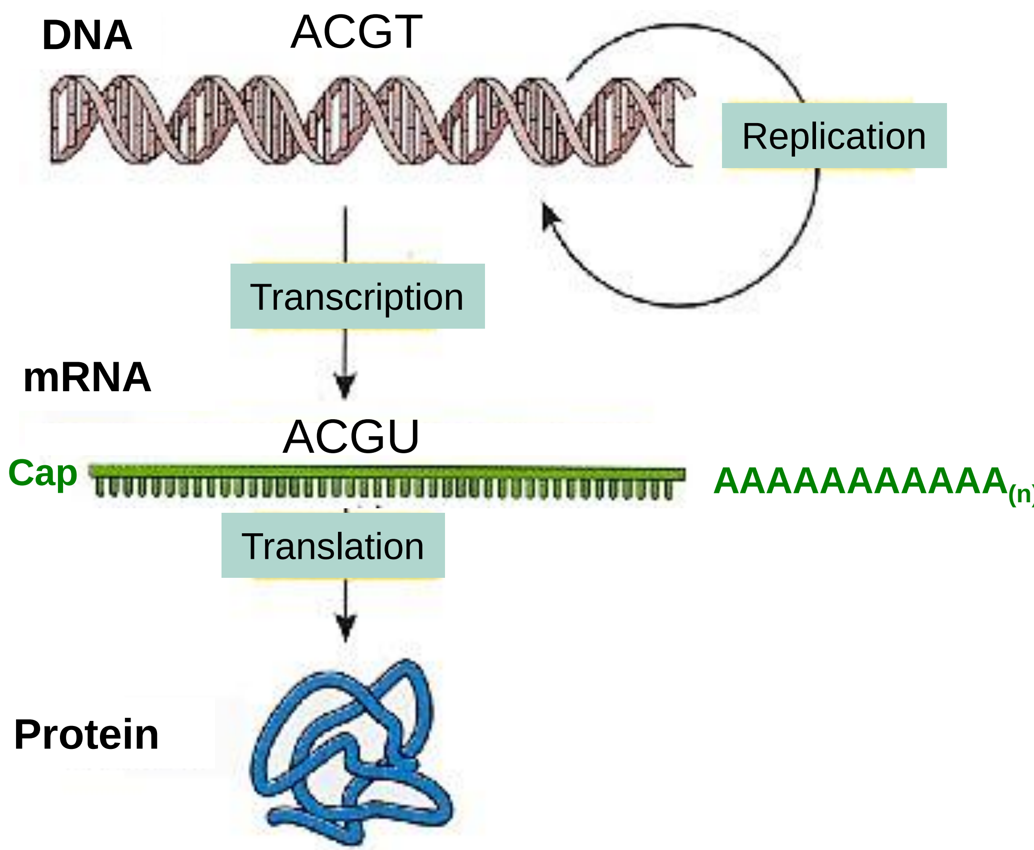
Vaccine preventable diseases (non-exhaustive list)

Disease	Pathogen	Type of vaccine	Injection	Adjuvant	Production
Tuberculosis	Mycobacterium tuberculosis	Attenuated bacteria	s.c.		bacilli Calmette-Guérin
Rubella (German measles)	Rubella virus	Attenuated virus **mRNA**	s.c.		Human embryonic lung cell line
Measles (Rubeola)	Measles virus	Attenuated virus **mRNA**	s.c.		Chick Embryo cells
Mumps	Mumps virus	Attenuated virus **mRNA**	s.c.		Chick Embryo cells
Chickenpox/Varicella-Zoster	Varicella Zoster virus	Attenuated virus **DNA**	s.c.		Human embryonic lung cell line
Smallpox (variola)	Variola major virus	Attenuated virus **DNA**	Prick		Animals
Rotavirus infection	Rotavirus	Attenuated virus **dsRNA**	Oral		Vero cells (monkey kidney epithelial cells)
Yellow fever	Yellow fever virus	Attenuated virus **mRNA**	s.c.		Eggs
Rabies	Rabies virus	Inactivated virus	i.m.		Vero cells (monkey kidney epithelial cells)
Swine Flu (2009 influenza A (H1N1) pandemic)	H1N1 influenza virus	Inactivated virus	i.m.	MF59 (squalene oil)	Eggs
Japanese encephalitis	Japanese encephalitis virus	Inactivated virus	i.m. or s.c.		Vero cells (monkey kidney epithelial cells)
Seasonal influenza	Influenza virus	Inactivated virus	i.m.	*Alum/MF59*	Eggs
Hepatitis A	Hepatitis A virus	Inactivated virus	i.m.	Aluminum hydroxide	MRC-5 cells
Cholera	Vibrio cholera	Inactivated/attenuated bacteria	Oral		Bacteria culture medium
Poliomyelitis	Polio virus	Inactivated/attenuated virus	s.c. or i.m /oral		Vero cells (monkey kidney epithelial cells)
Invasive Haemophilus influenzae disease	Haemophilus influenzae type b	Polysaccharide conjugated to tetanus toxoid or mutant of diphtheria toxin	i.m.	Aluminum hydroxide	Haemophilus influenzae type b
Meningococcal disease	Neisseria meningitidis bacteria	Polysaccharide conjugated to tetanus toxoid or mutant of diphtheria toxin	i.m.		Neisseria meningitidis bacteria
Invasive pneumococcal disease	Streptococcus pneumoniae	Polysaccharide conjugated to mutant of diphtheria toxin	i.m.		Streptococcus pneumoniae
Hepatitis B	Hepatitis B virus	Subunit: HBsAg	i.m.	Aluminum hydroxide	Yeast
Cervical cancer	Human papillomavirus	Subunit: VLPs	i.m.	Aluminum hydroxide	Vero cells (monkey kidney epithelial cells)
Pertussis	Bordetella pertussis	Pertussis antigens	i.m.	Aluminum hydroxide	Bordetella pertussis
Tetanus	Bacterium Clostridium tetani,	Tetanus toxoid	i.m.	Aluminum hydroxide	C tetani bacteria
Diphtheria	Corynebacterium diphtheriae/ulcerans	Subunit: Diphtheria toxoid	i.m.	Aluminum hydroxide	Corynebacterium diphtheriae/ulcerans

Colored: Basic vaccines suggested in Switzerland

In Cells

COVID-19 vaccine Format



20 amino acids

Stable	DNA	AstraZeneca Sputnik V Janssen
Transient	mRNA	BioNTech/Pfizer CureVac Moderna
Transient	Protein	Novavax

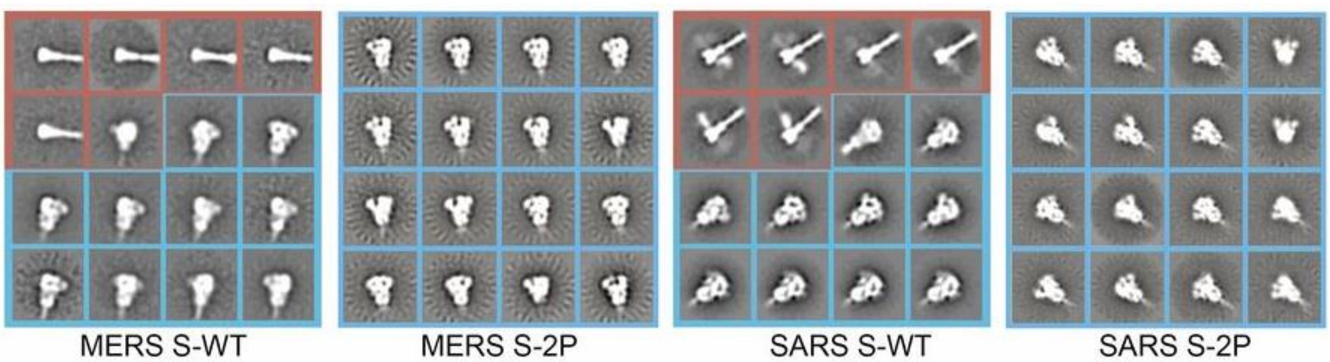
Der Informierte Arzt – March 2021 - Pascolo

Vaccines ordered by Switzerland

Platform	Company	Million doses reserved	Spike *	Efficacy (Jan 2020 SARS-CoV-2)	Efficacy (South African variant)	Dosing	Theoretical concerns
Purified protein	Novavax	6	PP	95%	Reduced	5µg 2x with 3 weeks interval	Induction of immunity against contaminants
Recombinant adenovirus	AstraZeneca	5.3	WT	Between 62% to 90%	Strongly reduced	ca. 2µg 2x with 4 weeks interval	Recombination Integration in genome (inducing transformation)
ivt mRNA	BioNTech /Pfizer	3	PP	95%	Slightly reduced	30µg 2x with 3 weeks interval	None
	CureVac	5	PP	48%	Not yet known	12µg 2x with 4 weeks interval	None
	Moderna	13.5	PP	94.1%	Slightly reduced	100µg 2x with 4 weeks interval	None

Der Informierte Arzt – March 2021 - Pascolo

* Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen.
Pallesen et al. Proc Natl Acad Sci U S A. 2017 Aug 29;114(35).
Proline instead of Lysine 986 and Valine 987



Vaccine formats (blue: aproved against SARS-CoV-2)

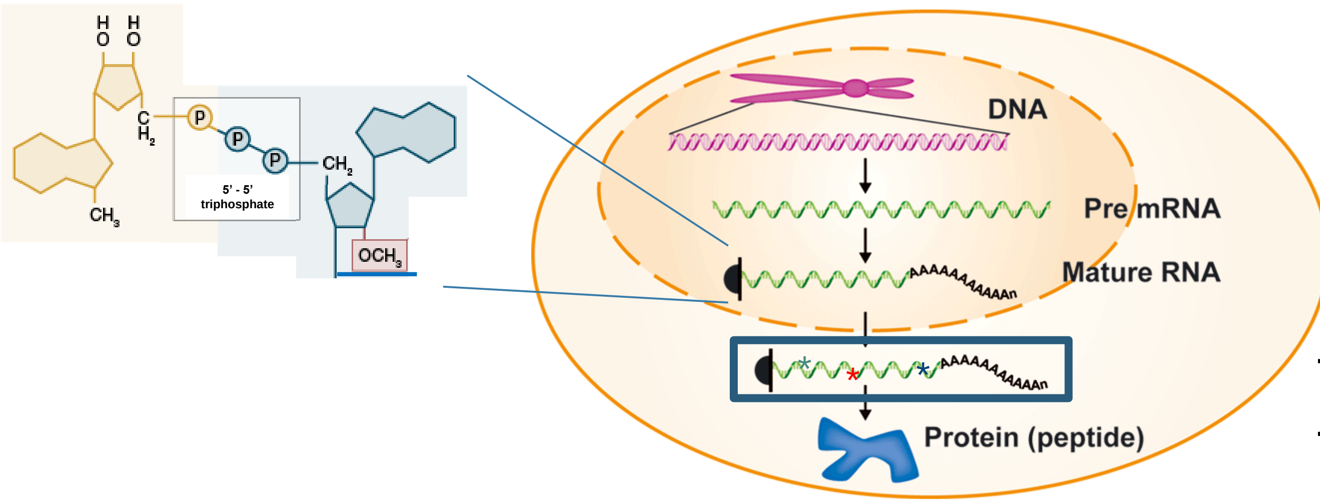
	Design	Upscaling	Re-using established GMP conditions	Theoretical Safety
Peptide	😐	😐	☹	☺
Plasmid DNA	☺	😐	☺	☹
Recombinant viral vector (adenovirus)	😐	😐	😐	☹
ivt mRNA	☺	☺	☺	☺
Proteins/ Sugars	😐	😐	😐	☺
Inactivated viruses	☺	😐	😐	☺
Attenuated viruses	😐	😐	😐	☹

☺ **Easy**
😐 **To be optimised / Not easy / Not garantied**
☹ **Difficult / Bad**

Adapted from Pascolo. Messenger RNA: The Inexpensive Biopharmaceutical. JMEST. Vol. 4 Issue 3, March - 2017

mRNA

COVID-19 vaccines

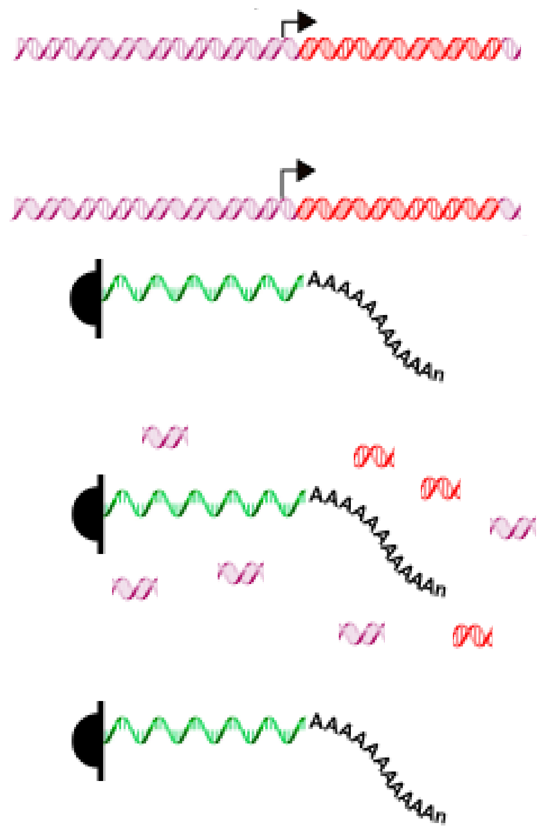


STABLE	AstraZeneca Sputnik V J&J
TRANSIENT	BioNTech/Pfizer CureVac Moderna
TRANSIENT	Novavax

Production of synthetic (ivt) mRNA

Starting material

- pDNA
- RNA polymerase
- Restriction enzyme
- DNase
- Nucleotides
- Buffers
- Cap analog



1- Linearisation

Restriction enzyme
Robust. Two hours

2- Transcription

T7/SP6 RNA polymerase
Robust. Two hours

3- Degradation of DNA

DNase
Robust. Two hours

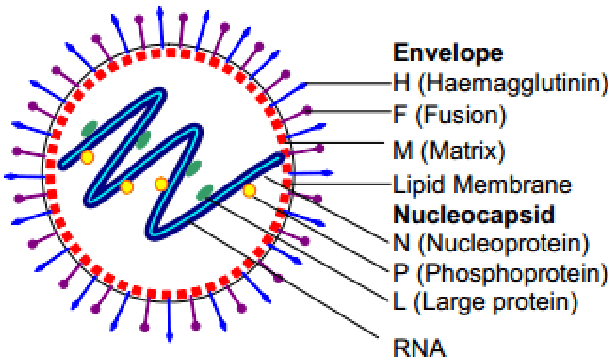
4- Purification of mRNA

LiCl precipitation:
Robust. Two hours

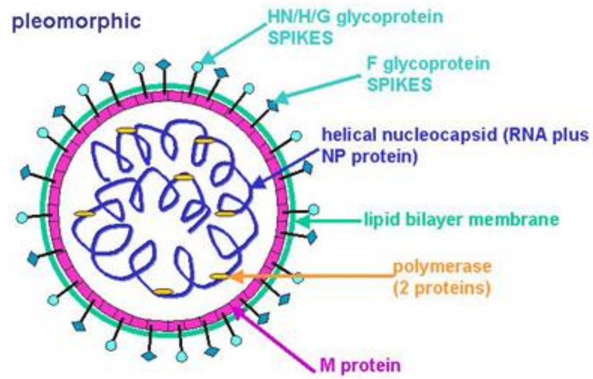
Pascolo. Messenger RNA: The Inexpensive Biopharmaceutical. JMEST. Vol. 4 Issue 3, March - 2017

mRNA vaccines: Natural (long used) and synthetic (newly approved)

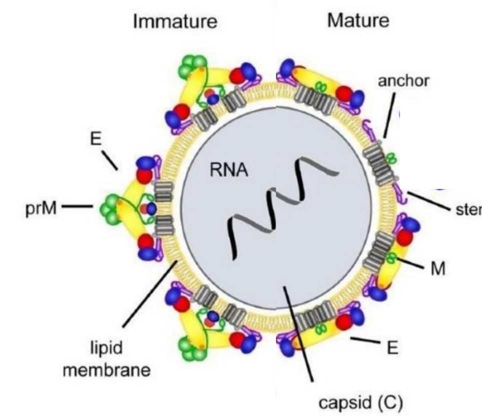
Natural mRNA vaccine



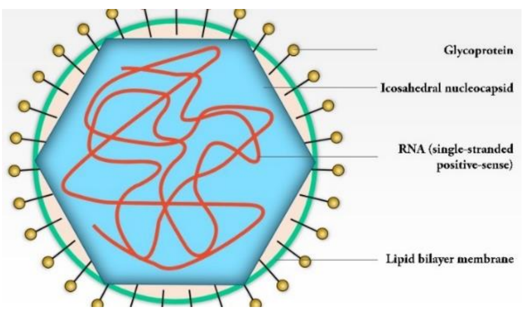
Measles (attenuated Virus)



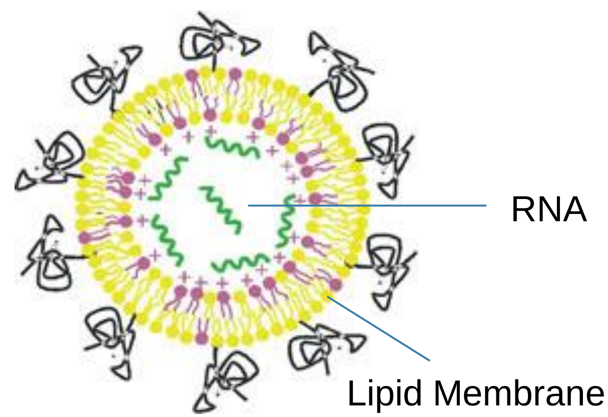
Mumps (attenuated Virus)



Yellow Fever (attenuated Virus)



Rubella (attenuated Virus)



Synthetic (ivt) mRNA vaccine

Facts on synthetic (ivt) mRNA vaccines

- **mRNA IS VERY STABLE (in the abscence of RNases!)**

- **RNA-liposome formulations may not be stable (aggregate, change size/form over time or by freeze/thaw)**

- **RNA in liposome is already an approved drug: Onpattro (Patisiran). Up to 30 mg i.v. every 3 weeks.** siRNA. Treatment of polyneuropathy in people with hereditary transthyretin-mediated amyloidosis.

- **ivt mRNA vaccines are vegan**

- **<u>1 million doses in 6 L and few hours</u> (viruses require 5000 L and few days/weeks for 1 million doses)**

ivt mRNA vaccines: Timelines

1869: In the kitchen of the castle of Tuebingen (Germany), Friedrich Miescher (1844-1895) isolates from the nuclei of human blood cells a new biological phosphate-rich substance that he names Nuclein.

1871: Back in Basel (Switzerland), he re-isolates it from salmon sperm. From such samples DNA and RNA were later on characterized

1889 Altman names the phosphate-rich product nucleic acid

1901 Kossel discovers nucleotides (A, C, G, T, U)

1929 Levene and Jacobs dissociate RNA and DNA

1943 Avery finds that DNA is the genetic material

1953 Complementarity of bases and structure of DNA: Watson and Crick

From the Portrait Collection of the University of Basel

ivt mRNA vaccines: Timelines

Discovery of mRNA: 1961

Artist: Caroline Schupbach

ivt mRNA vaccines: Timelines

Martinon et al use <u>mRNA in liposomes</u> to vaccinate mice against Influenza

Conry et al use <u>naked mRNA</u> to vaccinate mice against cancer

Hoerr et al use <u>naked and Protamine formulated mRNA</u> to vaccinate mice

Founding of CureVac

First injections of ivt mRNA in humans

1993 1995 2000 2000 2003 2006 2008 2010 2020

Manufacturing authorisation GMP production mRNA

First publication mRNA vaccine clinical study

Founding of BioNTech

Founding of Moderna

Approval first synthetic mRNA vaccine (BioNTech/Pfizer)

Artist: Caroline Schupbach

Martinon….Meulien. European Journal of Immunology 1993 “Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA”

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Hans-Georg Rammensee **Gunther Jung**
Ingmar Hörr **Florian von der Mülbe**
Steve Pascolo

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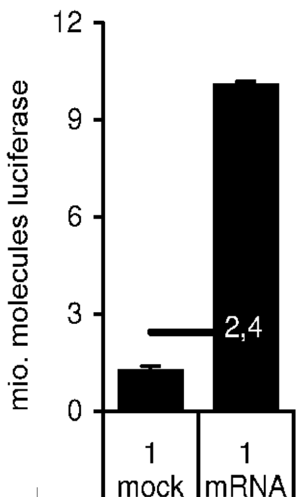

Probst….Pascolo. Gene Therapy 2007 "Spontaneous cellular uptake of exogenous messenger RNA in vivo is nucleic acid-specific, saturable and ion dependent"

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Artist: Caroline Schupbach

Ozlem Tureci CMO and Ugur Sahin CEO/CSO BioNTech

ivt mRNA vaccines against SARS-CoV-2

In liposomes, intramuscular, coding Spike

Moderna: PseudoU mRNA. Started March 16
> 25 µg, 100 µg, or 250 µg.

BioNTech/Pfizer: PseudoU (BNT162b) and U (BNT162a) mRNA. Started April 23
> 1µg up to 100µg

CureVac: U mRNA. Started June.
> 2µg up to 12µg

Safe and well tolerated although there were some side effects

ALL VOLUNTEERS SEROCOVERTED – NEUTRALISING ANTIBODIES AFTER BOOST

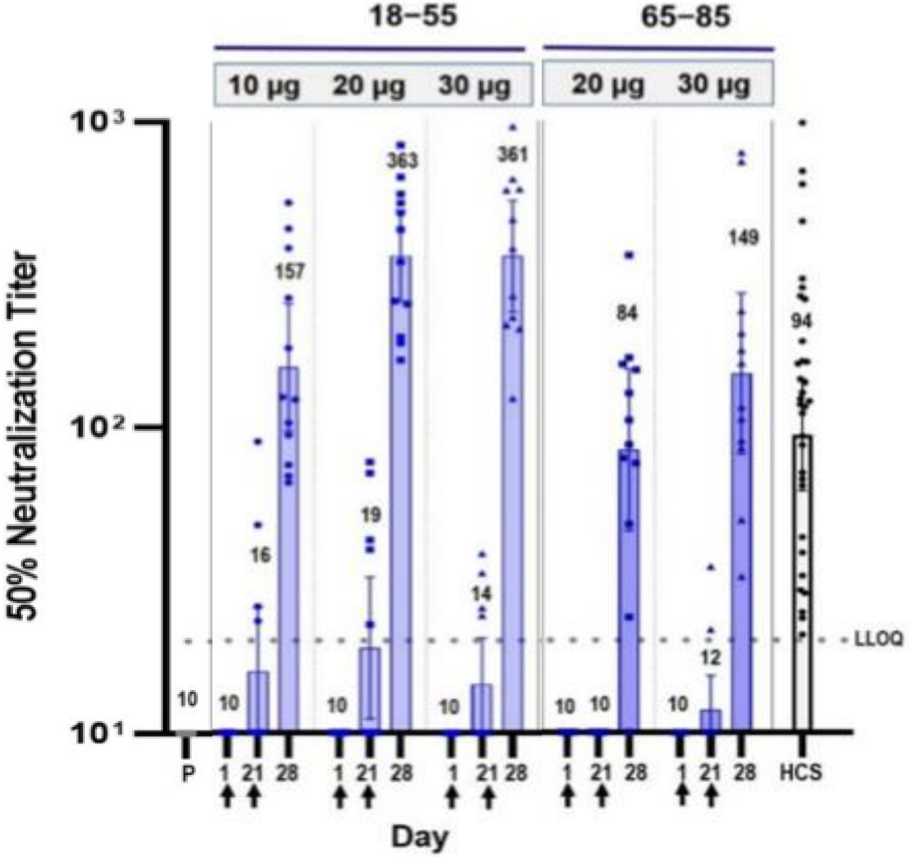
Approved: 100 µg for Moderna and 30 µg for BioNTech/Pfizer. CureVac failed with 12 µg

Over 95% protection against COVID-19 and 94% protection against infection by SARS-CoV-2 (BioNTech/Pfizer results in Israel)

Variants: "With the BNT162b2 vaccine, the effectiveness of two doses was 93.7% among persons with the alpha variant and 88.0% among those with the delta variant. With the ChAdOx1 nCoV-19 vaccine, the effectiveness of two doses was 74.5% among persons with the alpha variant and 67.0% among those with the delta variant.
Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant | NEJM **July 2021**

ivt mRNA vaccines against SARS-CoV-2

BioNTech/Pfizer. https://www.nejm.org/doi/full/10.1056/NEJMoa2027906
October 14, 2020



mRNA platform @ URPP since January 2017

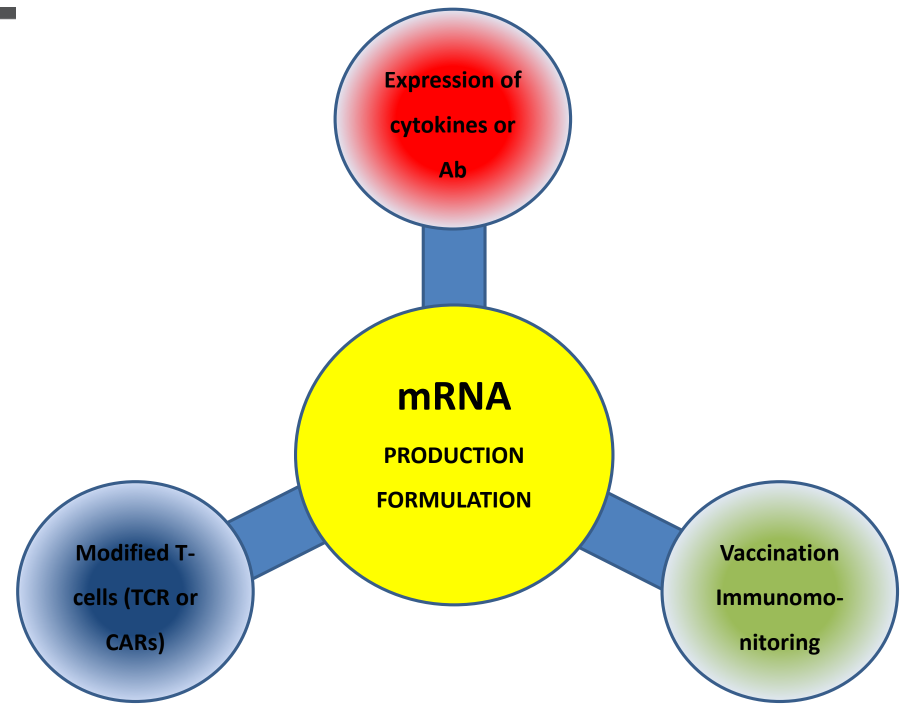
https://www.cancer.uzh.ch/en/Research/mRNA-Platform.html

University of Zurich[UZH]

URPP Translational Cancer Research

University of Zurich » URPP Translational Cancer Research » Research » **mRNA Platform**

Messenger RNA Platform

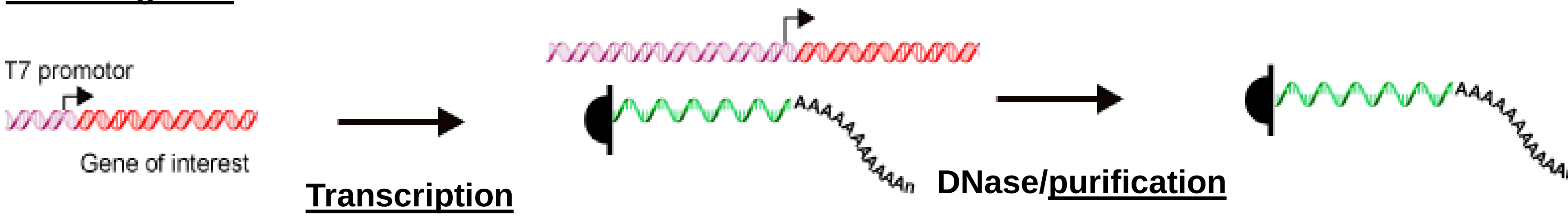


https://www.cancer.uzh.ch/en/Research/mRNA-Platform.html

mRNA platform @ URPP

Production & Optimisation of mRNA (coding luciferase)

PCR Fragment

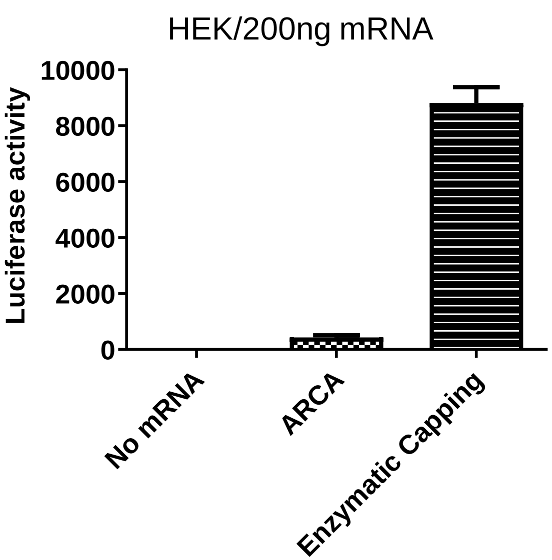


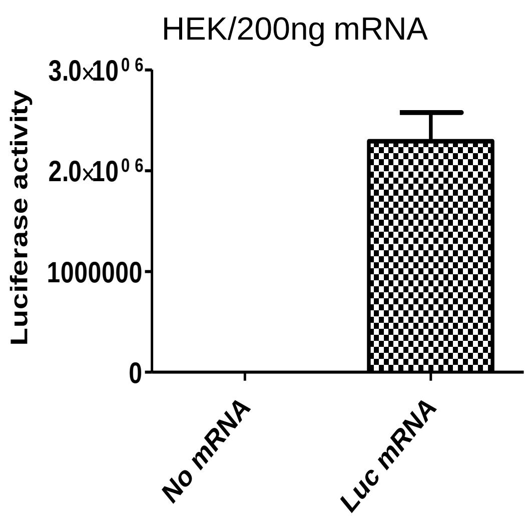
Feb 2017

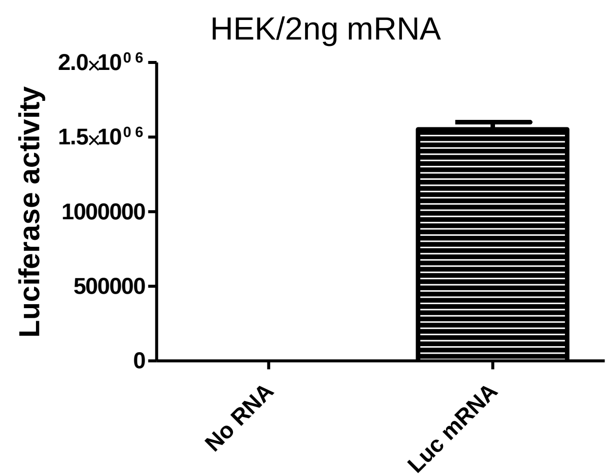
200x

Dec 2017

Jul 2019

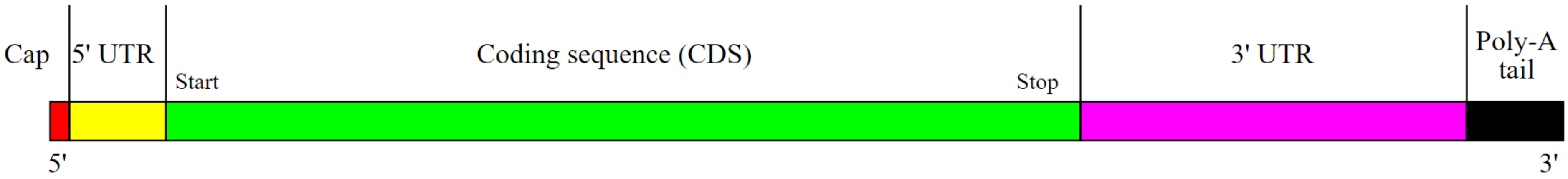






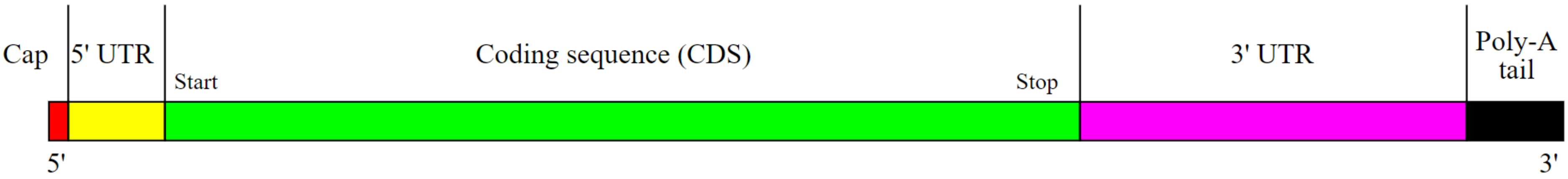
/10 production cost (ca 0.4 CHF per microgram)

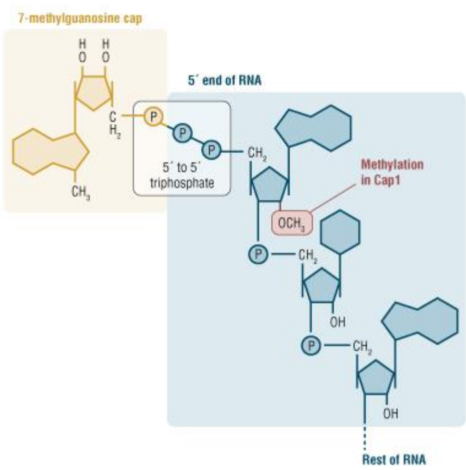
mRNA platform @ URPP
Production & Optimisation of mRNA

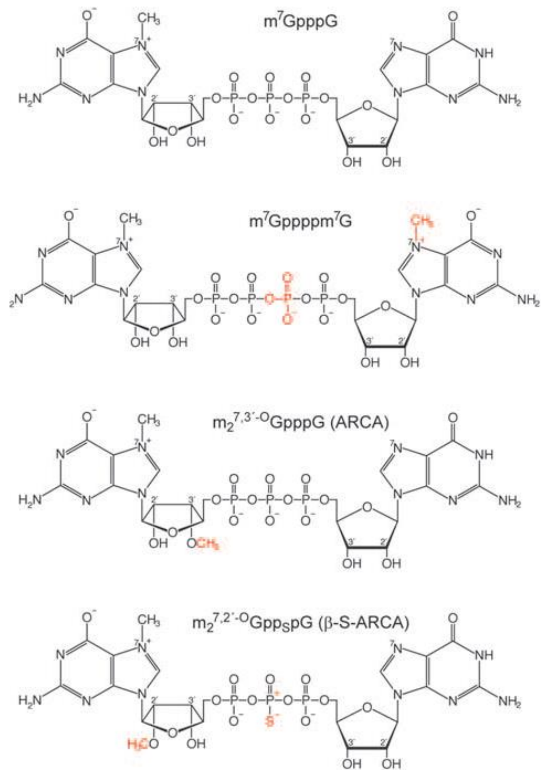


mRNA platform @ URPP

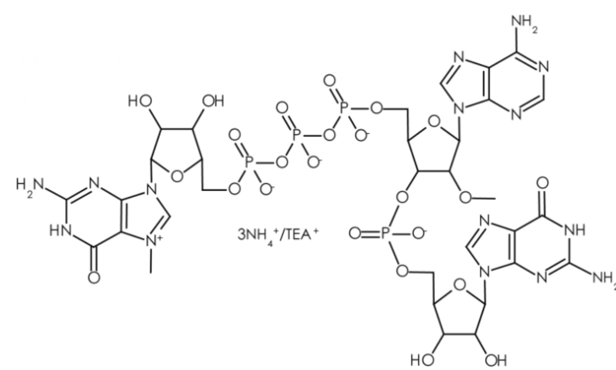
Production & Optimisation of mRNA







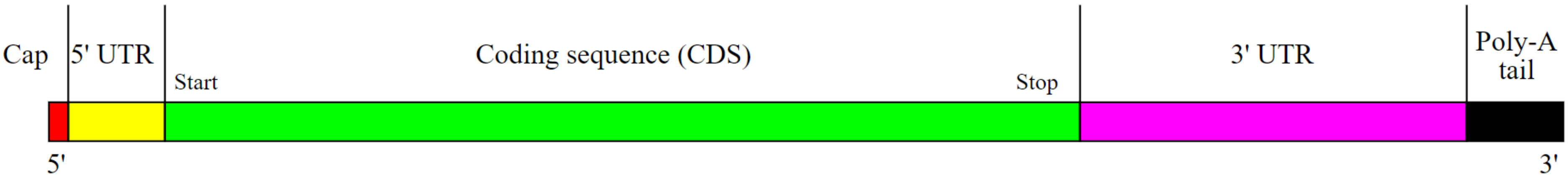
CleanCap® Reagent AG 2017



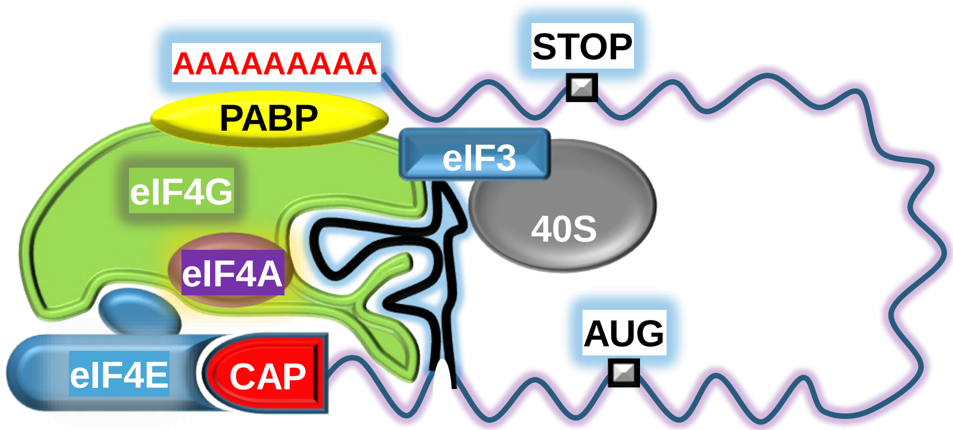
Kuhn, Diken, Kreiter, Selmi, Kowalska, Jemielity, Darzynkiewicz, Huber, Türeci, Sahin. Phosphorothioate cap analogs increase stability and translational efficiency of RNA vaccines in immature dendritic cells and induce superior immune responses in vivo. Gene Ther. 2010 Aug;17(8):961-71.

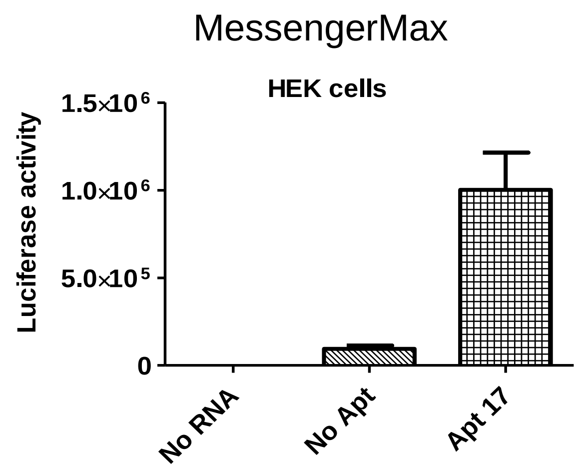
mRNA platform @ URPP

Production & Optimisation of mRNA



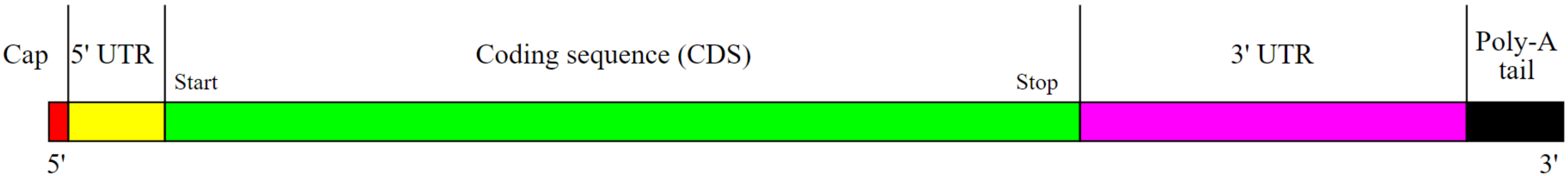
Globin RNA are very stable: 5’ UTR (ca. 60 bases) and 3’ UTR (ca. 150 bases)





Tusup, M., Kundig, T., Pascolo, S. (2018). An eIF4G-recruiting aptamer increases the functionality of in vitro transcribed mRNA. *EPH - International Journal of Medical and Health Science (ISSN: 2456 - 6063)*, *4*(6), 29-37.

mRNA platform @ URPP
Production & Optimisation of mRNA



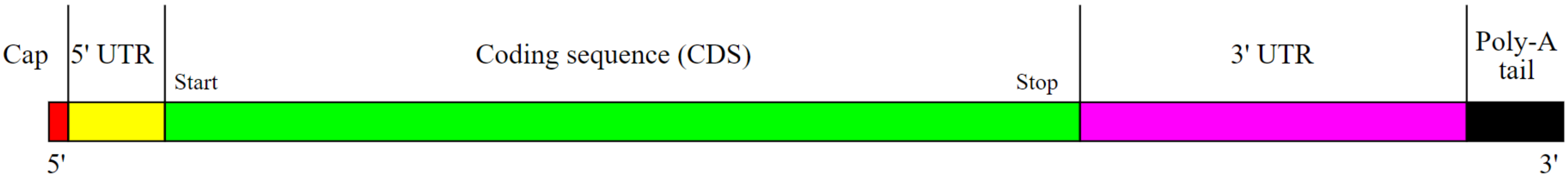
Codon optimisation can be used

- By controlling the speed of translation, rare codons are used to slow translation between structural domains allowing time for each domain to fold properly.
- Secondary structures may be created

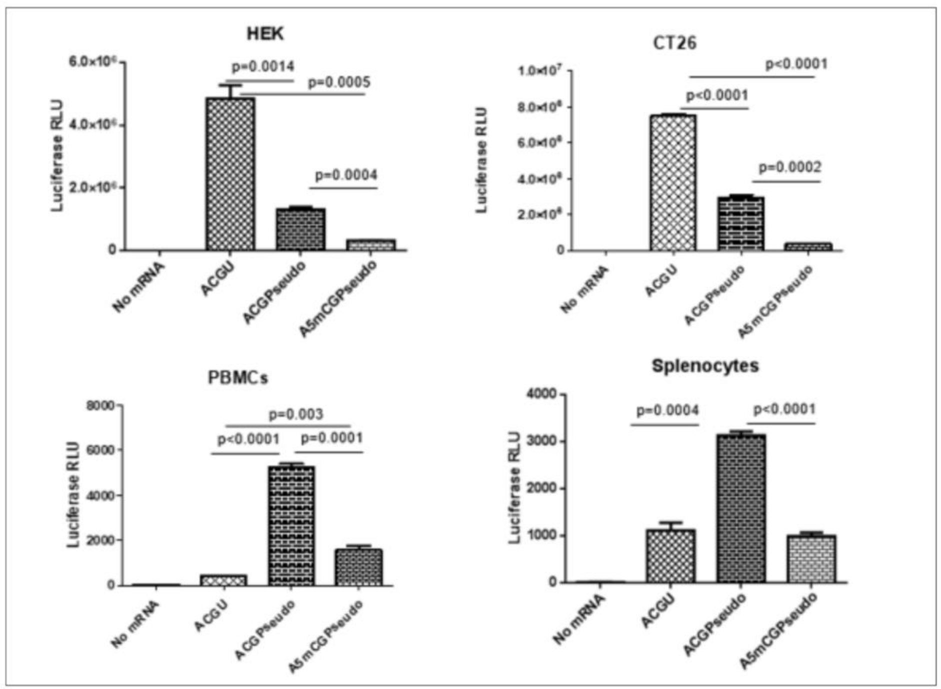
-> Codon optimization only for xenogenic mRNAs

Tusup M, French LE, De Matos M, Gatfield D, Kundig T, Pascolo S. Design of in vitro Transcribed mRNA Vectors for Research and Therapy. Chimia (Aarau). 2019 May 29;73(6):391-394.

mRNA platform @ URPP
Production & Optimisation of mRNA

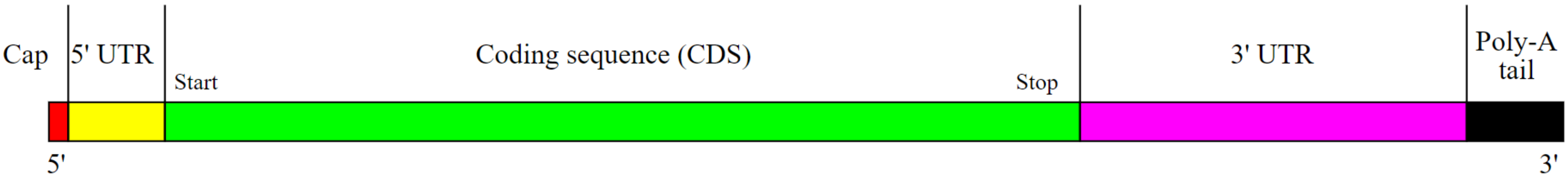


U versus PseudoU versus 1-methyl-PseudoU



Tusup, M., French, L., Guenova, E., Kundig, T., Pascolo, S. Optimizing the Functionality of in vitro-Transcribed mRNA
BJSTR 2018; 7 (2) : 5845-5850;

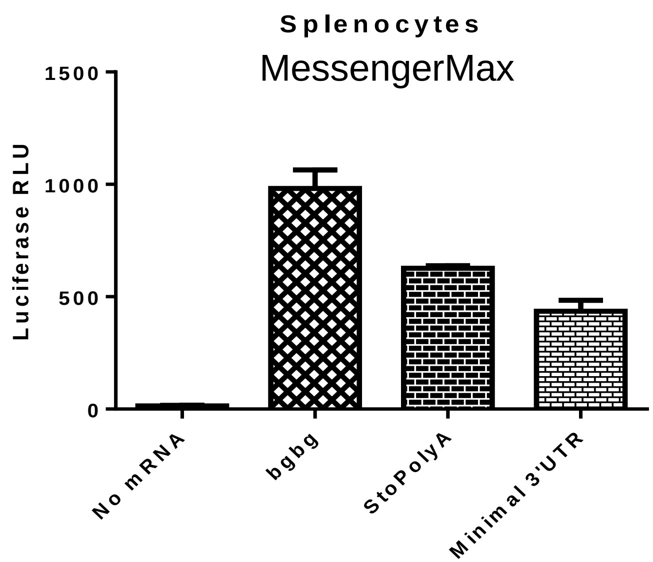
mRNA platform @ URPP
Production & Optimisation of mRNA



Globin RNA are very stable: 5’ UTR (ca. 60 bases) and 3’ UTR (ca. 150 bases)

Beta globin 3’UTR < beta globin 3’UTR tandem < “mtRNR1” + “AES” 3’UTR

Holtkamp, S. et al. Blood (2006). Orlandini von Niessen, A. G. et al. Mol Ther (2018).



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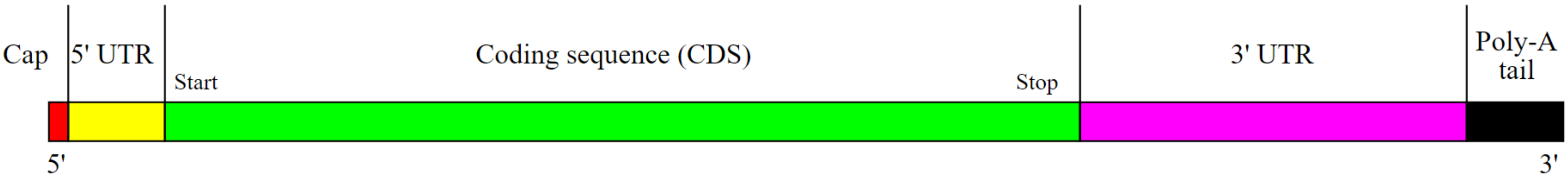
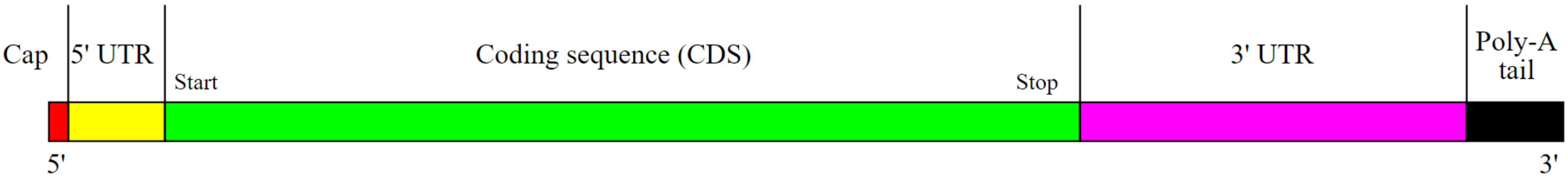


90 residues or more (encoded by DNA) or treatment with poly-A polymerase

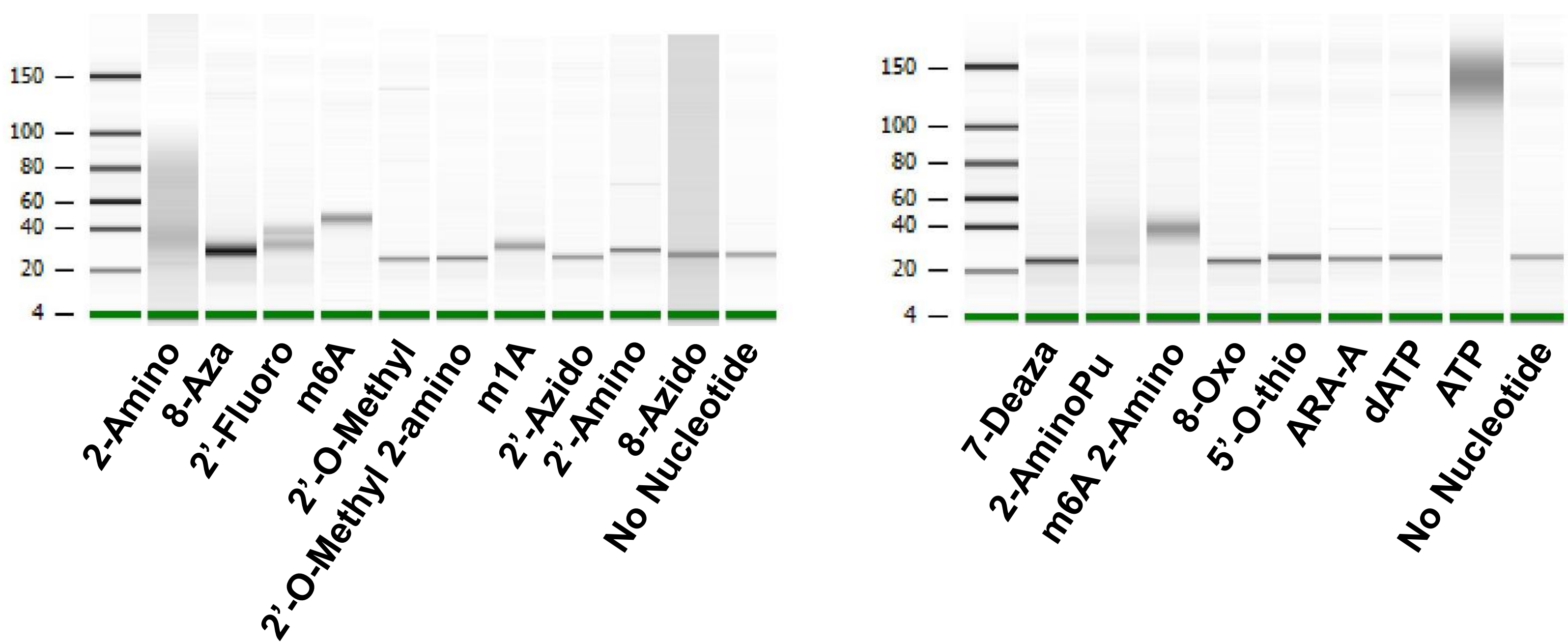
Tusup, M., French, L., Guenova, E., Kundig, T., Pascolo, S. Optimizing the Functionality of in vitro-Transcribed mRNA BJSTR 2018; 7 (2) : 5845-5850;

mRNA platform @ URPP

Production & Optimisation of mRNA



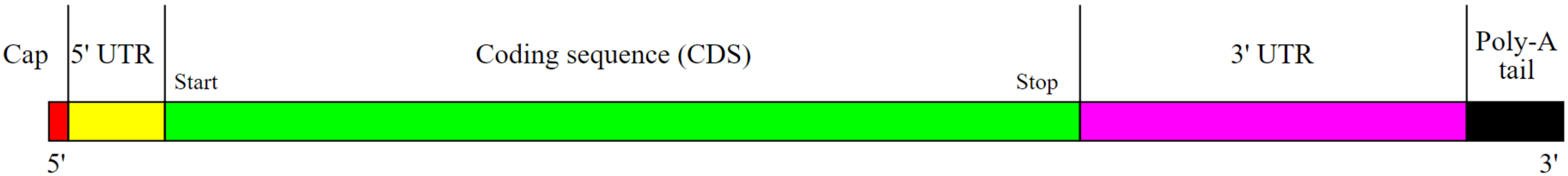
Enzymatic poly-adenylation of an RNA oligonucleotide



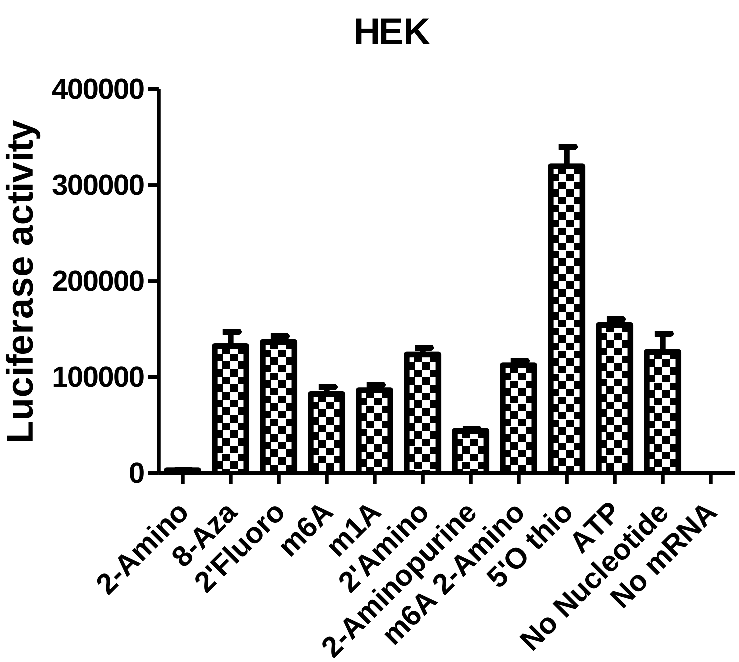
Tusup M., *et al* unpublished

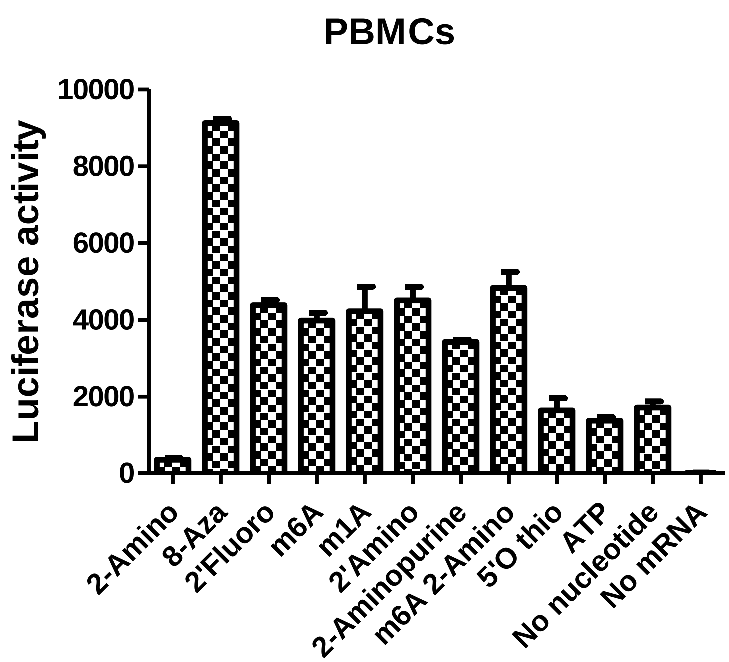
mRNA platform @ URPP

Production & Optimisation of mRNA



Enzymatic poly-adenylation of an mRNA coding Luciferase nd having 90 A residues

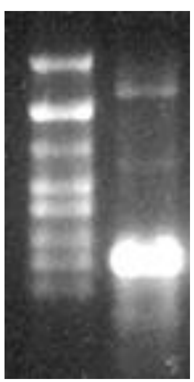


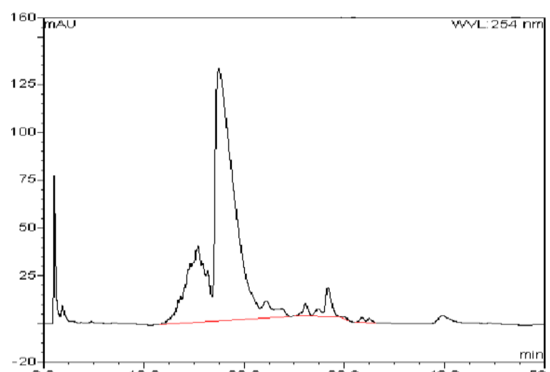


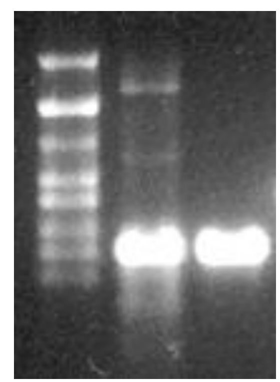
Tusup M., *et al* unpublished

mRNA platform @ URPP
Production & Optimisation of mRNA

- Separation of mRNA by size
- Fraction collection of peak of interest





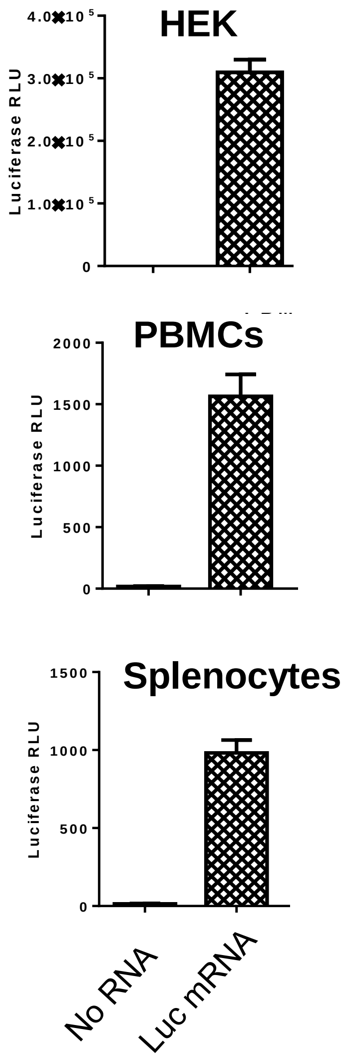


Messenger RNA-based vaccines. Pascolo S. Expert Opin Biol Ther. 2004 Aug;4(8):1285-94.

mRNA platform @ URPP

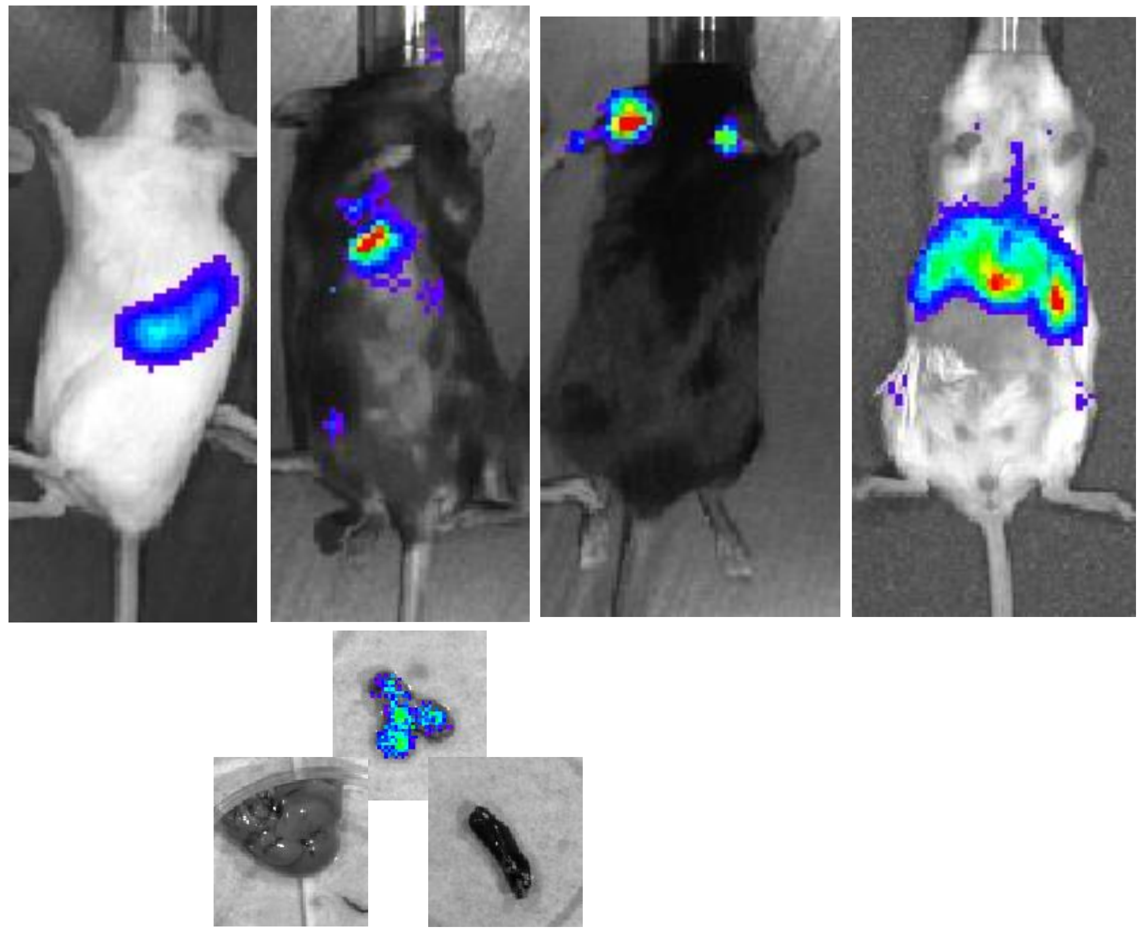
Formulation of mRNA (coding luciferase)

in vitro



in vivo

Liposome (i.v.) Liposome (i.v.) Naked (i.d.) Liposome (i.v.)



Synthetic mRNA: Tomorrow

- **Vaccines against (all) viruses**
- **Vaccines against cancer (shared tumor antigens or individualised vaccines against mutations)**
- **Vaccines against allergies**

Infection	Phase	Status	Drug treatment	NCT number
SARS-CoV-2	I	Active	BNT162b1 + placebo	NCT04523571
	I	Active	CVnCoV vaccine + placebo	NCT04449276
	III	Active	mRNA-1273 + placebo	NCT04470427
	I/II/III	Recruiting	BNT162b1 + BNT162b2	NCT04368728
	II	Active	CVnCoV	NCT04515147
	I	Active	mRNA-1273	NCT04283461
	II	Active	mRNA-1273 + placebo	NCT04405076
	II	Not yet recruiting	2 doses of BNT162b2 or one dose of BNT162b2s01	NCT04949490
Rabies	I	Completed	CV7201 mRNA encoding the rabies virus glycoprotein	NCT02241135
	I	Active	Rabipur®	NCT03713086
HIV-1	I/II	Completed	mRNA-transfected autologous DCs+/− autologous DCs with no mRNA transfection	NCT00833781
	I	Terminated	TriMix mRNA+/−HIV mRNA	NCT02413645
	II	Completed	iHIVARNA-01 + TriMix+/−Placebo	NCT02888756
Zika virus	I	Completed	mRNA-1325 + placebo	NCT03014089
	I	Active	mRNA-1893 + placebo	NCT04064905
Tuberculosis	I	Completed	GSK 692342	NCT01669096
Ebola virus	I	Completed	two separate mRNAs encoding two Zaire strain Ebola glycoproteins, respectively	NCT02485912
Influenza	I	Completed	VAL-506440 + placebo	NCT03076385
Influenza	I/II	Recruiting	mRNA1010 + placebo	NCT04956575
Cytomegalovirus	I	Completed	mRNA-1647 + placebo	NCT03382405
Cytomegalovirus	II	Recruiting	mRNA-1647 + placebo	NCT04232280
Respiratory syncytial virus (RSV) vaccine	I	Recruiting	mRNA-1345 + placebo	NCT04528719
Human Metapneumovirus and Human Parainfluenza Infection	I	Completed	mRNA-1653 + placebo	NCT03392389
Human Metapneumovirus and Human Parainfluenza Infection	I	Recruiting	mRNA-1653 + placebo	NCT04144348

Tumor type	Phase	Status	Drug treatment	NCT number
Non-small cell lung cancer (NSCLC)	I/II	Recruiting	BI 1361849 (CV9202) + Durvalumab+/−Tremelimumab	NCT03164772
	I/II	Recruiting	Personalized mRNA vaccine encoding neoantigen	NCT03908671
	I/II	Recruiting	Suppressor of cytokine signaling (SOCS) 1, MUC1 and Survivin mRNA-loaded DC + cytokine-induced killer cells	NCT0268868
Melanoma	I	Completed	mRNA+GM-CSF	NCT00204607
	I/II	Completed	DCs transfected with hTERT, survivin and p53	NCT00978913
	i	Completed	Dendritic cells electroporated with mRNA encoding gp100 and tyrosinase	NCT00940004
	I/II	Completed	TriMix-DC	NCT01066390
	I	Completed	DCs loaded with mRNA encoding tumor-associated antigens gp100 and tyrosinase+/ −cisplatinum	NCT02285413
	I/II	Completed	mRNA coding for melanoma associated antigens+GM-	NCT00204516
	I	Completed	CSF mRNA-transfected DCs + IL-2	NCT01278940
	I	Completed	autologous dendritic cell vaccine by mRNA	NCT01530698
	II	Completed	Electroporation Autologous dendritic cell vaccine	NCT00243529
	I/II	Recruiting	mRNA-4157 + pembrolizumab	NCT03897881
	I	Active	Autologous Langerhans-type dendritic cells electroporated with mRNA encoding a tumor-associated antigen	NCT01456104
	II	Active	Lipo-MERIT	NCT02410733
	I	Terminated	Dendritic cells - transfected with hTERT-, survivin- and tumor cell-derived mRNA+ex vivo T cell expansion and reinfusion+Temozolomide	NCT00961844
	I	Terminated	(NCI)-4650, an mRNA-based, personalized cancer	NCT03480152
	I/II	Terminated	vaccine Autologous dendritic cells with mRNA	NCT0092901
Ovarian Cancer	I	Recruiting	W_ova1 + carboplatin/paclitaxel	NCT04163094
	I/II	Terminated	DC-006 vaccine	NCT01334047
	I	Terminated	DCs loaded with TERT-mRNA and survivin-peptide	NCT01456065
Prostate cancer	I	Completed	mRNA-transfected dendritic cells	NCT01278914
	II	Completed	DCs transfected with PSA, PAP, survivin and hTERT mRNA+docetaxel	NCT01446731
	I/II	Completed	DC loaded with protamine/mRNA encoding keyhole limpet hemocyanin (KLH) + DC loading with MHC I binding peptides, NY-ESO-1 and MUC1 PepTivator®	NCT02692976
	I	Active	Dendritic cell vaccine	NCT01197625
	II	Withdrawn	Human telomerase reverse transcriptase mRNA (hTERT	NCT01153113
	I	Terminated	mRNA) transfected dendritic cell	
	I/II	Completed	CV9104	NCT02140138
	I	Completed	Peptide vaccine+montanide ISA-51+/−GM-CSF+/−imiquimod+/−mRNA/protamin	NCT02452307
Gastrointestinal Cancer	1/II	Completed	CEA mRNA-loaded DCs	NCT00228189
	I/II	Completed	Personalized mRNA vaccine encoding neoantigen	NCT03468244
	I/II	Recruiting	Adenovirus-transfected autologous DCs + CIK cells	NCT02693236

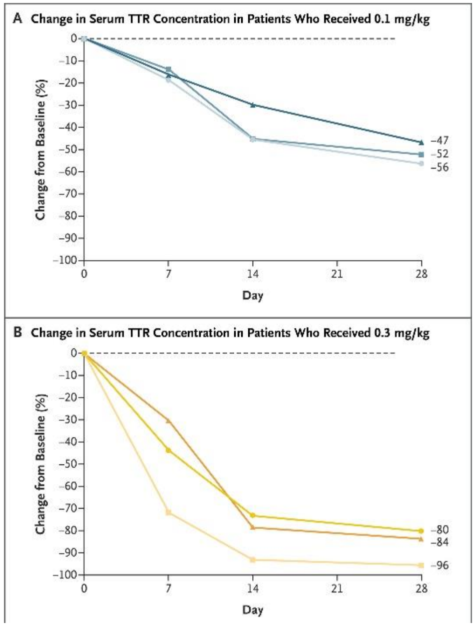
Meisel and Pascolo “mRNA Vaccines Against Infectious Diseases and Cancer”, submitted

Synthetic mRNA: Tomorrow

- Vaccines against (all) viruses
- Vaccines against cancer (shared tumor antigens or individualised vaccines against mutations)
- Vaccines against allergies

- Expression of therapeutic proteins: erythropoïétine, antibodies, bispecifics, etc
- Regeneration: blood vessels, retina, skin, muscles, neurones…
- Reprogramming cells: iPSC, CAR-T cells
- Modifying genomes (Meganucleases, TALEN, CRISPR/CAS)

CRISPR-Cas9 In Vivo Gene Editing for Transthyretin Amyloidosis
Gillmore, et al. Lebwohl. N Engl J Med 2021; 385:493-502

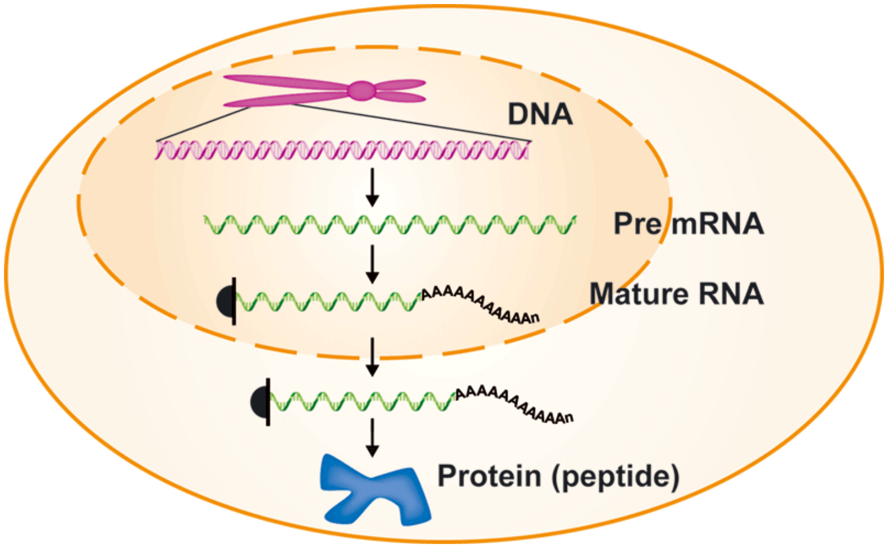


- Tolérisation spécifique du système immunitaire

[A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis.](#)
Krienke, et al. Sahin. Science. 2021 Jan 8;371(6525):145-153.

Beyond mRNA: Chemically synthesized RNA oligonucleotides

Enzymatically synthesized RNA (>100 b)



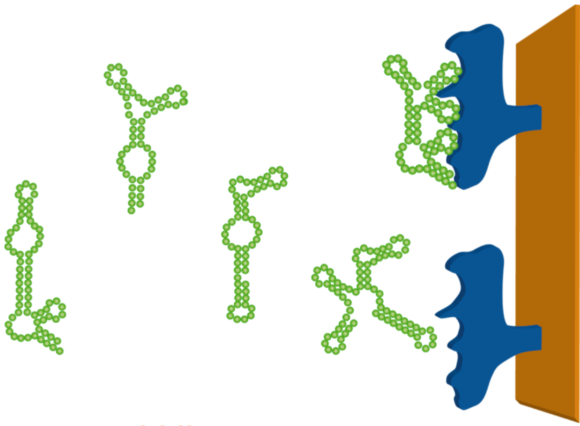
Messenger RNA

cRNA libraries

Bring proteins

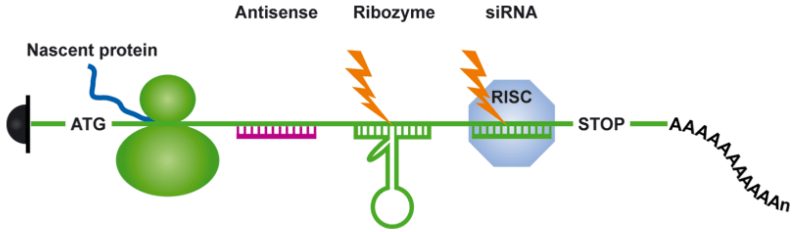
Chemically synthesized RNA (<100 b)

Aptamer (Macugen)



Antisense (Fomivirsen CMV)/ribozyme

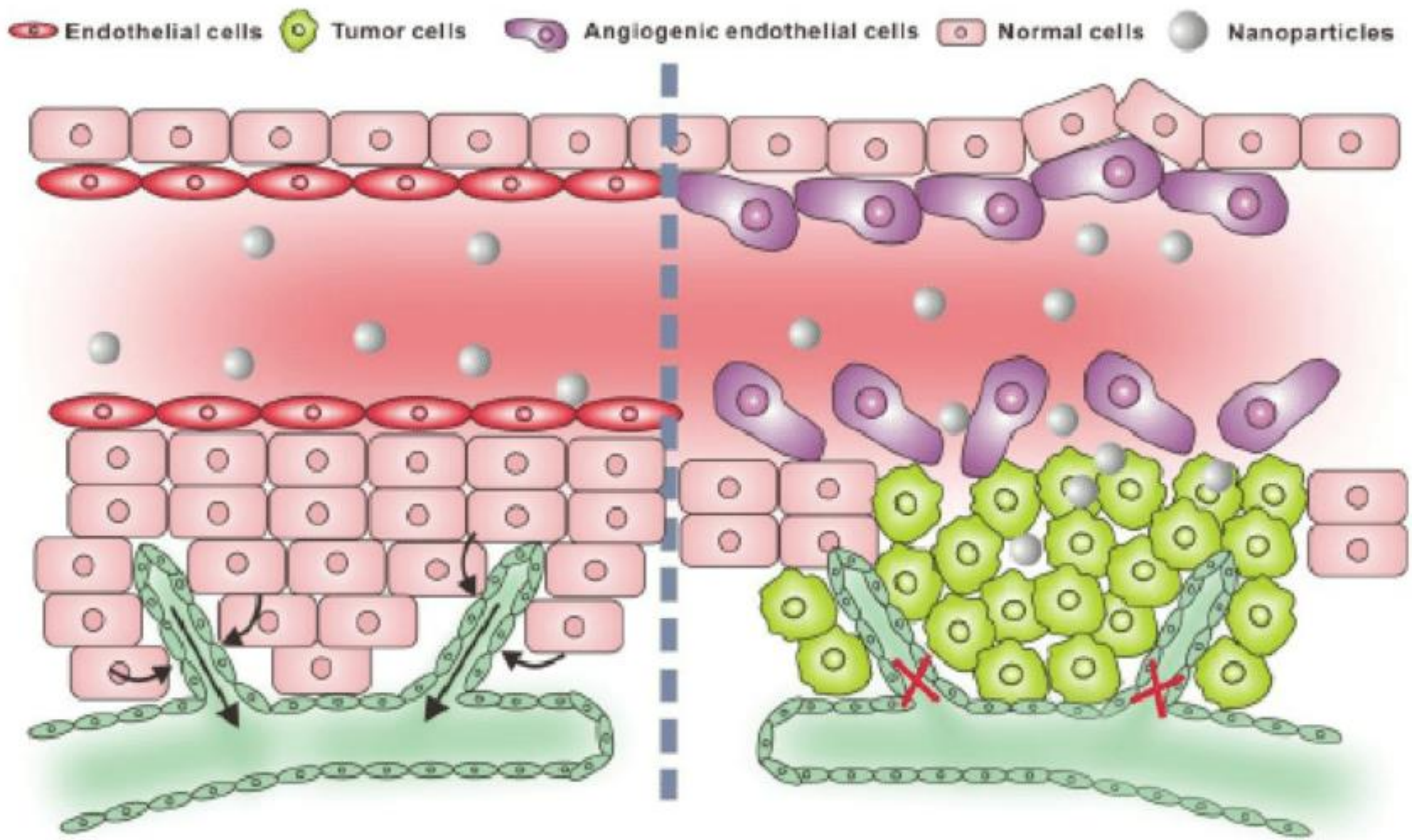
siRNA (Onpattro hereditary transthyretin-mediated amyloidosis)



Block a protein

Immunostimulation (TLR or RIG-I)

Enhanced Permeability and Retention (EPR) effect



From:
Xie X1, Zhang Y1, Li F1, Lv T1, Li Z1, Chen H1, Jia L1, Gao Y1.
Challenges and Opportunities from Basic Cancer Biology for Nanomedicine for targeted drug delivery
Curr Cancer Drug Targets. 2019;19(4):257-276. doi: 10.2174/1568009618666180628160211.

Enhanced Permeability and Retention (EPR) effect

Organic nanoparticles: Lipoplexe (liposomes or micelles), polyplexes, lipopolyplexes

Nab-paclitaxel (Abraxane®) [36] and pegylated liposomal doxorubicin (Doxil®/Caelyx®) [37] are the first-generation nanomedicine by EPR effects

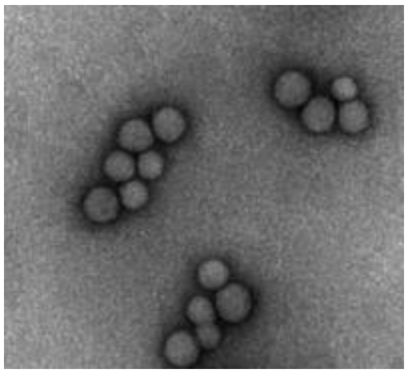
Optimal characteristics for EPR

- Size: less than 200nm, more than 10nm
- Charge: neutral or negative
- Surface: pegylated to avoid uptake by the reticular endothelial system (RES)
- Shape: rods, discs, hemispheres, ellipsoids may target tumors more effectively than spherical nanoparticles

Protamine-RNA nanoparticles

RNA mixed with Protamine generates nanoparticles of defined sizes

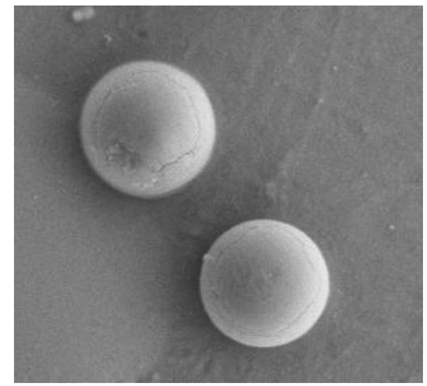
Protamine and RNA diluted in water (no salt)



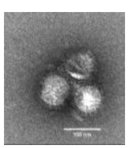
0.2 µm

+

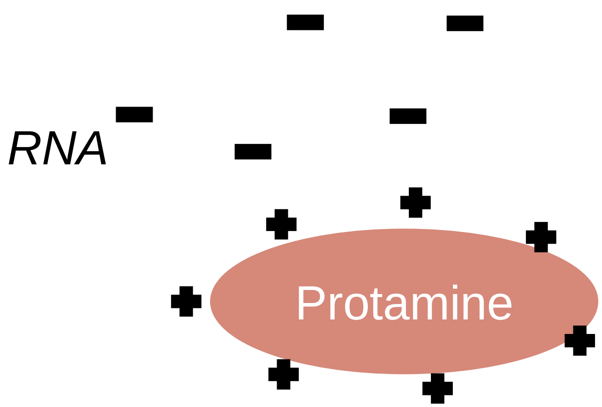
Protamine and RNA diluted in Ringer (125mM salt)

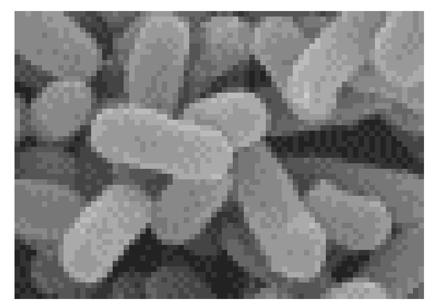


1 µm



Influenza virus





E. coli

Rettig, *et al.* Blood. 2010

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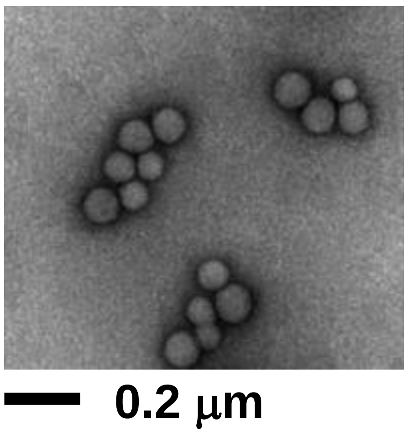
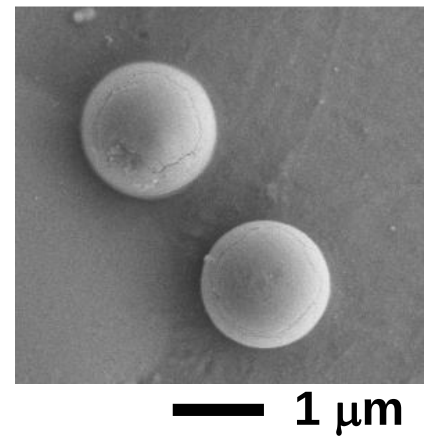
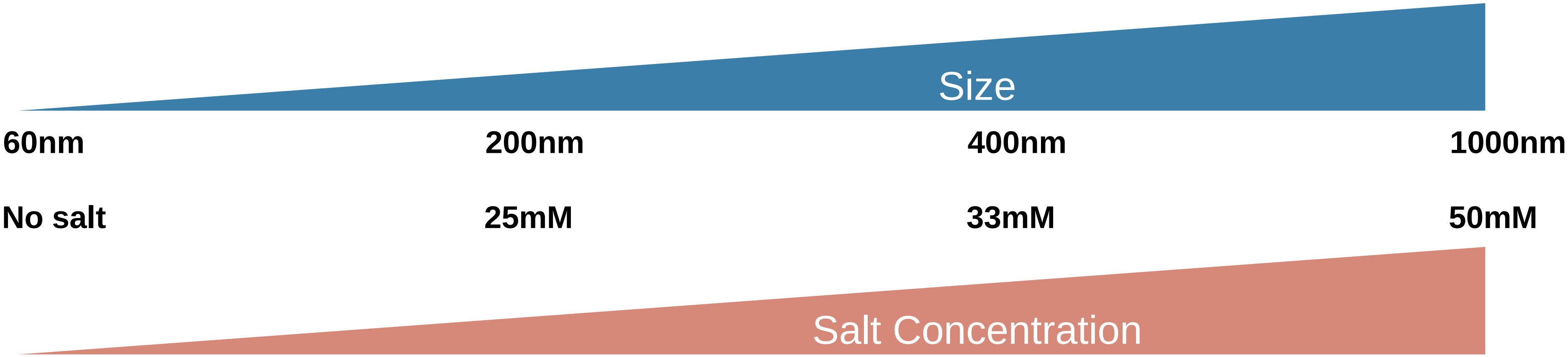


Protamine and RNA diluted in Ringer (125mM salt)





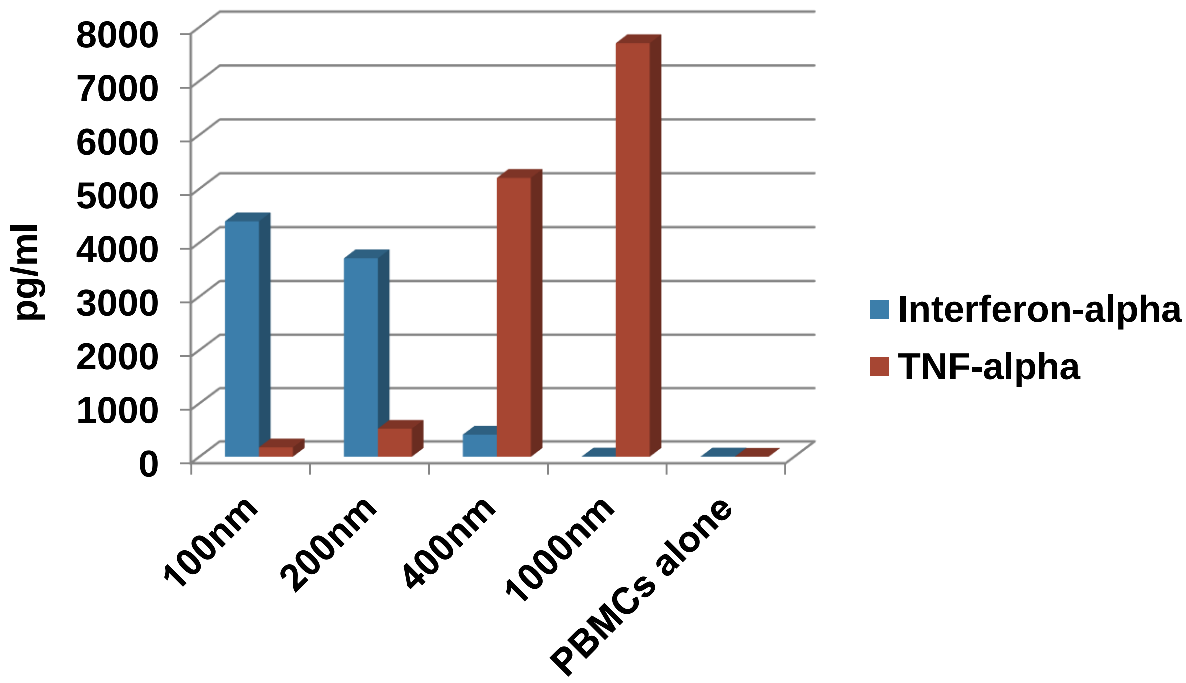
Rettig, *et al.* Blood. 2010

Protamine-RNA nanoparticles

RNA mixed with Protamine generates nanoparticles of defined sizes

Differential immunostimulation of human PBMCs *in vitro*

Human blood cells incubated 18 hours with Protamine-RNA nanoparticles (2.5 micrograms per ml)



Rettig, Haen, Bittermann, von Boehmer, Curioni, Kramer, Knuth and Pascolo. Particle size and activation threshold: a new dimension of danger signaling. Blood 2010

Tusup & Pascolo. Generation of Immunostimulating 130 nm Protamine-RNA nanoparticles. Methods Mol Biol. 2017.

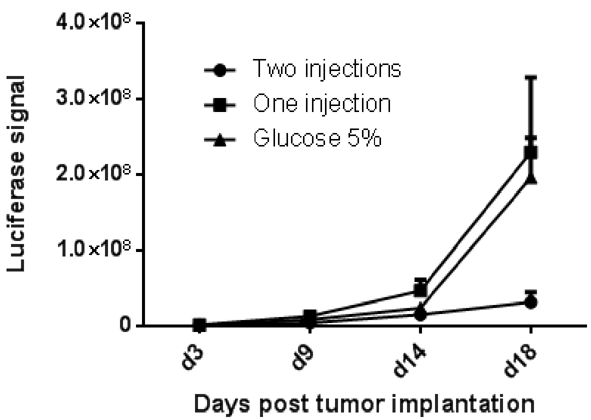
Protamine-RNA nanoparticles

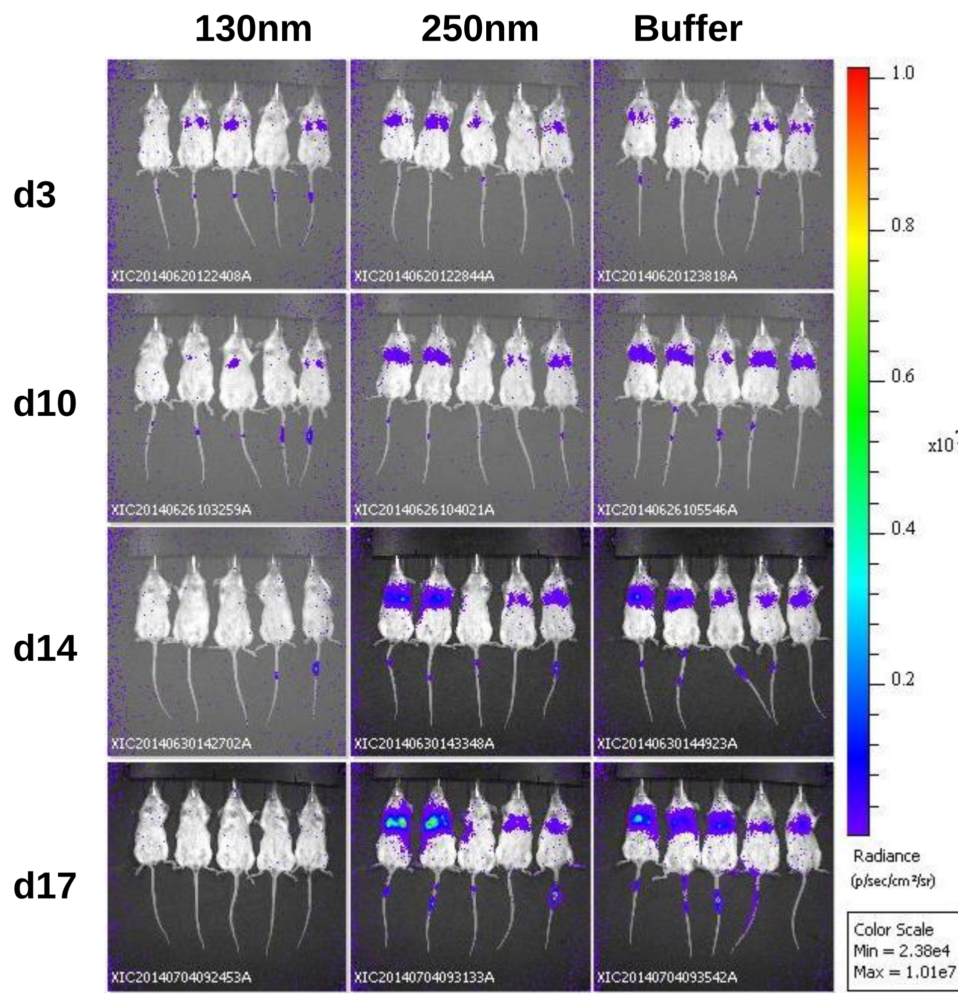
RNA mixed with Protamine generates nanoparticles of defined sizes

Injection schedule in mice

Two intravenous injections of 130nm particles can cure established lung tumors

BALB/c mice injected intra-venous with
D0: CT26-luciferase
D3: 2x Protamine-mRNA particles
D10: 2x Protamine-mRNA particles





Protamine-RNA nanoparticles

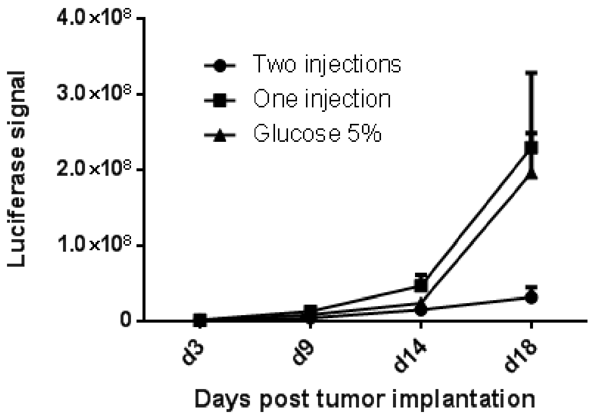
RNA mixed with Protamine generates nanoparticles of defined sizes

Injection schedule in mice

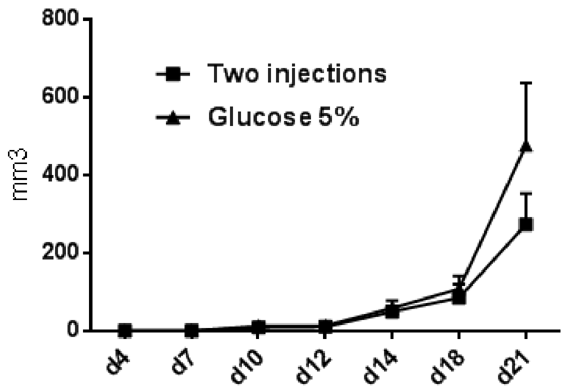
Two intravenous injections of 130nm particles can cure established lung tumors

However: no significant efficacy on established sub-cu tumors

CT26 intravenous



CT26 sub-cutaneous



Protamine-RNA nanoparticles

RNA mixed with Protamine generates nanoparticles of defined sizes

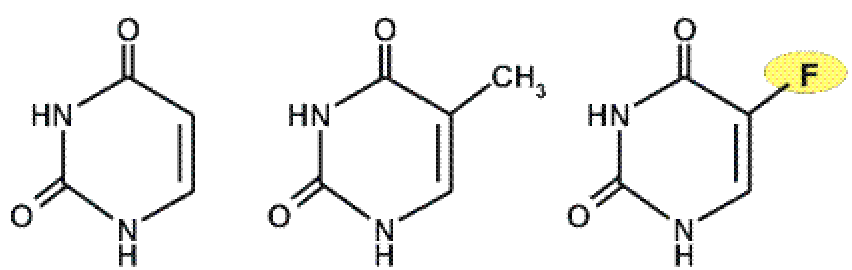
Purine / pyrimidine analogs

Nelarabine
Mercaptopurine

Cladribine
Clofarabine
Fludarabine
Pentostatine

A C G U

Azacitidine
Cytarabine
Decitabine
Gemcitabine



Uracile Thymine 5-Fluoro-Uracile

5FU / Capecitabine

Protamine-RNA nanoparticles

RNA mixed with Protamine generates nanoparticles of defined sizes

Injection schedule in mice

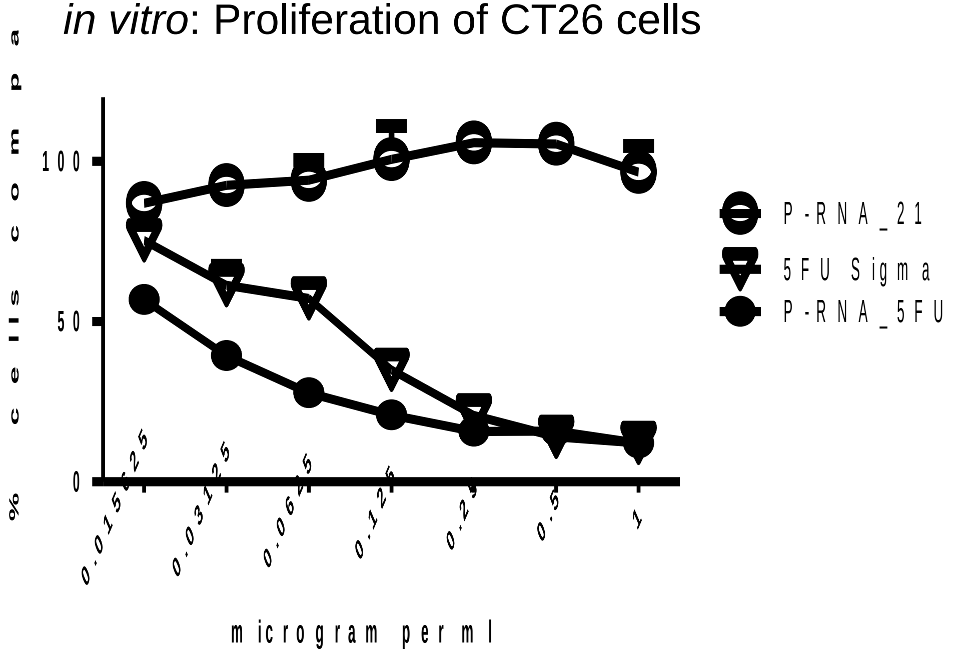
Two intravenous injections of 130nm particles can cure established lung tumors

However: no significant efficacy on established sub-cu tumors

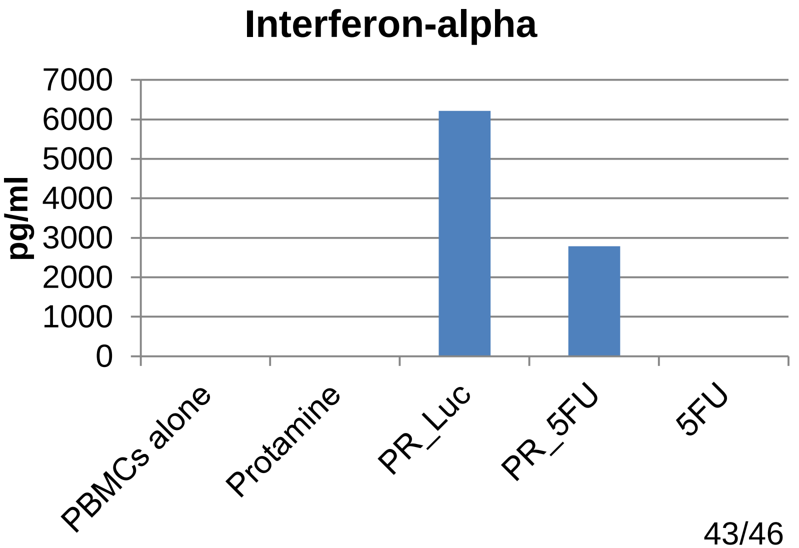
5FU containing RNA: IMMUNOCHEMOTHERAPEUTIC RNA OLIGO

5‘ A.G.U(5F).G.U(5F).U(5F).A.U(5F).U(5F).C.U(5F).U(5F).G.U(5F).A.U(5F).G.G.U(5F).U(5F).G 3‘

in vitro: Proliferation of CT26 cells



Stimulation hPBMCs



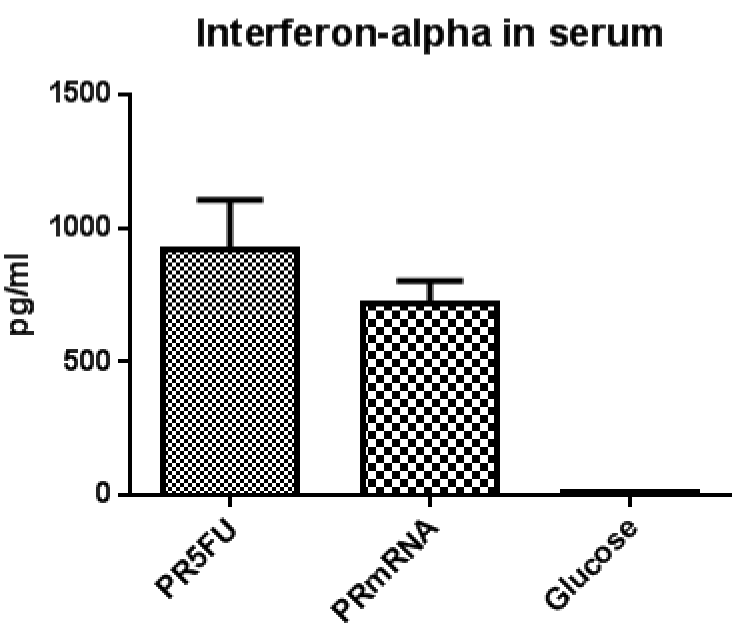
Protamine-RNA nanoparticles

RNA mixed with Protamine generates nanoparticles of defined sizes

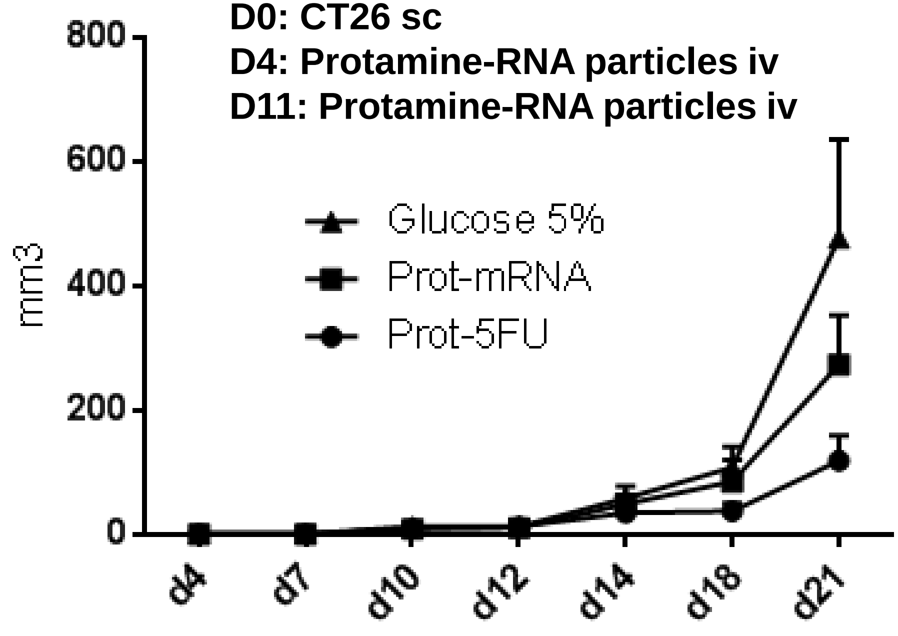
Immunochemotherapeutic RNA:
5‘ A.G.U(5F).G.U(5F).U(5F).A.U(5F).U(5F).C.U(5F).U(5F).G.U(5F).A.U(5F).G.G.U(5F).U(5F).G 3‘

Immunostimulating RNA: mRNA coding Luciferase

in vivo: Tumor bearing mice, 20 μg RNA + 20 μg Protamine

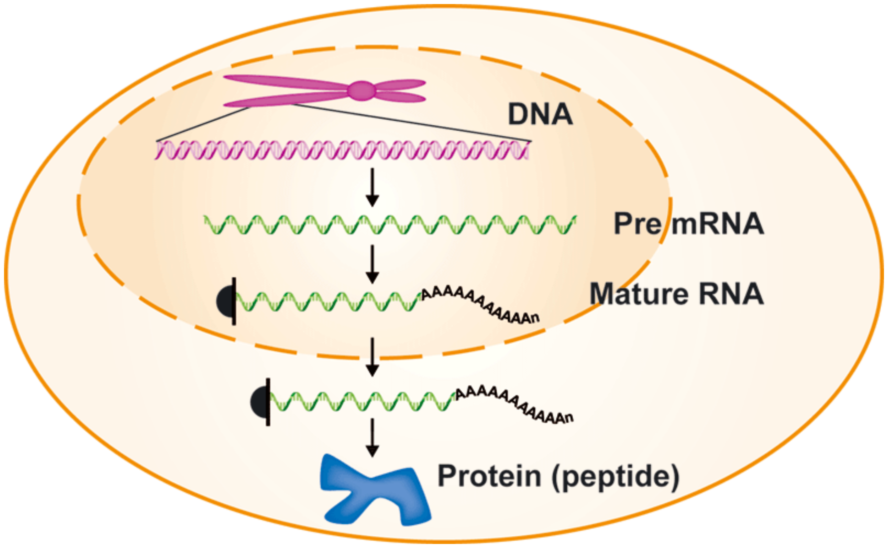


BALB/c mice injected with
D0: CT26 sc
D4: Protamine-RNA particles iv
D11: Protamine-RNA particles iv



Beyond mRNA: Chemically synthesized RNA oligonucleotides

Enzymatically synthesized RNA (>100 b)

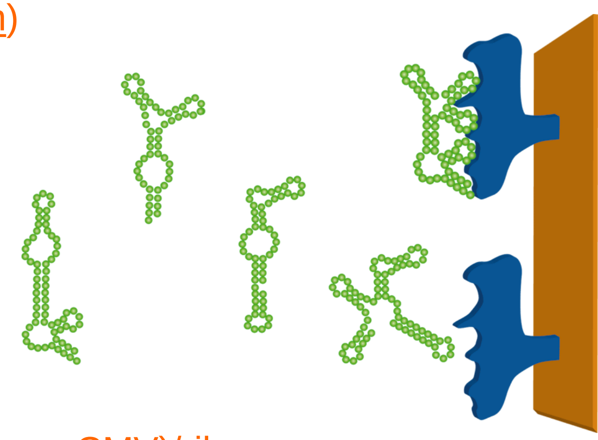


Messenger RNA

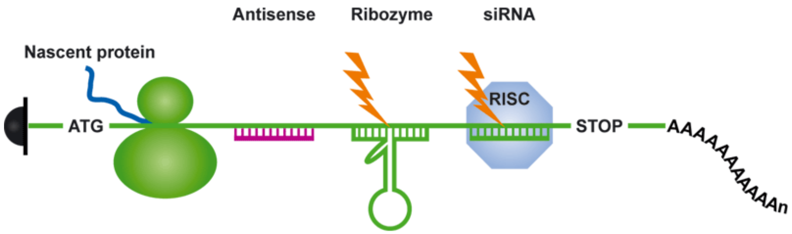
cRNA libraries

Chemically synthesized RNA (<100 b)

Aptamer (Macugen)



Antisense (Fomivirsen CMV)/ribozyme
siRNA (Onpattro hereditary transthyretin-mediated amyloidosis)



Bring proteins

Immunostimulation (TLR or RIG-I) Immunochemotherapy

Block a protein

Thanks for your attention

Synthetic Messenger RNA-Based Vaccines: from Scorn to Hype.
Pascolo S.
Viruses. 2021 Feb 9;13(2):270. doi: 10.3390/v13020270.

Steve.pascolo@usz.ch

Swiss National Science Foundation NRP78
“An optimised prophylactic mRNA vaccine against coronavirus disease 2019”
EU grant «NEWmRNA»
Monique Dornonville de la Cour Stiftung
UZH URPP «Translational Cancer Research»
UZH Stiftung für wissenschaftliche Forschung