Developing mRNA for therapy

Katalin Karikó, PhD
University of Pennsylvania, Philadelphia

2022 Laureate of the Science for the Future Solvay Prize

INTERNATIONAL SOLVAY INSTITUTES BRUSSELS

Solvay Prize Lecture
March 29, 2022

CHEMISTRY FOR THE FUTURE Solvay Prize

Visiting Brussels

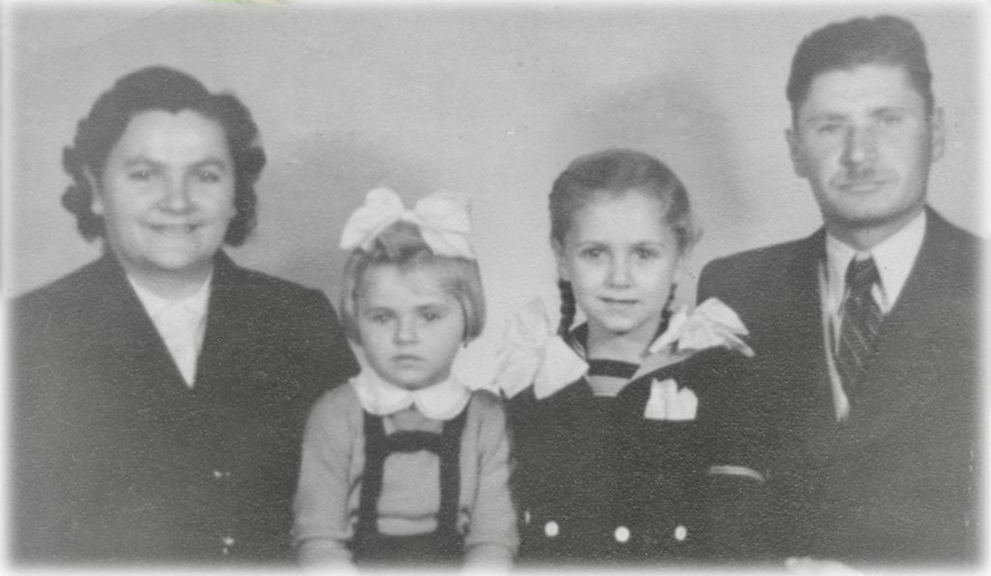
1977

Visiting Brussels

1977

2022

1955-60s growing up in Hungary



1968-69 competing in science


EMLÉKLAP

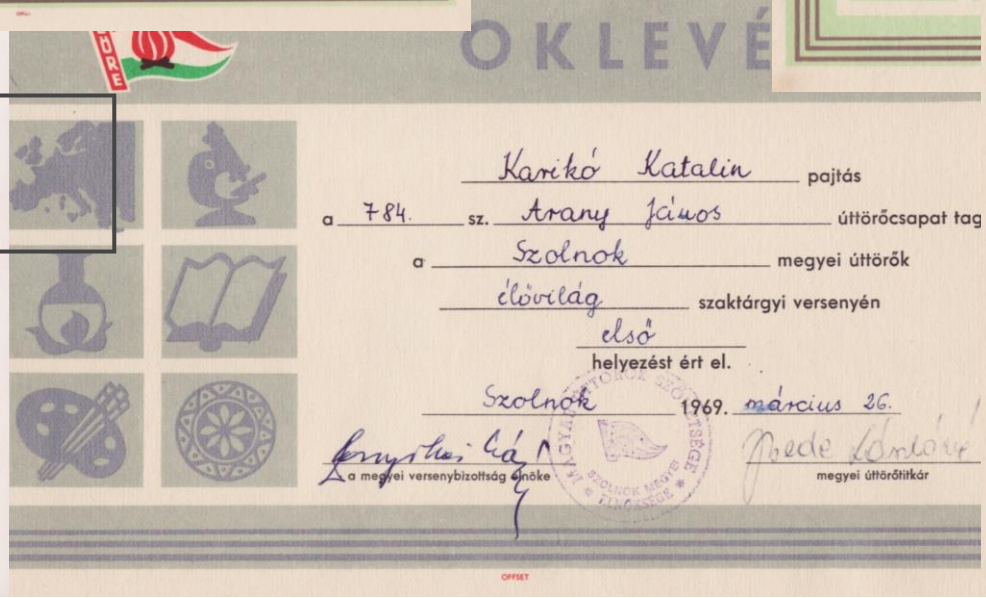
KARIKÓ KATALIN pajtás
a 784. sz. Arany János úttörőcsapat tagja
a Kisújszállás járási (városi, kerületi)
úttörő élővilág I. h. szaktárgyi versenyen részt vett.
Kisújszállás 1968. III. hó 26.
a versenybizottság elnöke
úttörőtitkár


OKLEVÉL

Karikó Katalin pajtás
a 784. sz. Arany János úttörőcsap
a Türkeve-Kisújszállás járási (városi, kerületi)
úttörők kémia szaktárgyi versenyén
hatodik
helyezést ért el.


OKLEVÉL

Karikó Katalin
pajtás
a 784.sz. Arany János Úttörőcsapat tagja
a KISUJSZÁLLÁS--TURKEVE városi
uttörők TERMÉSZETISMERET szaktárgyi versenyén
első
helyezést ért el
Kisujszállás, 1969. február 11-én
MÜv.csop.vez
úttörőtitkár


OKLEVÉ

Karikó Katalin pajtás
a 784. sz. Arany János úttörőcsapat tag
a Szolnok megyei úttörők
élővilág szaktárgyi versenyén
első
helyezést ért el.
Szolnok 1969. március 26.
a megyei versenybizottság elnöke
megyei úttörőtitkár


EMLÉKLAP

Karikó Katalin pajtás
a 784. sz. Arany János úttörőcsapat tagja
az úttörők természetismeret szaktárgyi versenyének
országos döntőjén részt vett.
Budapest, 1969. junius 1o.
az országos versenybizottság elnöke
a Magyar Úttörők Szövetsége Országos Elnöksége főtitkára

1968 – Csillebérc - Camp for 7th graders, winners of the regional biology competition

1960-70s

Albert Tóth
biology teacher

Zoltán Csobay
chemistry teacher

Móricz Zsigmond High School
Kisújszállás

Albert Szent-Györgyi

János Selye

1973-85

University of Szeged
1973-78

Biological Research Center
Hungarian Academy of Sciences

The Lipid Lab

Biological Research Center
Hungarian Academy of Sciences

Tibor Farkas

Éva Kondorosi

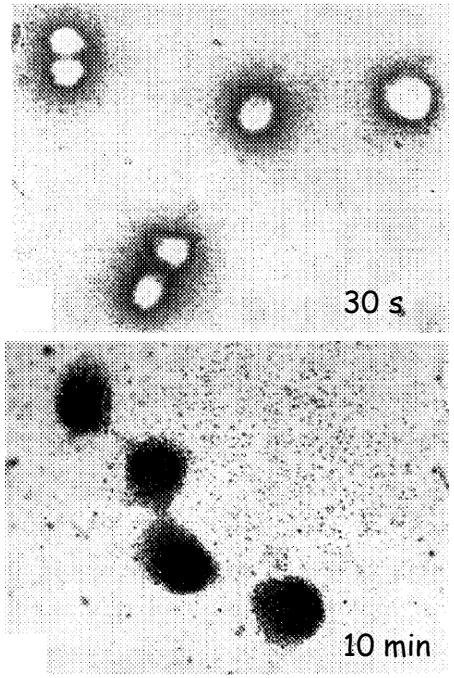
Ernö Duda

Acta Biochim. et Biophys. Acad. Sci. Hung. Vol. 20 (3–4), pp. 203–211 (1985)

Liposome Mediated DNA-transfer into Mammalian Cells

G. Somlyai, É. Kondorosi, K. Karikó,* E. G. Duda

Institute of Biochemistry and *Institute of Biophysics, Biological Research Center, Szeged, Hungary



The RNA Lab

Biological Research Center
Hungarian Academy of Sciences

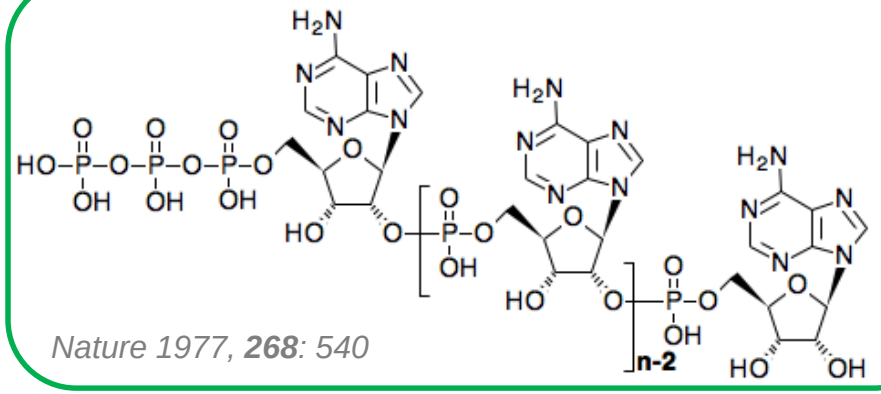
Jenö Tomasz

THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 251, No. 16, Issue of August 25, pp. 5043–5053, 1976
Printed in U.S.A.

Mechanism of Formation of Reovirus mRNA 5′-terminal Blocked and Methylated Sequence, m^7GpppG^mpC

(Received for publication, March 26, 1976)

YASUHIRO FURUICHI, S. MUTHUKRISHNAN, JENÖ TOMASZ*, AND AARON J. SHATKIN
*From the Roche Institute of Molecular Biology, Nutley, New Jersey 07110, and the *Institute of Biophysics, Szeged, Hungary*



*Nature 1977, **268**: 540*

2’-5’ oligoadenylate, 2-5A

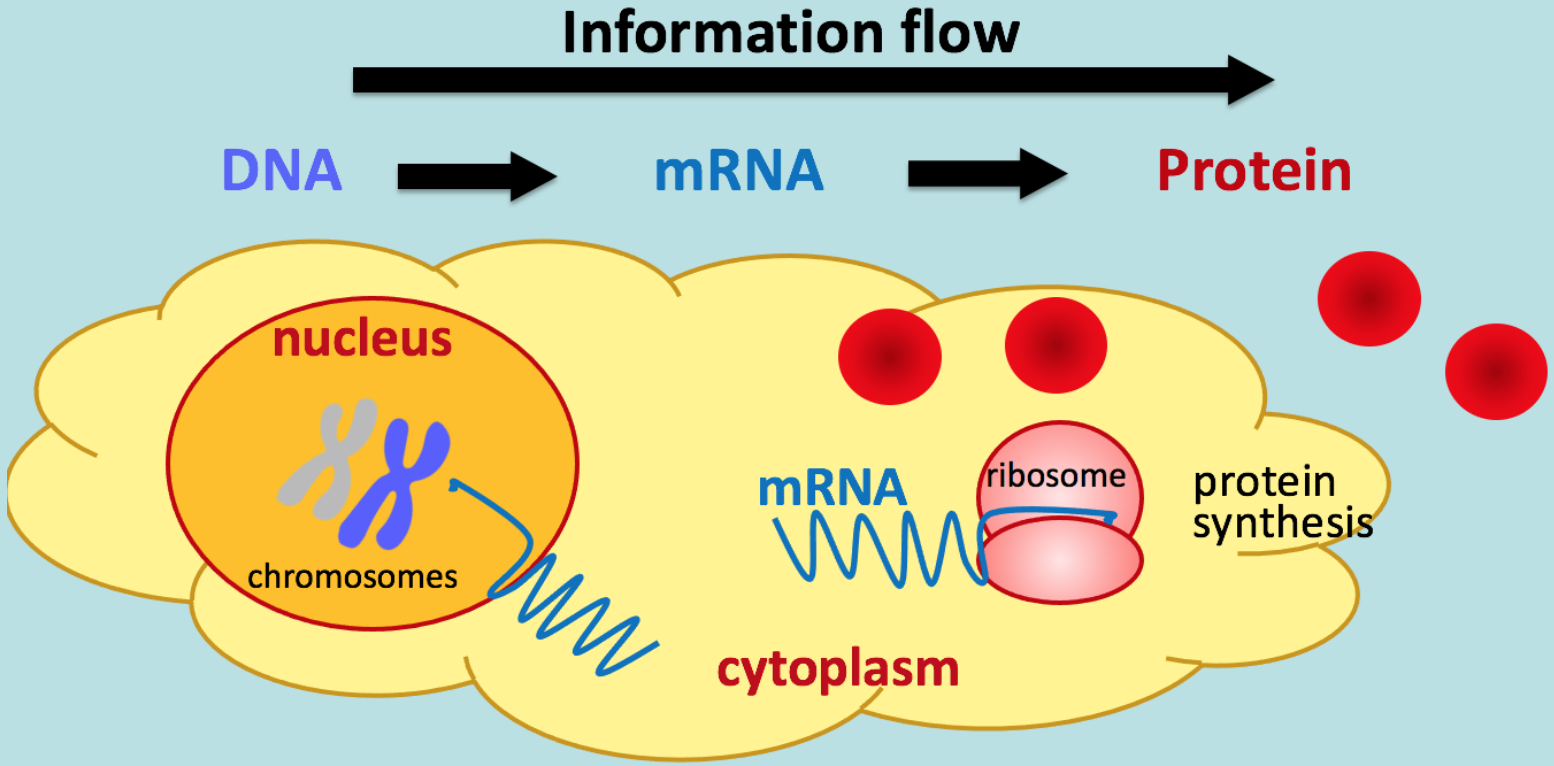
János Ludwig

Milestones of mRNA development for therapy

- **1961 Discovering mRNA**
- 1978 Isolated mRNA delivery into mammalian cells
- 1984 Synthesizing mRNA in vitro
- 1990s - Optimizing performance of the mRNA
- 2010s - Optimizing formulation for mRNA delivery
- **2021 FDA approval of COVID-19 LNP-mRNA vaccine**

1961 - Discovery of mRNA

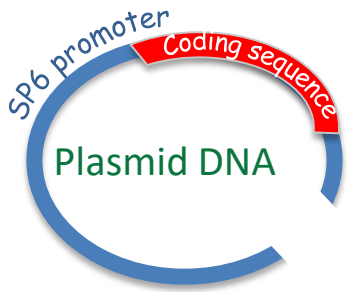
mRNA: the labile intermediate carrying the message from the DNA to ribosome



Nature 1961 , **190**: 576
Nature 1961 , **190**: 581

1984 – Synthesizing mRNA in test tube

The first in vitro synthesis of mRNA and its translation into a functional protein



Transcription/capping

+ SP6 RNA Polymerase - 1984
+ ATP, GTP, UTP, CTP
+ capping enzyme

In vitro-transcribed mRNA (IVT mRNA)

Harvard University
Douglas Melton
Paul Krieg

Nucleic Acids Res. 1984, 12: 7035, 7057

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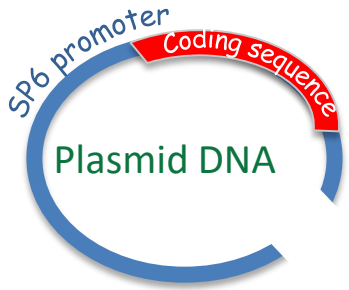

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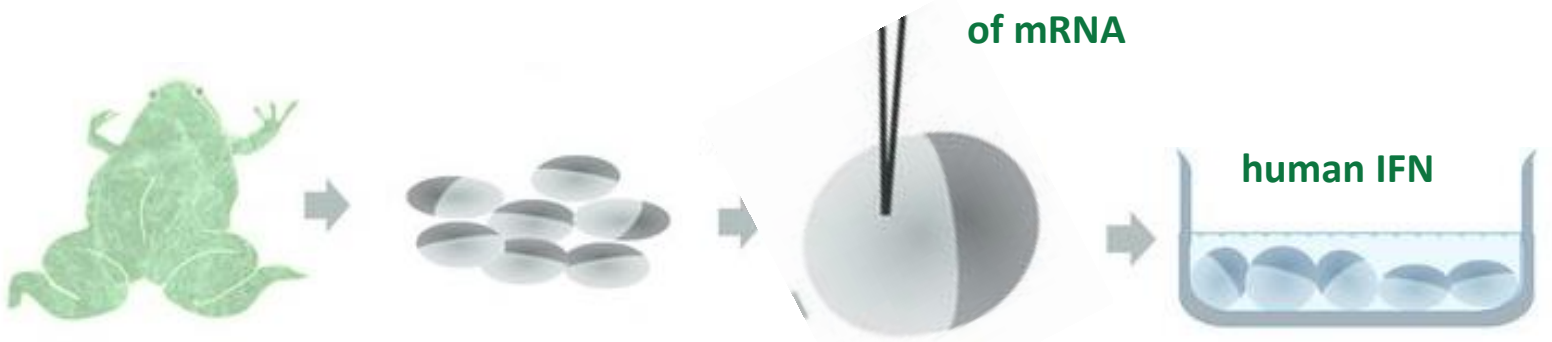
In vitro-transcribed mRNA (IVT mRNA)

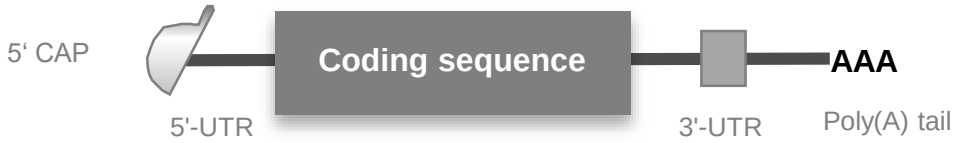
Harvard University
Douglas Melton
Paul Krieg

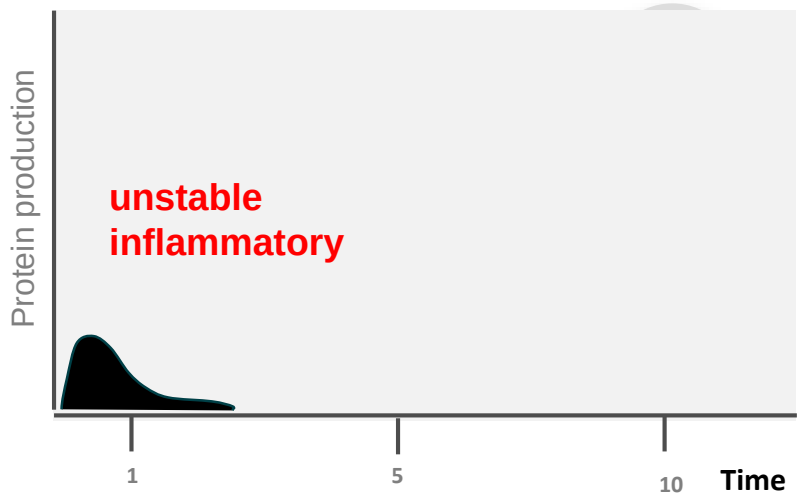
microinjection of mRNA



Nucleic Acids Res. 1984, 12: 7035, 7057

Challenges for the human use of mRNA in 1990s

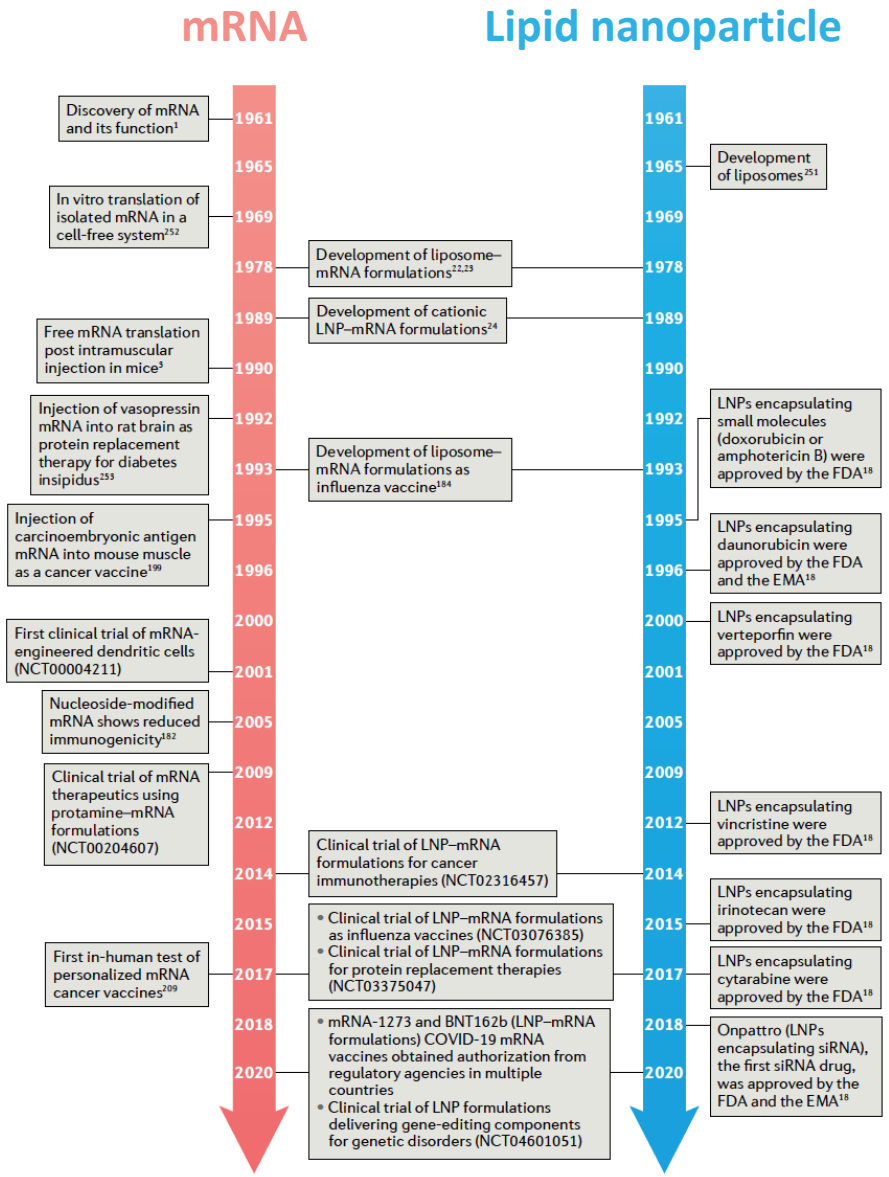




mRNA

- unstable, degrade
- amount of translated protein is too little
- cause inflammation

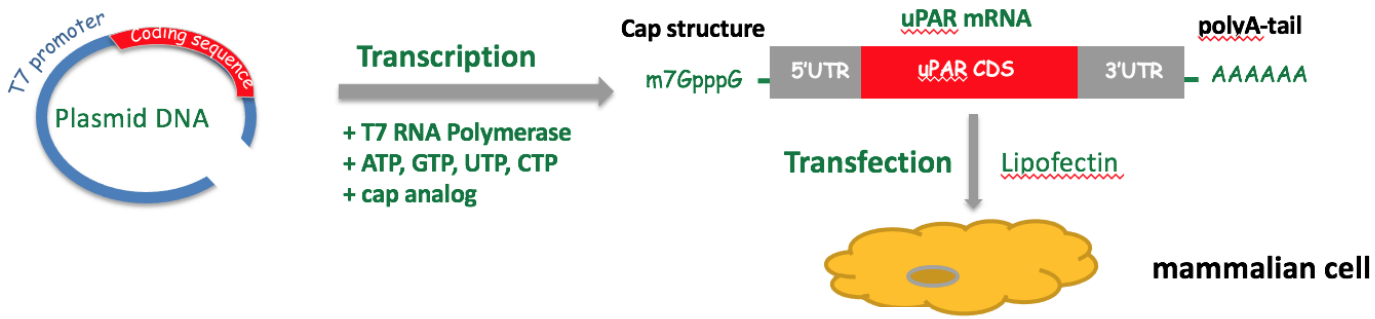
Timeline of key milestones for mRNA and lipid nanoparticle development

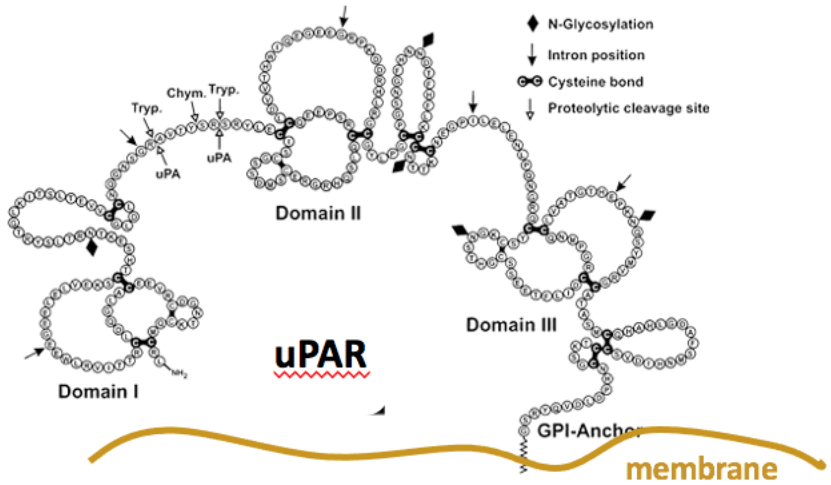


Nature Reviews Materials(2021) **6**:1078

Developing mRNA for therapy

Medical School of University of Pennsylvania, Philadelphia





In vitro-transcribed mRNA

iNOS

Catalase, SOD-1, SOD-2,
Glutathione Peroxidase, Ph-GPx
RNase1, DNase, apyrase, NT5E,

Biochimica et Biophysica Acta 1369 (1998) 320–334

Phosphate-enhanced transfection of cationic lipid-complexed mRNA and plasmid DNA

Katalin Karikó [a,*], Alice Kuo [b], Elliot S. Barnathan [b], David J. Langer [a]

Journal of Neuroscience Methods 105 (2001) 77–86

In vivo protein expression from mRNA delivered into adult rat brain

Katalin Karikó *, Jason M. Keller, Valerie A. Harris, David J. Langer, Frank A. Welsh

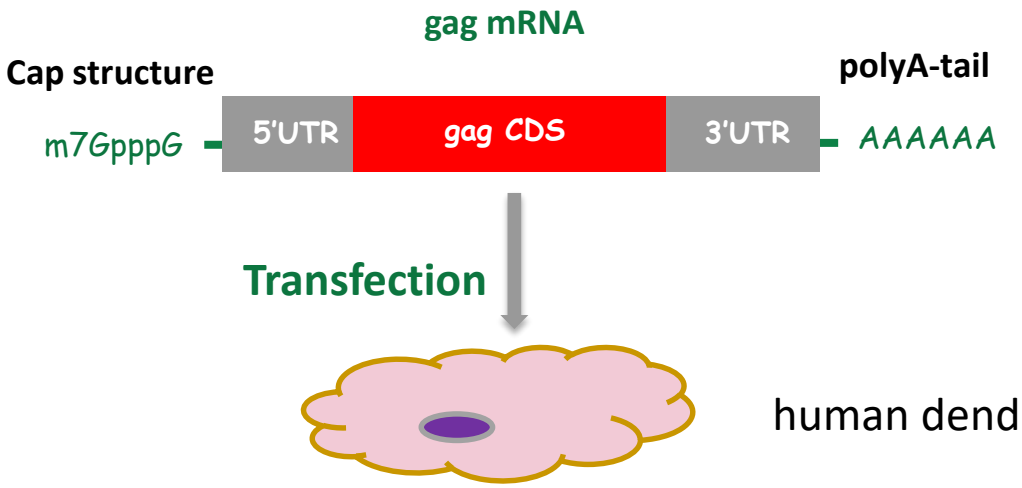
Gene Therapy (1999) 6, 1092–1100

Overexpression of urokinase receptor in mammalian cells following administration of the in vitro transcribed encoding mRNA

K Karikó[1], A Kuo[2] and ES Barnathan[2]

1998-2000 - Evaluating gag mRNA in human dendritic cells

Drew Weissman



J Immunol 2000; 165:4710-4717

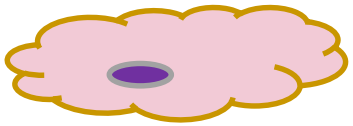
HIV Gag mRNA Transfection of Dendritic Cells (DC) Delivers Encoded Antigen to MHC Class I and II Molecules, Causes DC Maturation, and Induces a Potent Human In Vitro Primary Immune Response[1]

Drew Weissman,[2]* Houping Ni,* David Scales,* Annie Dude,* John Capodici,* Karen McGibney,* Asha Abdool,* Stuart N. Isaacs,* Georgetta Cannon,* and Katalin Karikó†

2005 - Natural RNAs are not equally potent activators of DCs

Isolated RNA

Transfection



Monocyte-derived human DCs

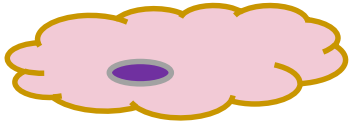
Inflammatory response
TNF-α

Immunity 2005, 23: 165

2005 - Natural RNAs are not equally potent activators of DCs

Isolated RNA

Transfection



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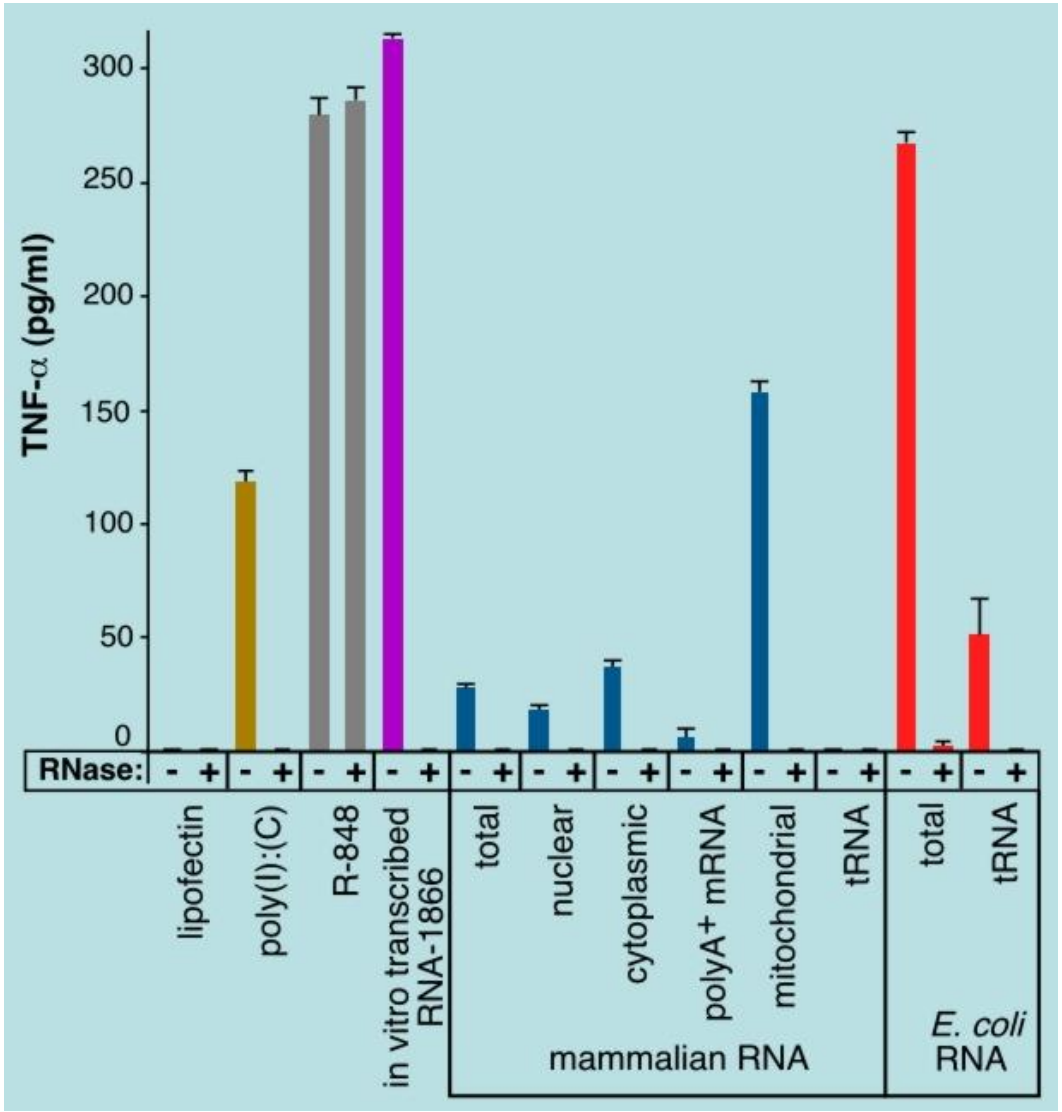
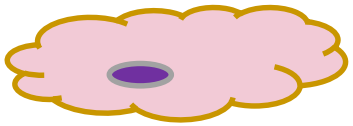


Immunity 2005, 23: 165

2005 - Natural RNAs are not equally potent activators of DCs

Isolated RNA

Transfection



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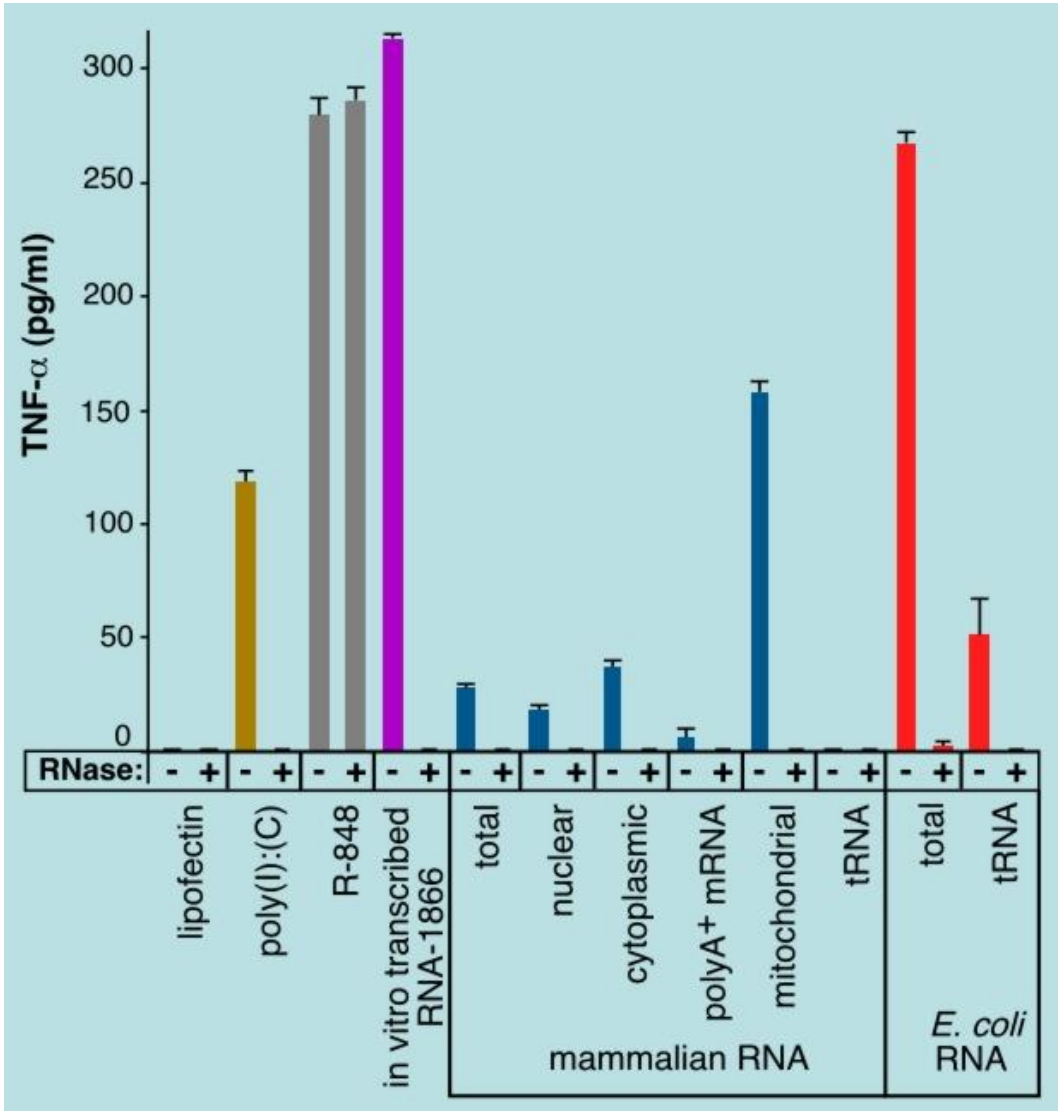
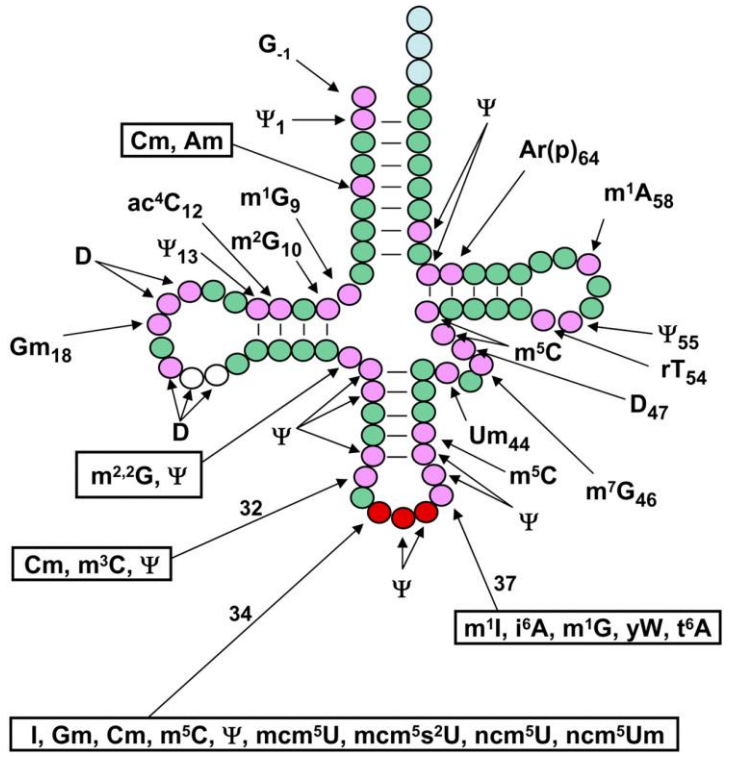
TNF-α



J Immunol 2000, 165: 4710

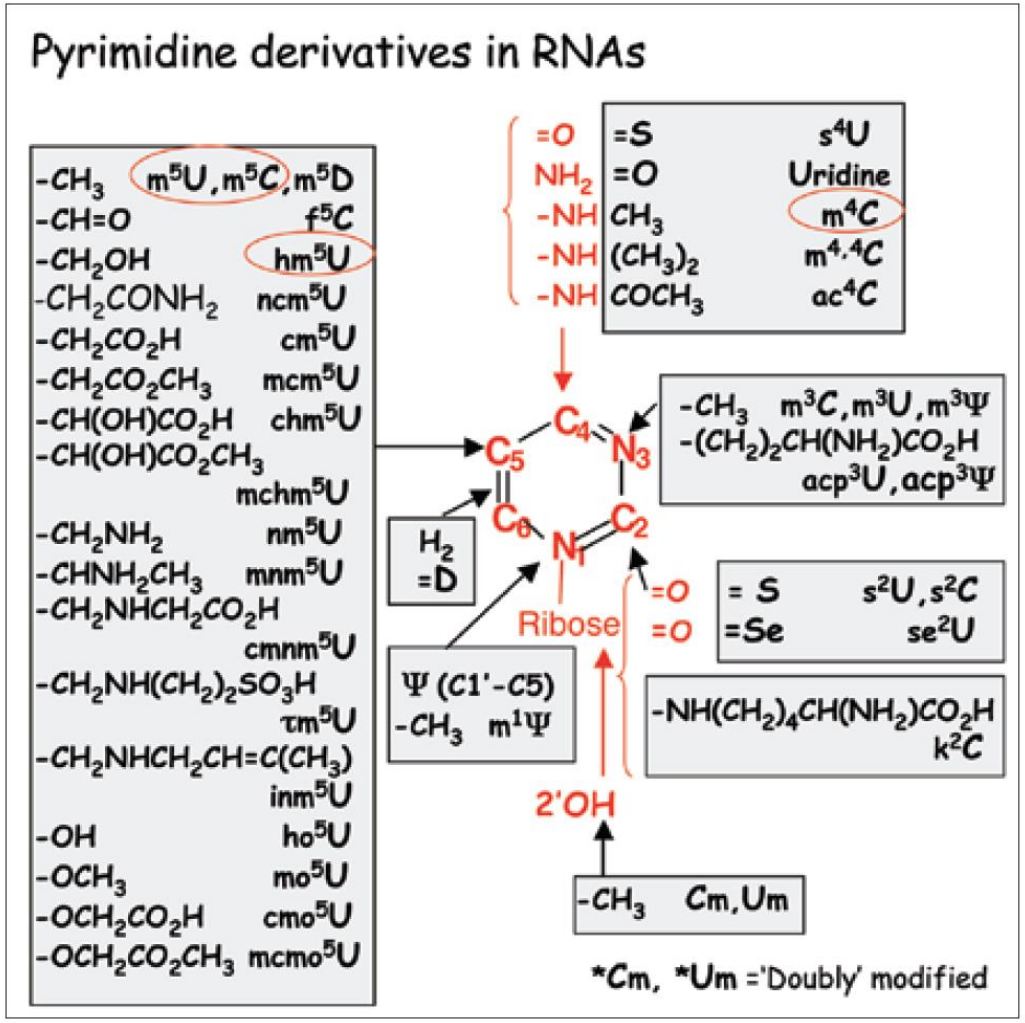
tRNA

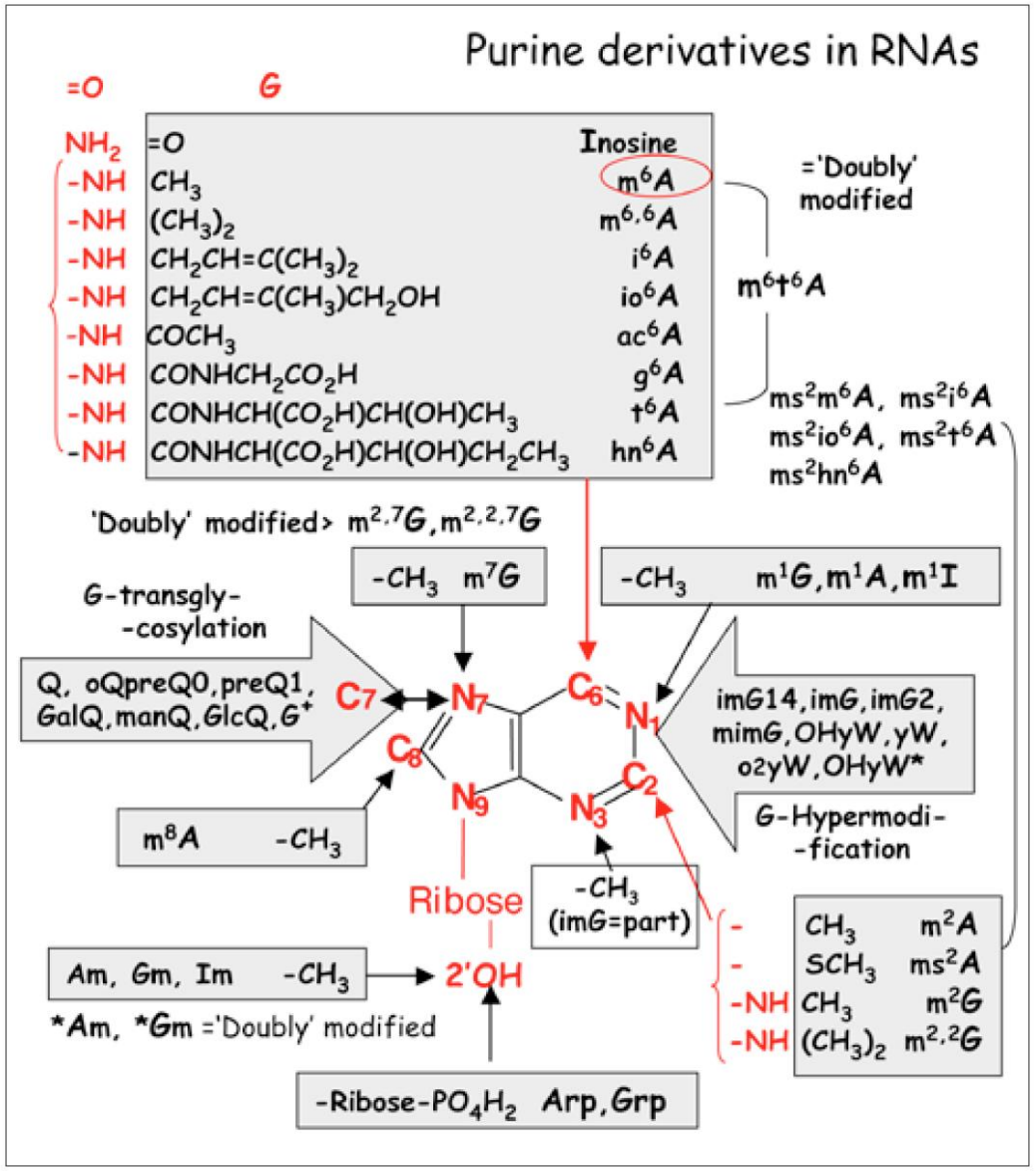


tRNA is enriched in modified nucleosides

Immunity 2005, 23: 165

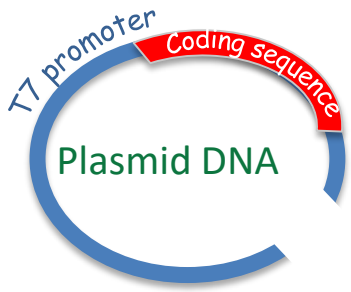
100+ Naturally-occurring modified nucleosides in RNA





DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution, edited by Henri Grosjean

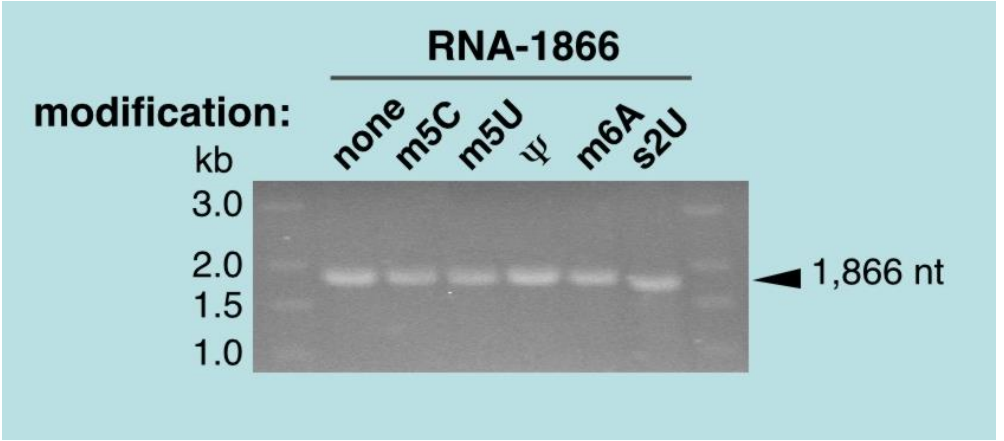
2005 - Incorporation of modified nucleotides into RNA by in vitro transcription



Transcription

+ T7 RNA Polymerase
+ cap analog
+ ATP, GTP, UTP, CTP

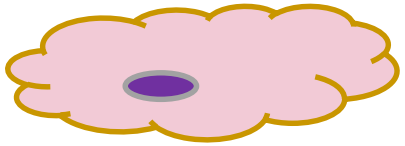
NTP	
m5C m5U Ψ m6A s2U	yes
m1A m1G m7G 2'-*O*-Met-C 2'-*O*-Met-U	no



Immunity 2005, 23: 165

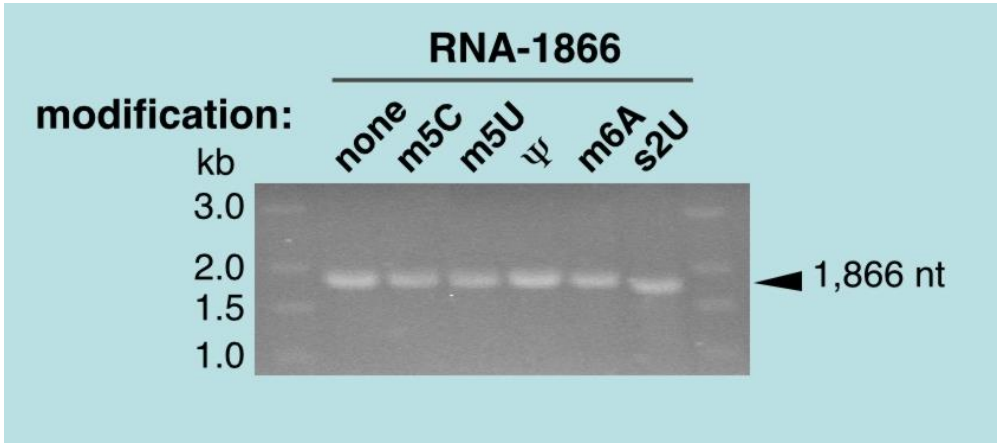
2005 – Synthesizing modified mRNA – Measurement of inflammatory response

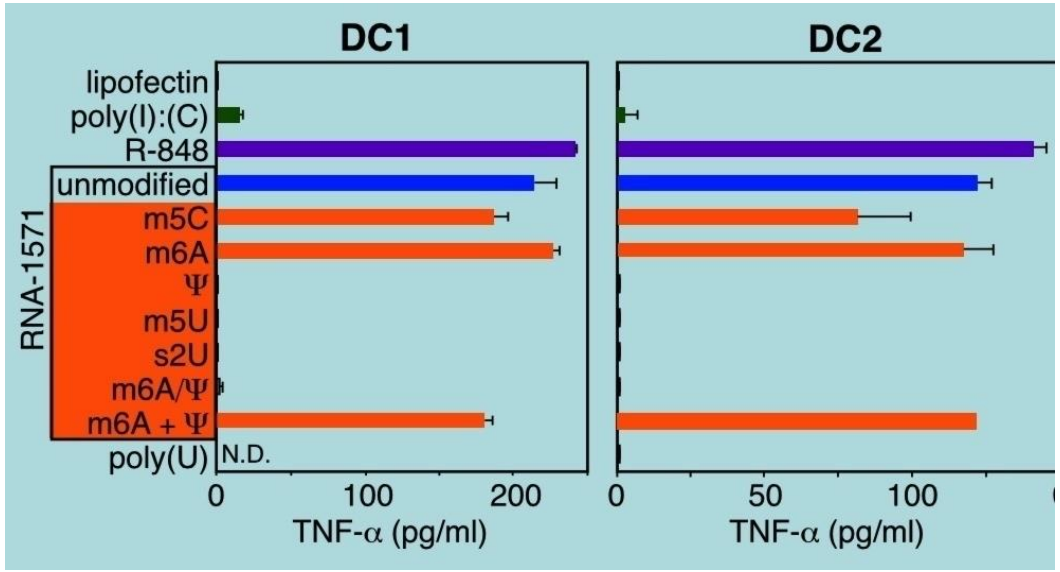
Transfection



Isolated primary human DCs

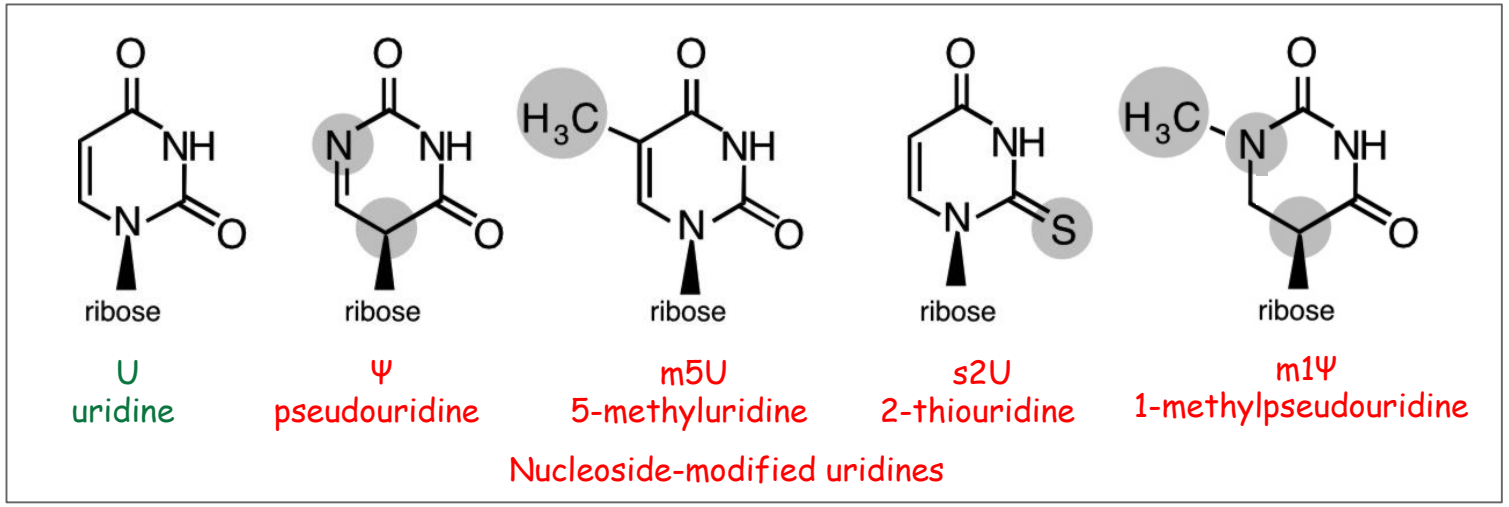
inflammatory response

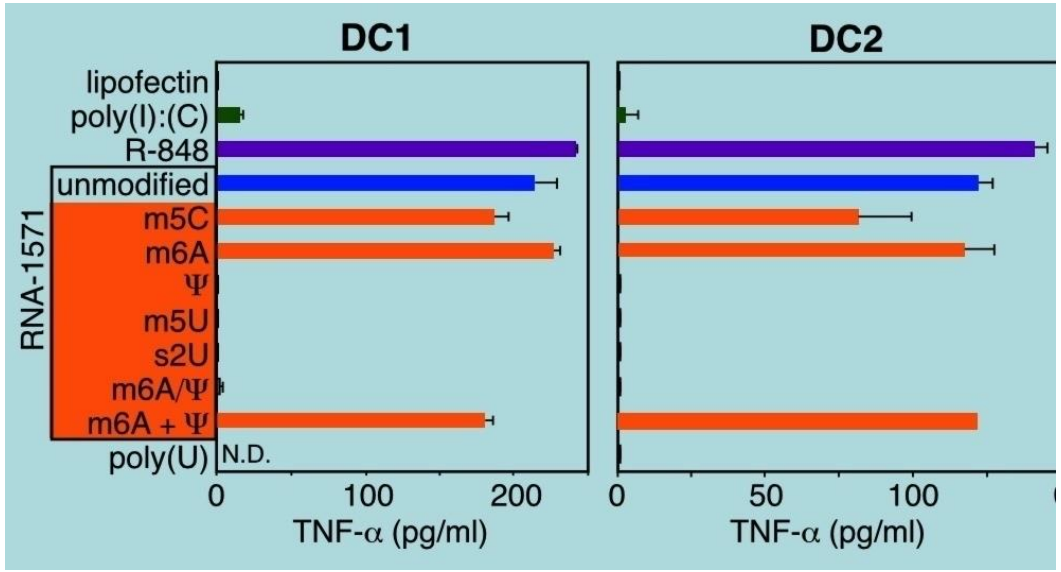




Immunity 2005, 23: 165

2005-08 Modified uridine-containing mRNA is non-immunogenic, Ψ-mRNA translates the best





Immunity 2005, 23: 165

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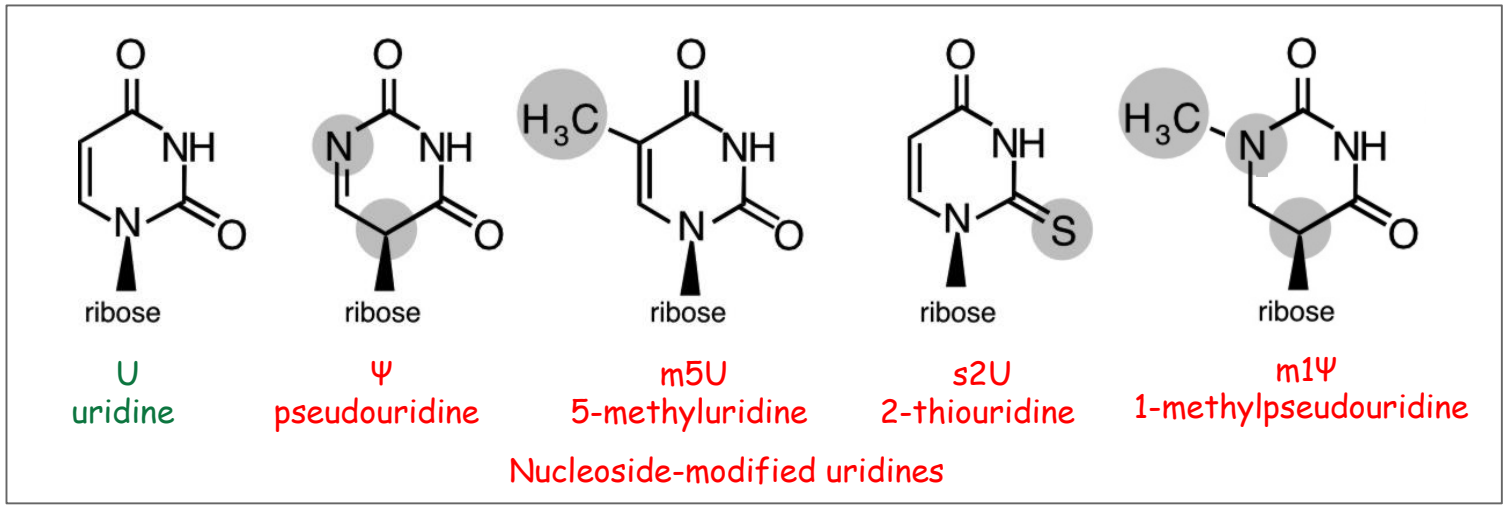
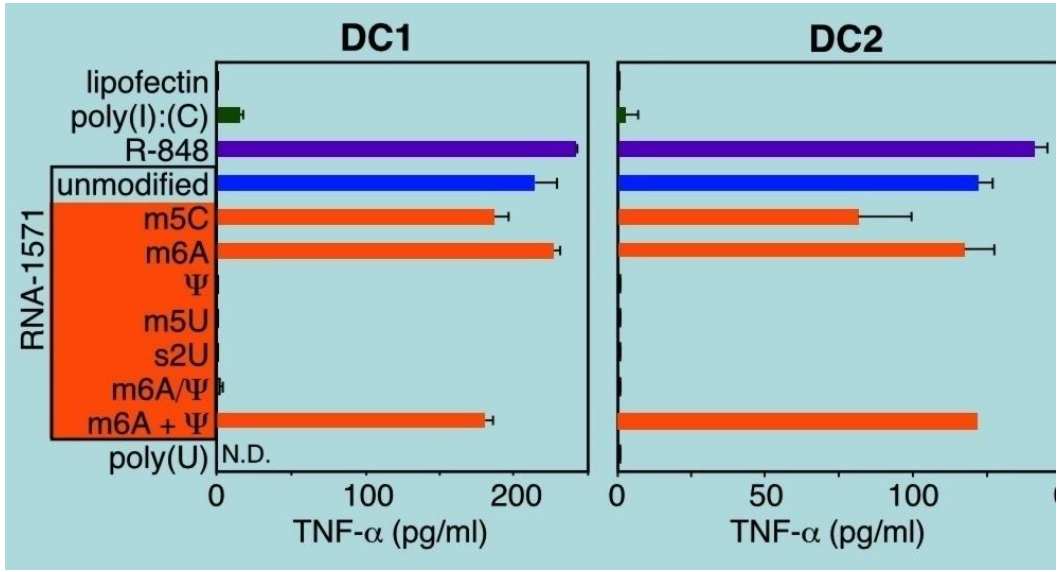
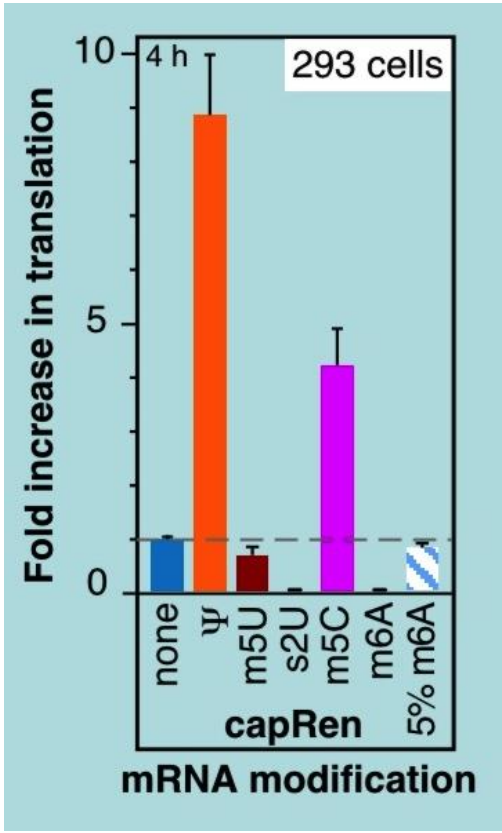


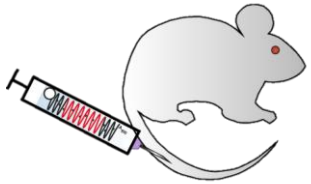


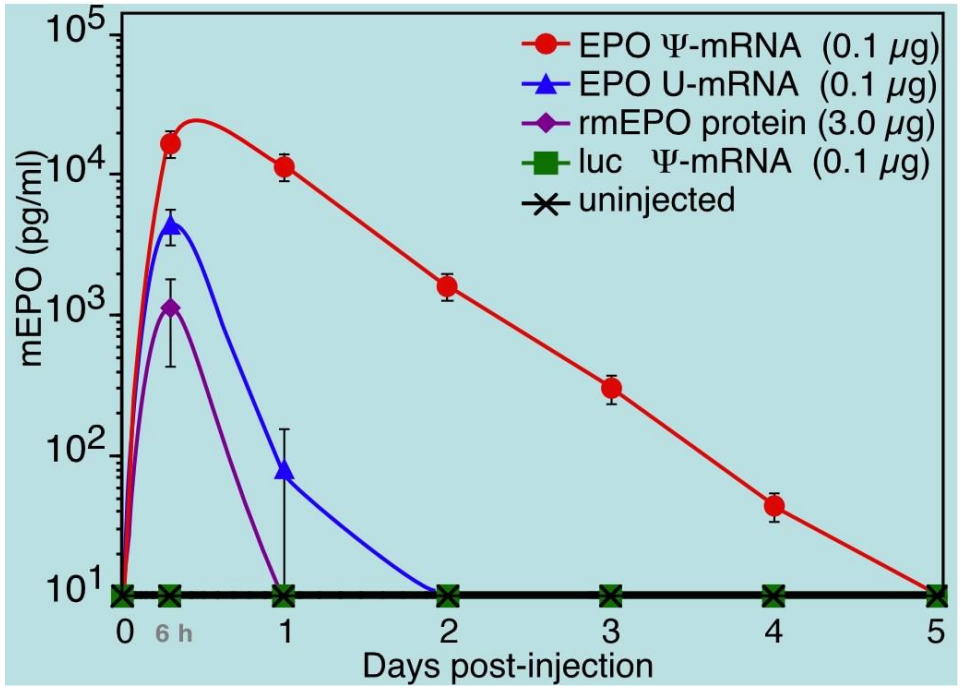
Immunity 2005, 23: 165

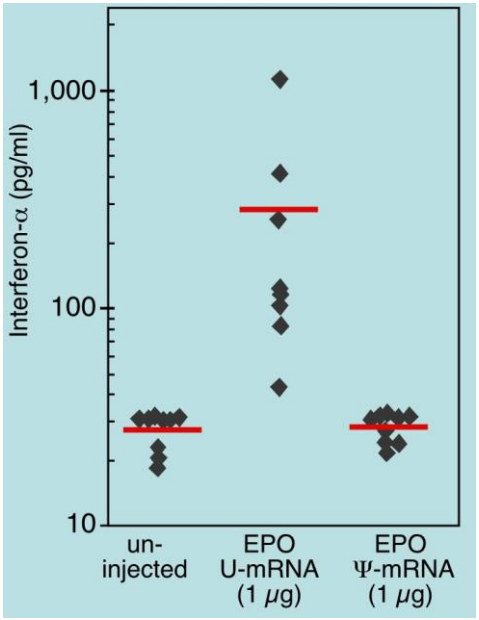


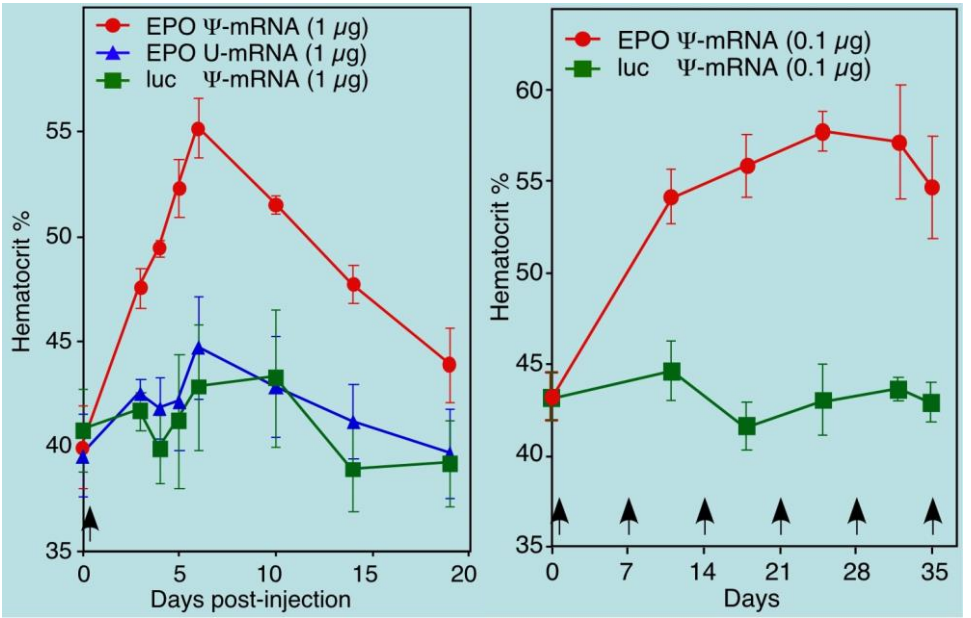
Mol. Therapy 2008, 16: 1833

2012 Pseudouridine-modified mRNA: non-inflammatory, translates into functional EPO







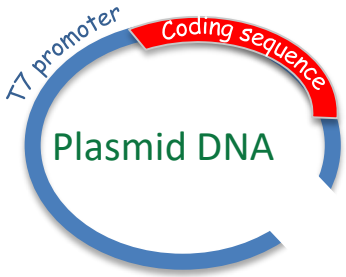


Mol. Therapy 2012, 20: 948

2013 BioNTech

Sahin U, Karikó K, Türeci Ö. **mRNA based therapeutics - developing a new class of drugs,**
Nature Reviews Drug Discovery 2014, 13: 759

Further optimizing IVT mRNA



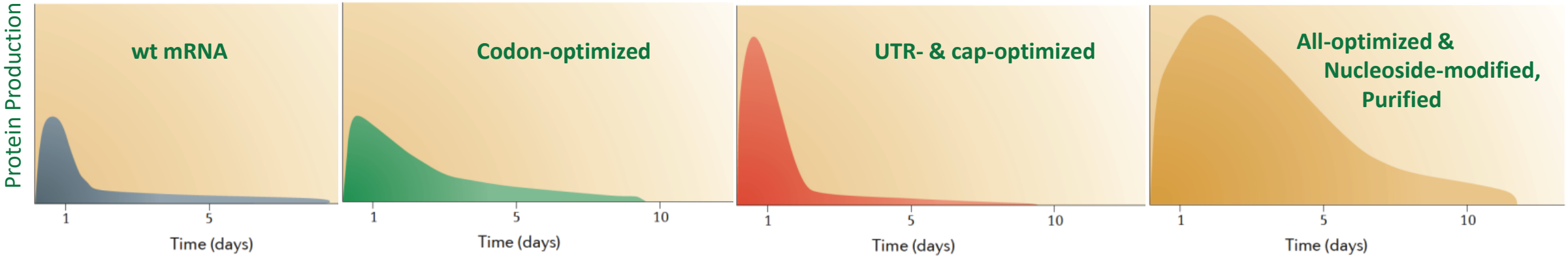
Transcription

+ T7 RNA polymerase
+ NTP
+ cap1 analog

Cap1 structure

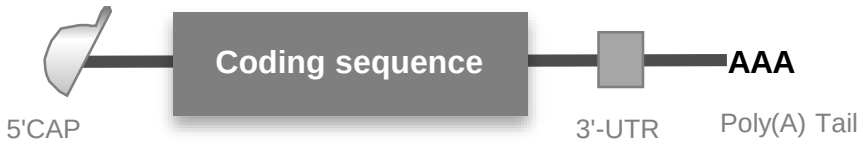
polyA-tail

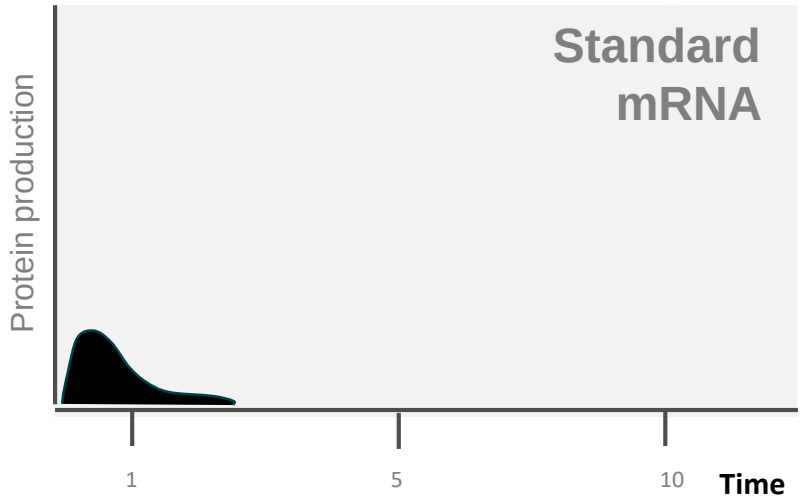
In vitro-transcribed mRNA



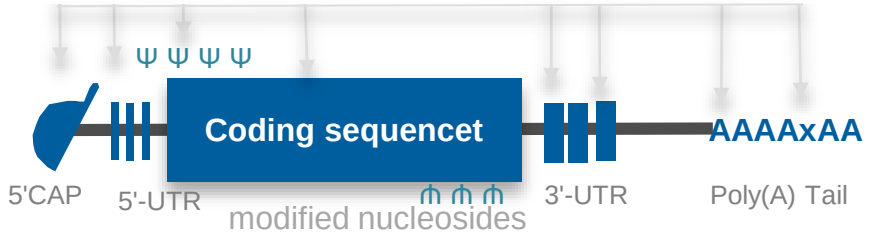
Optimizing mRNA performance by multiple modifications

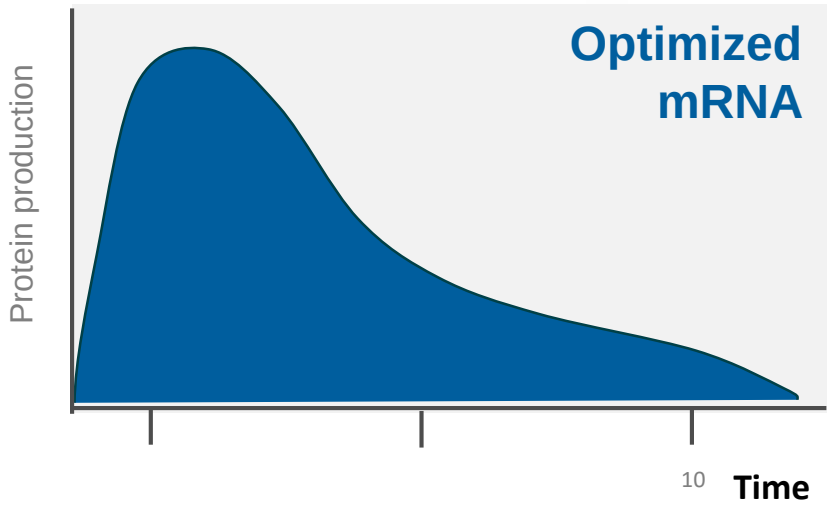
The path to the development of a new class of active substances





Modification of the mRNA structural elements





Combination of modifications of the structural mRNA components plus nucleoside modification makes an extreme increase in antigen production.

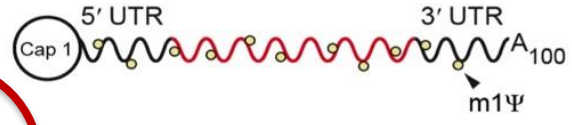
Sahin U, Karikó K, Türeci Ö. mRNA based therapeutics - developing a new class of drugs, Nat Rev Drug Disc, 2014.
Karikó, K et al D. (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 23, 165-175;
Holtkamp S et al (2006), Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. *Blood* 108, 2006.

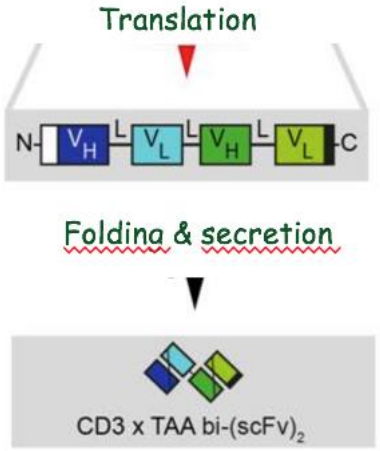
Therapeutic efficacy of CD3xCLDN6 RiboMAB-encoding mRNA

nature medicine
VOLUME 23 | NUMBER 7 | JULY 2017

Elimination of large tumors in mice by mRNA-encoded bispecific antibodies

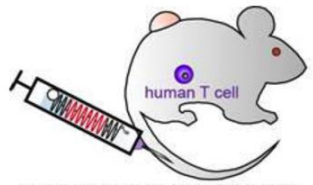
Christiane R Stadler[1], Hayat Bähr-Mahmud[1], Leyla Celik[1], Bernhard Hebich[1,5], Alexandra S Roth[1,5], René P Roth[1,5], Katalin Karikó[1], Özlem Türeci[2] & Ugur Sahin[1,3,4]

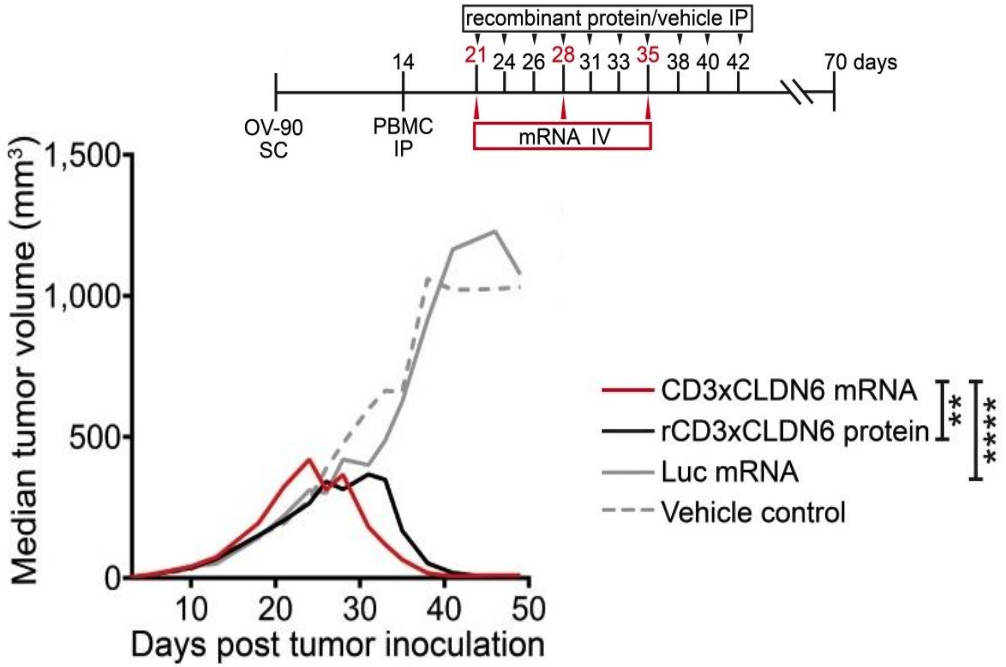




LNP-formulated mRNA (3 µg)
or 200 µg/kg rec. protein

Humanized NSG mice





- Elimination of advanced xenograft tumors upon three weekly treatments of mice with 3 µg RiboMAB-encoding mRNA
- mRNA (3 injections) as effective as the corresponding recombinant bsAb (10 injections)

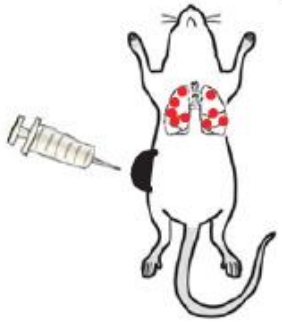
Intratumor injection of mRNA for cancer treatment

Science Translational Medicine

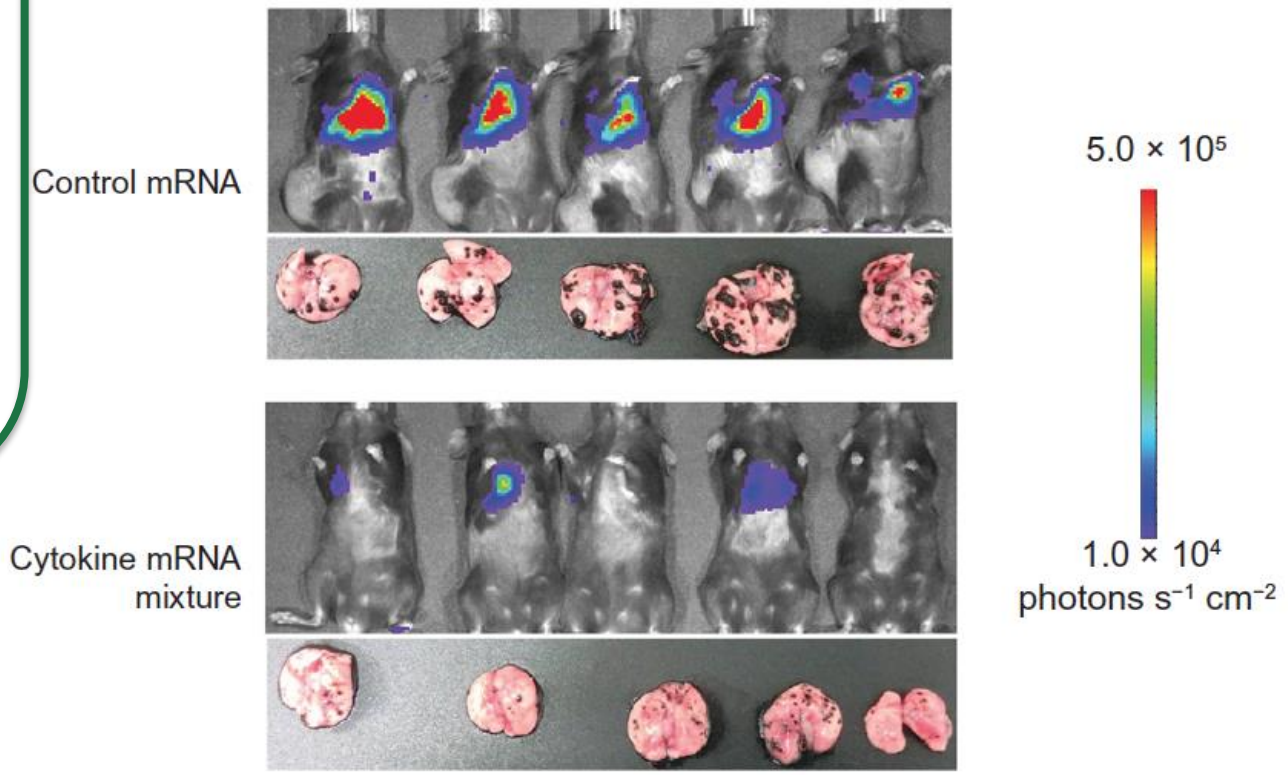
Local delivery of mRNA-encoding cytokines promotes antitumor immunity and tumor eradication across multiple preclinical tumor models

Christian Hotz[1†], Timothy R. Wagenaar[2*†], Friederike Gieseke[1], Dinesh S. Bangari[2], Michelle Callahan[2], Hui Cao[2], Jan Diekmann[1], Mustafa Diken[1,3], Christian Grunwitz[1], Andy Hebert[2], Karl Hsu[2], Marie Bernardo[2], Katalin Karikó[1], Sebastian Kreiter[1,3], Andreas N. Kuhn[1], Mikhail Levit[2], Natalia Malkova[2], Serena Masciari[2], Jack Pollard[2], Hui Qu[2], Sue Ryan[2], Abderaouf Selmi[3], Julia Schlereth[1], Kuldeep Singh[2], Fangxian Sun[2], Bodo Tillmann[1], Tatiana Tolstykh[2], William Weber[2], Lena Wicke[1], Sonja Witzel[3], Qunyan Yu[2], Yu-An Zhang[2], Gang Zheng[2], Joanne Lager[2‡], Gary J. Nabel[2§], Ugur Sahin[1,3*†], Dmitri Wiederschain[2†‖]

Sci. Transl. Med. **13**, eabc7804 (2021)



interleukin-12 single chain mRNA
interferon-alpha mRNA
GM-CSF mRNA
IL-15 sushi mRNA



ClinicalTrials.gov Identifier: NCT03871348

Induction of tolerization with autoantigen-encoding mRNA

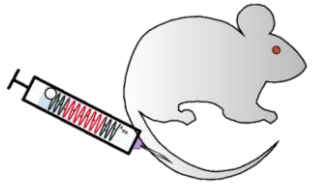
Science

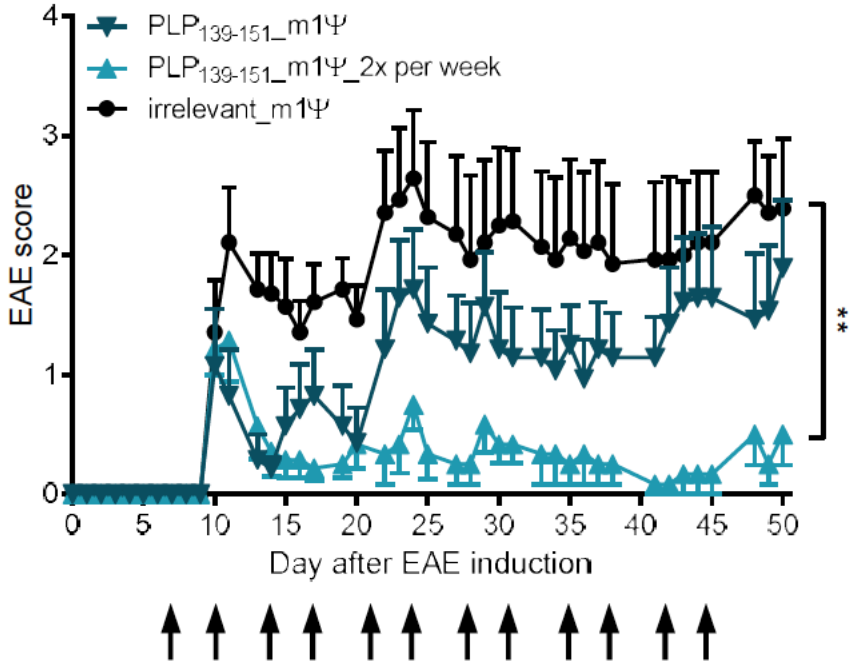
MULTIPLE SCLEROSIS

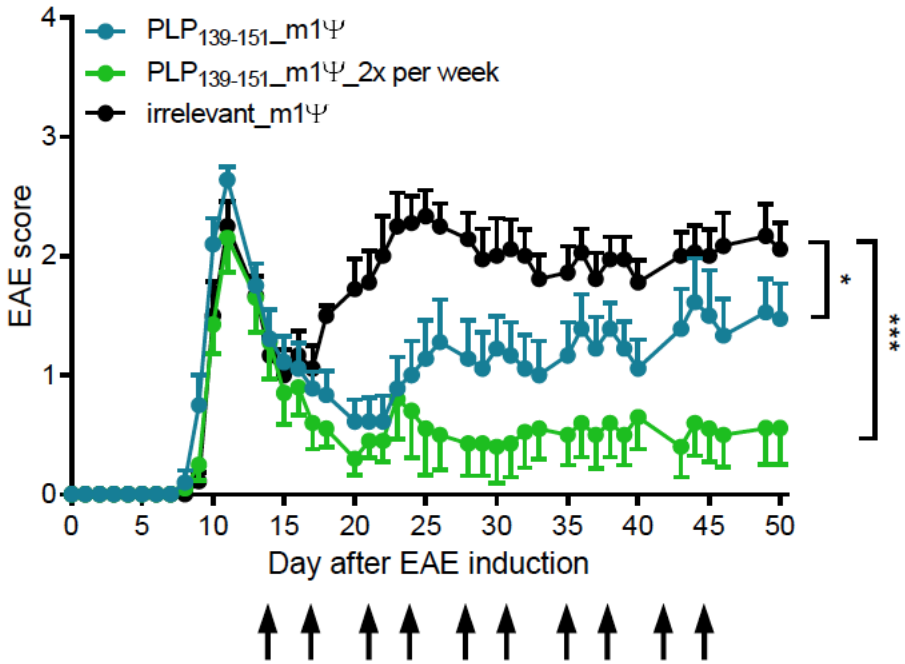
A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis

Christina Krienke[1,2], Laura Kolb[1]*, Elif Diken[1]*, Michael Streuber[1], Sarah Kirchhoff[1], Thomas Bukur[1], Özlem Akilli-Öztürk[1], Lena M. Kranz[3], Hendrik Berger[3], Jutta Petschenka[1,4], Mustafa Diken[1,3], Sebastian Kreiter[1,3], Nir Yogev[5,6], Ari Waisman[2,5], Katalin Karikó[3], Özlem Türeci[3,7], Ugur Sahin[1,2,3]†

mouse models of multiple sclerosis





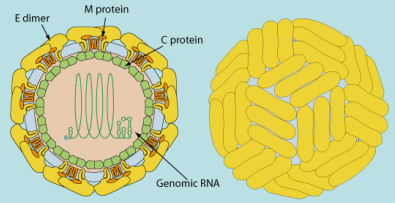


Science (2021) **371**: 145

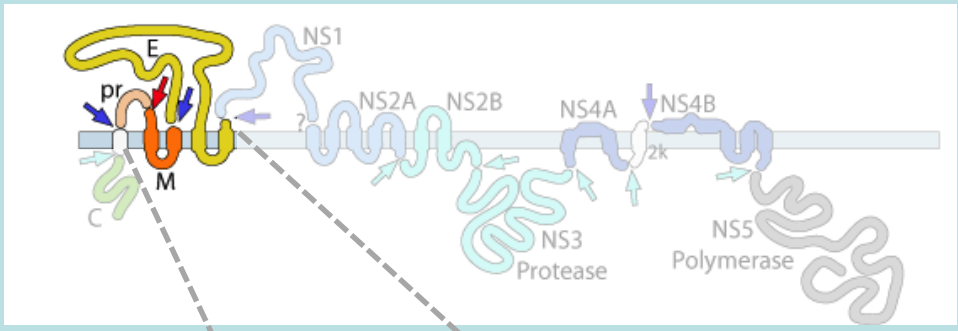
2017- Generating m1Ψ-mRNA encoding glycoproteins of Zika virus

Norbert Pardi
University of Pennsylvania

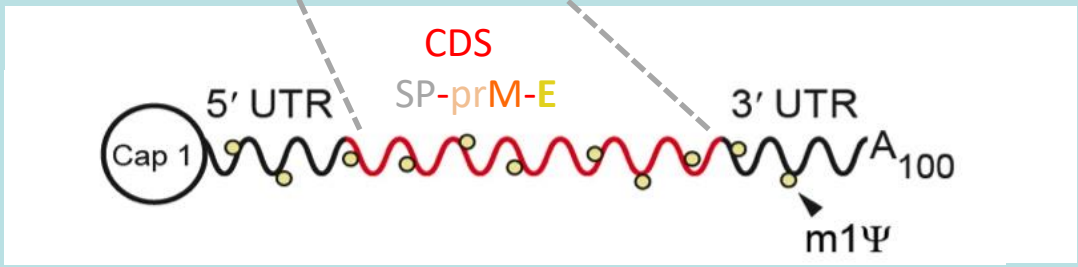
Zika virus



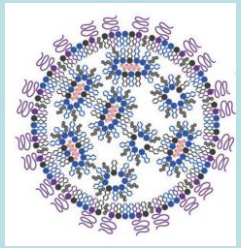
ZIKV protein



IVT m1Ψ-mRNA SP-prM-E



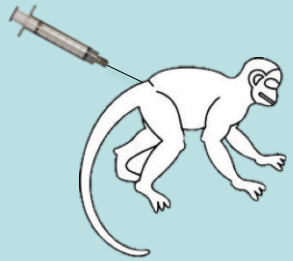
LNP-formulated mRNA



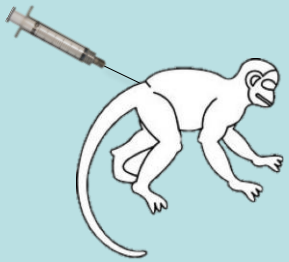
80-100 nm

Nature 2017, 543: 248

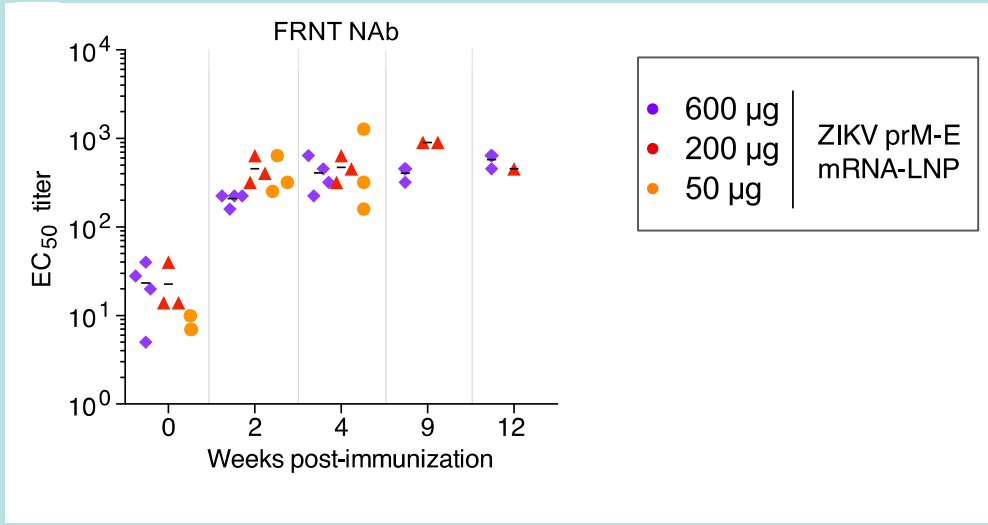
2017 ZIKV modRNA-LNP protects macaques from ZIKV challenge

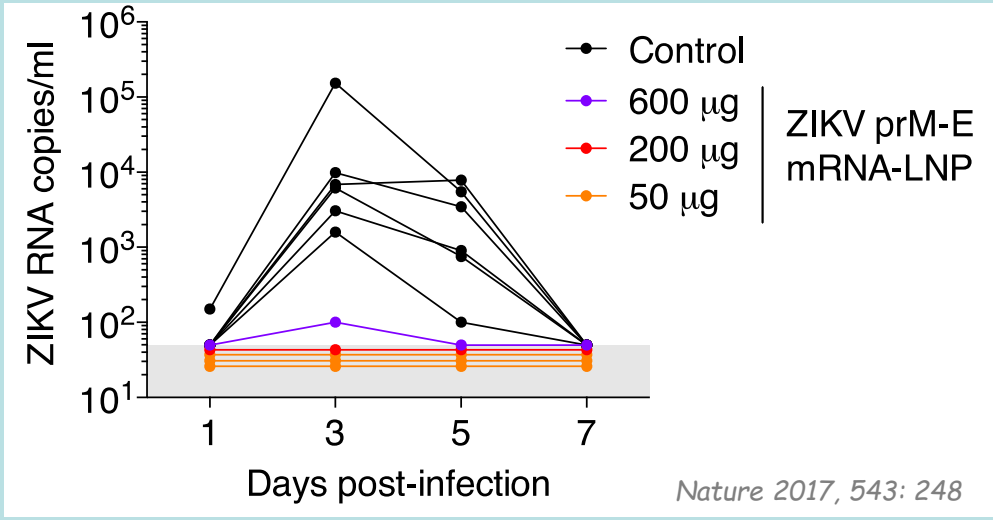


Vaccination i.d.: 50, 200 or 600 µg
ZIKV prM-E m1Ψ-mRNA-LNP
50 µg ~ 0.02 mg/kg



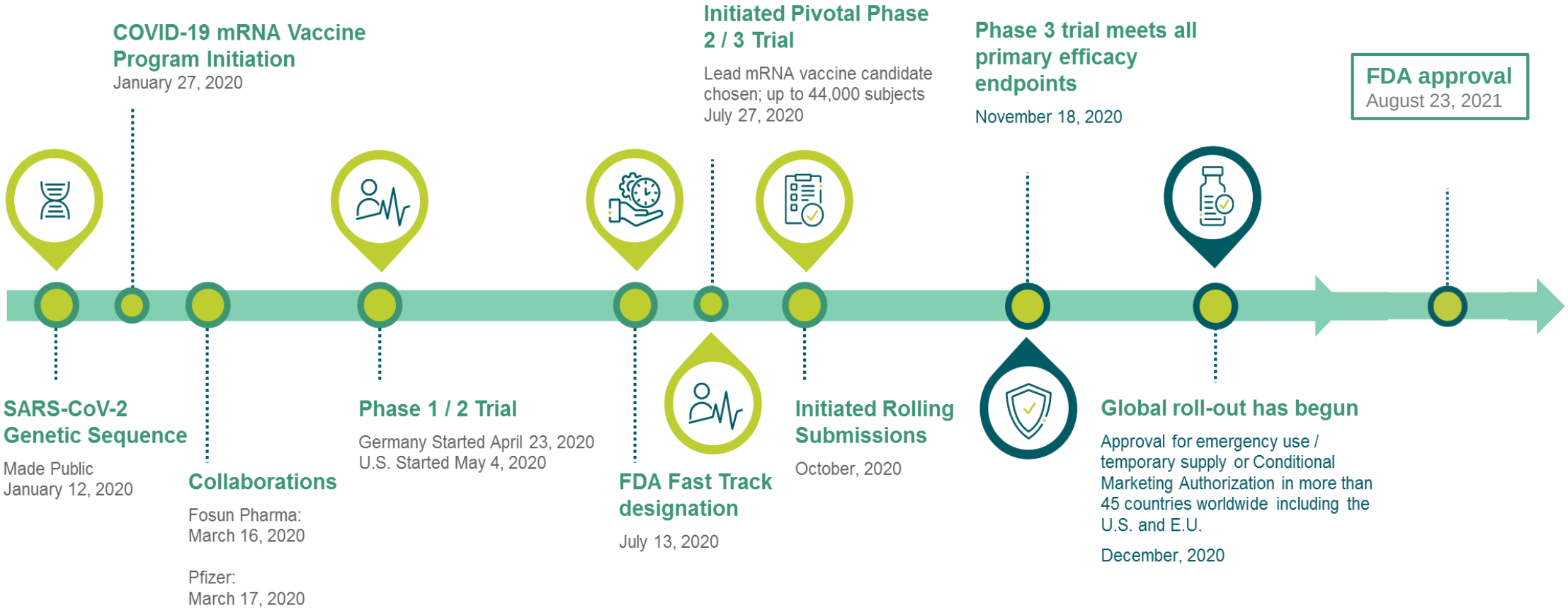
Challenge i.d.: 10,000
$TCID_{50}$
live ZIKV PRVABC59





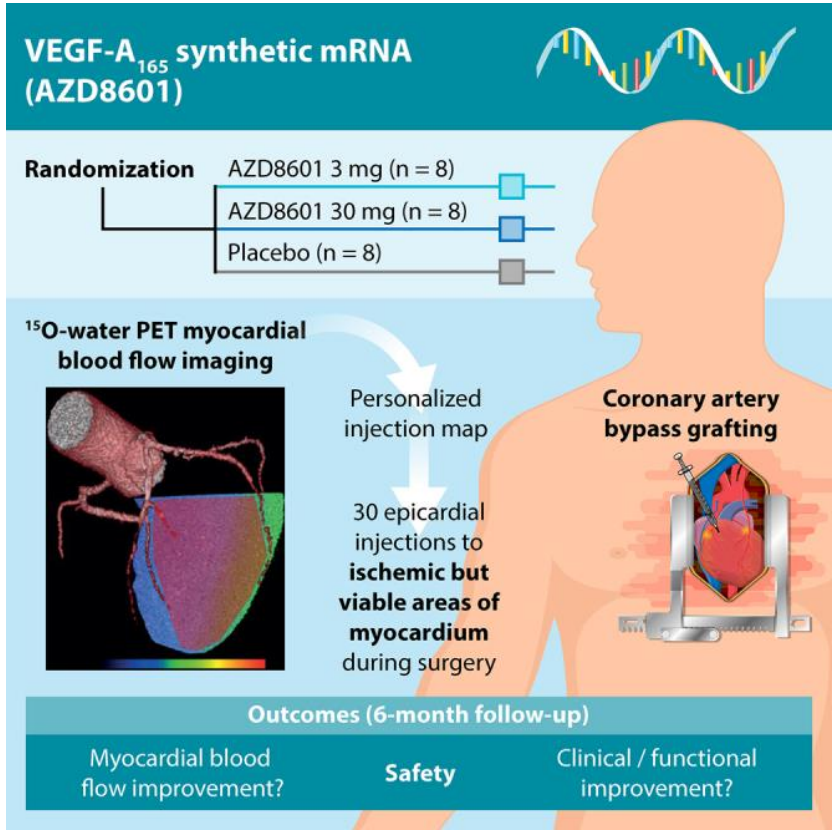
Nature 2017, 543: 248

2020 Clinical development of BNT162b2 covid-19 mRNA vaccine



BIONTECH Pfizer

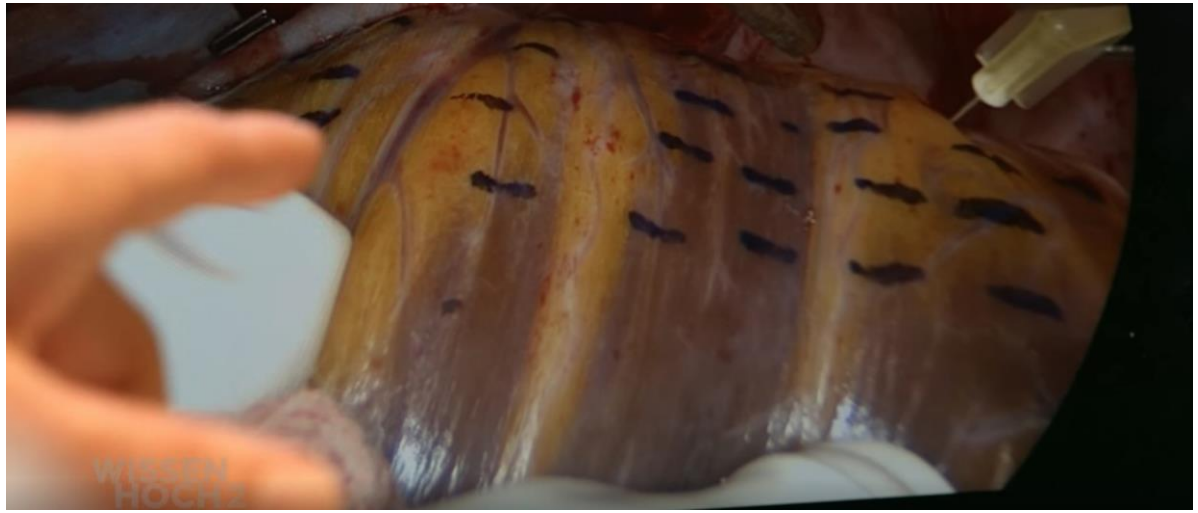
VEGF-A mRNA treatment of heart failure



ClinicalTrials.gov Identifier: NCT03370887

Synthetic mRNA Encoding VEGF-A in Patients Undergoing Coronary Artery Bypass Grafting: Design of a Phase 2a Clinical Trial - *Molecular Therapy: Methods & Clinical Development* 2020, 18:464-472

First patient injected:
February 5, 2018

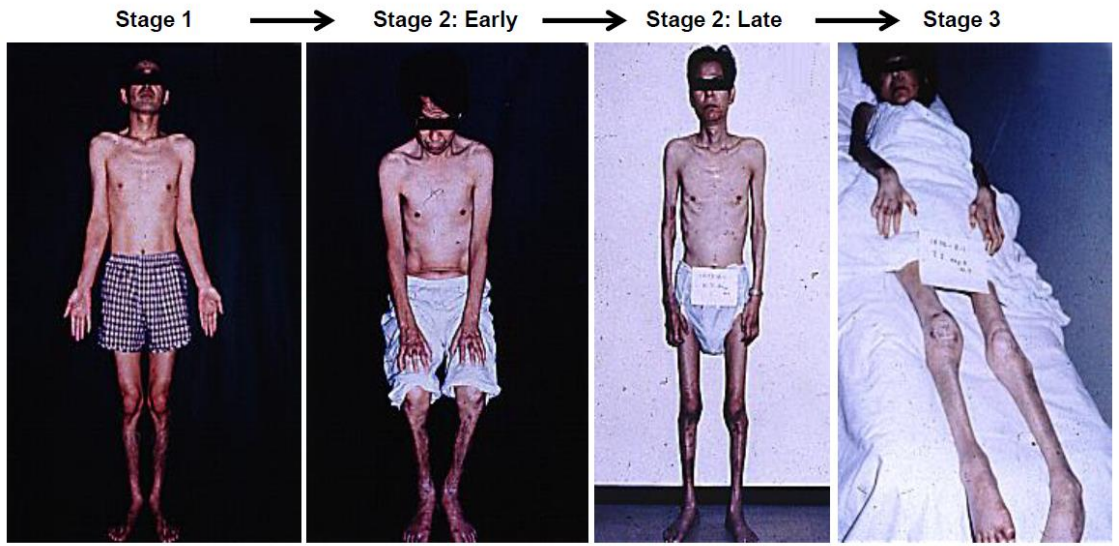


January 2022

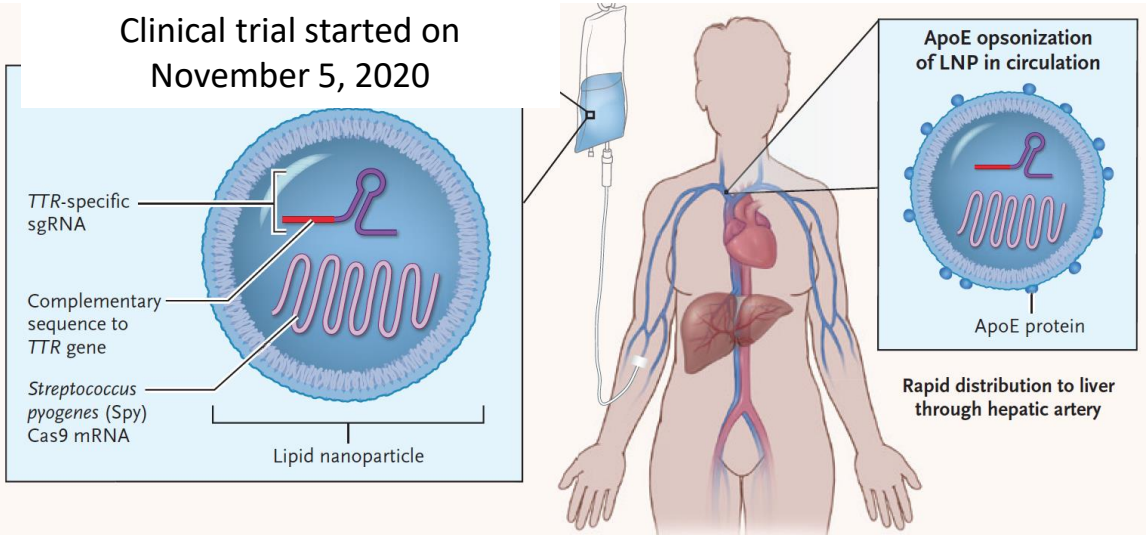
ClinicalTrials.gov Identifier: NCT03370887

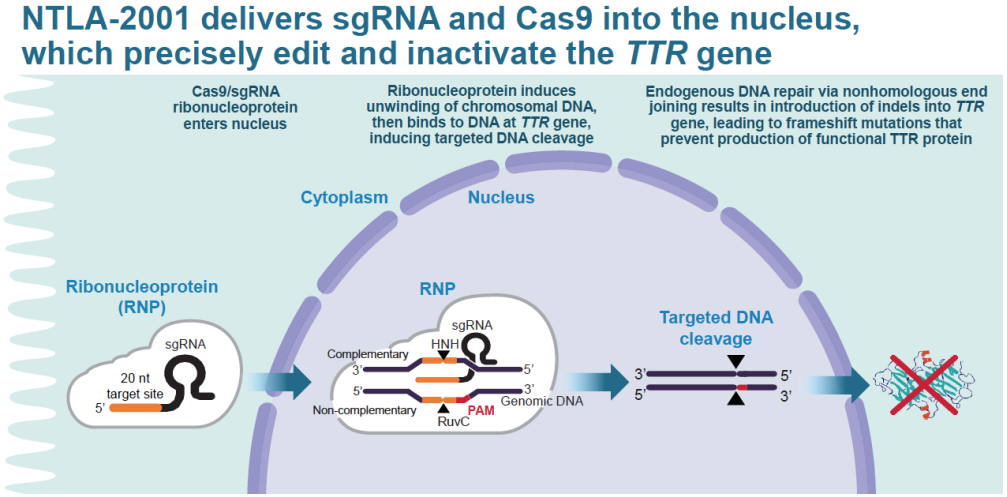
Phase 2 study of mRNA therapeutic that encodes for vascular endothelial growth factor-A (VEGF-A) (AZD8601) met the primary endpoint of safety and tolerability

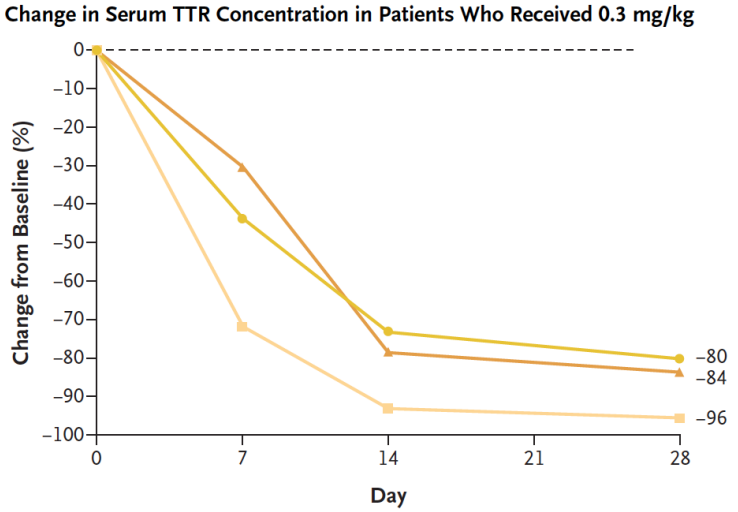
CAS-9 mRNA for treatment of patients suffering from ATTR – by Intellia



Clinical trial started on November 5, 2020







N Engl J Med 2021; 385:493-502

Thanks to all of those who have helped me on my journey


GO USA
London 2012
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