# *Developing mRNA for Therapy and Vaccine: The Story of An Immigrant*

**Katalin Karikó, PhD**

University of Pennsylvania Perelman School of Medicine
University of Szeged, Hungary


**April 8, 2023**

# 1955-60s growing up in Hungary


# 1973-85



University of Szeged
1973-78

Biological Research Center
Hungarian Academy of Sciences

# The Lipid Lab

**Biological Research Center**
**Hungarian Academy of Sciences**

**Research Institute for Fisheries**
**Szarvas, Hungary**

Reprinted from *Lipids*, vol. 16, no. 6 (June 1981), p. 418-422.

## Incorporation of [1-$^{14}$C] Acetate into Fatty Acids of the Crustaceans *Daphnia magna* and *Cyclops strenus* in Relation to Temperature

**T. FARKAS**, Institute of Biochemistry, and **K. KARIKO**, Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, H-6701 Szeged, Hungary, and
**I. CSENGERI**, Fisheries Research Institute, H-5541 Szarvas, Hungary

# The Lipid Lab

**Biological Research Center**
**Hungarian Academy of Sciences**

**Tibor Farkas**

**Éva Kondorosi**

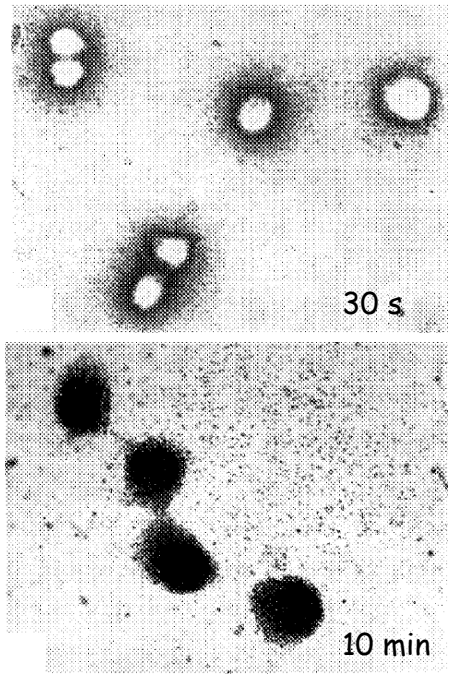
**Ernö Duda**

*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**

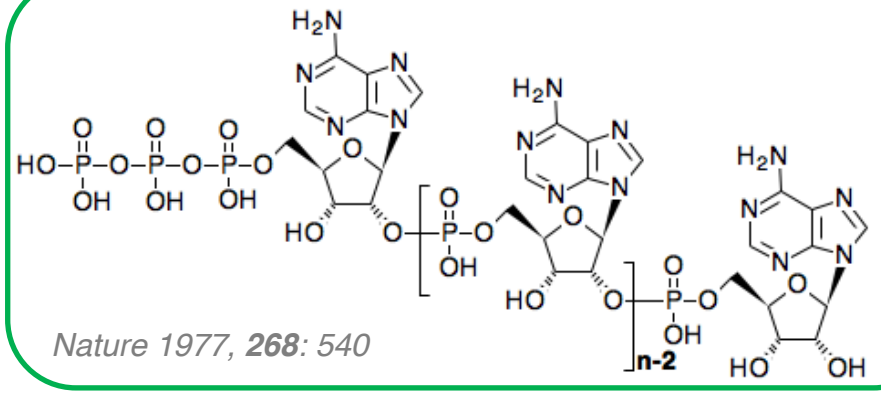
Jenö Tomasz

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*



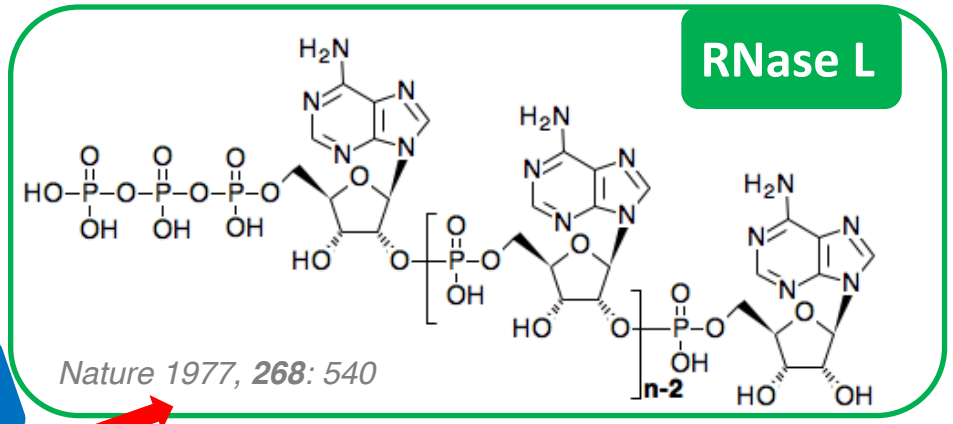
*Nature 1977, **268**: 540*

**2’-5’ oligoadenylate, 2-5A**

János Ludwig

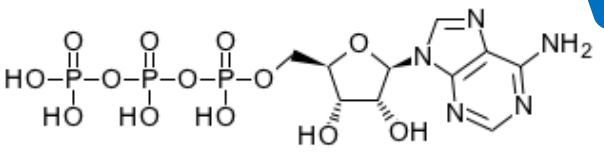
# 2-5A – The antiviral molecule

**Biological Research Center**
**Hungarian Academy of Sciences**
**Szeged, Hungary**

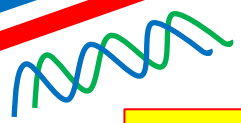



**2'-5' oligoadenylate, 2-5A**

**OAS**



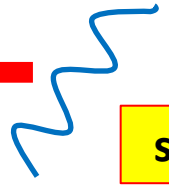
**ATP**



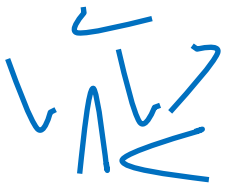
**dsRNA**

**RNase L**

**RNase L**



**ssRNA**



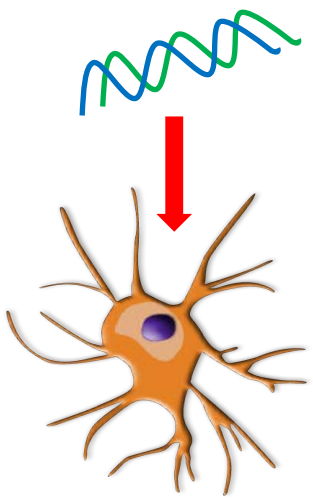
**degraded RNA**

**Viral replication**

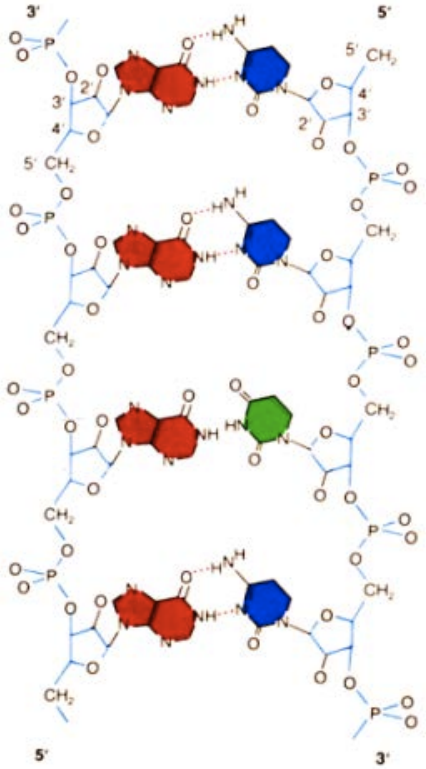
# The nucleoside Lab

**Temple University & Hahnemann University**
**Philadelphia**

**dsRNA**
**polyI:polyC**



**interferon**



**mismatched dsRNA**
**Ampligen**
**polyI:polyC$_{12}$U**

THE LANCET, JUNE 6, 1987

## CLINICAL, IMMUNOLOGICAL, AND VIROLOGICAL EFFECTS OF AMPLIGEN, A MISMATCHED DOUBLE-STRANDED RNA, IN PATIENTS WITH AIDS OR AIDS-RELATED COMPLEX

WILLIAM A. CARTER[1]
ISADORE BRODSKY[1]
MICHAEL G. PELLEGRINO[1]
HORACE F. HENRIQUES[3]
DAVID M. PARENTI[3]
RICHARD S. SCHULOF[3]
W. EDWARD ROBINSON[4]
DAVID J. VOLSKY[5]
HELENE PAXTON[7]
KATALIN KARIKÓ[8]
ROBERT J. SUHADOLNIK[8]
DAVID R. STRAYER[1]
MARK LEWIN[1]
LEO EINCK[2]
GARY L. SIMON[3]
ROCHELLE G. SCHEIB[3]
DAVID C. MONTEFIORI[4]
WILLIAM M. MITCHELL[4]
DEBORAH PAUL[6]
WILLIAM A. MEYER, III[7]
NANCY REICHENBACH[8]
DAVID H. GILLESPIE[1]

**i.v. 250 mg dsRNA 2x week for 18 weeks**

# 1961 - Discovery of mRNA

576 NATURE May 13, 1961 Vol. 190

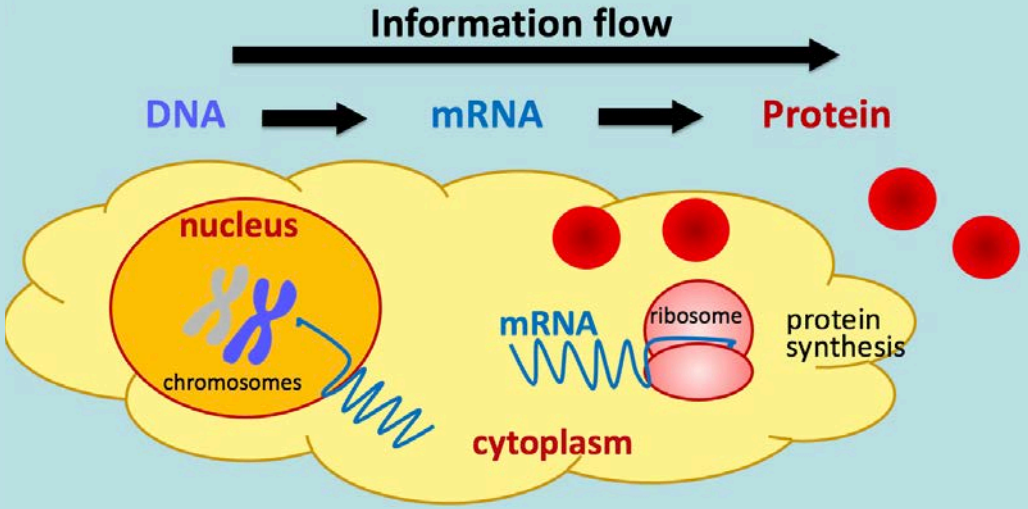
## AN UNSTABLE INTERMEDIATE CARRYING INFORMATION FROM GENES TO RIBOSOMES FOR PROTEIN SYNTHESIS

By Dr. S. BRENNER
Medical Research Council Unit for Molecular Biology, Cavendish Laboratory, University of Cambridge
Dr. F. JACOB
Institut Pasteur, Paris
AND
Dr. M. MESELSON
Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena, California

## UNSTABLE RIBONUCLEIC ACID REVEALED BY PULSE LABELLING OF *ESCHERICHIA COLI*

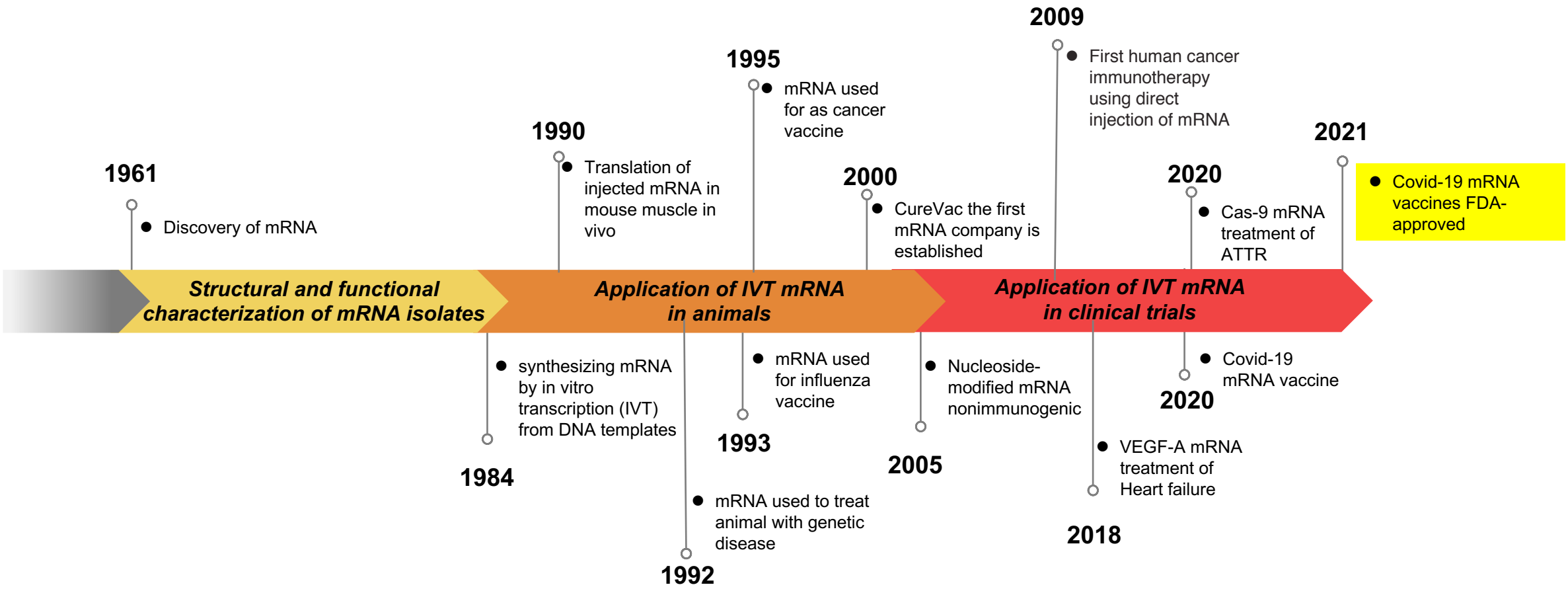
By Drs. FRANCOIS GROS and H. HIATT
The Institut Pasteur, Paris
Dr. WALTER GILBERT
Departments of Physics, Harvard University
AND
Dr. C. G. KURLAND, R. W. RISEBROUGH and Dr. J. D. WATSON
The Biological Laboratories, Harvard University

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

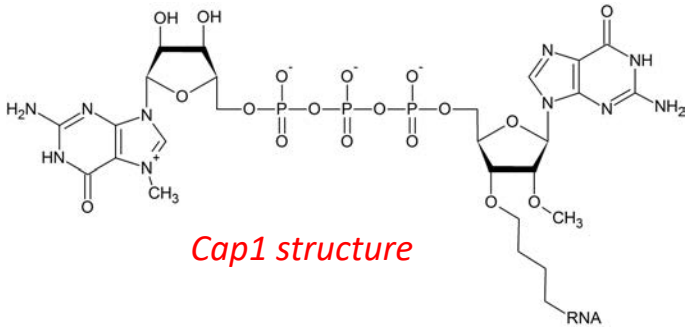



Nature 1961 , **190**: 576
Nature 1961 , **190**: 581

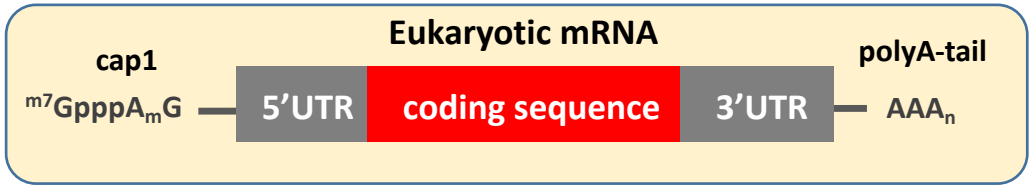
# Milestones of mRNA development for therapy



# Structural characterization of mRNA isolates: 5’-end cap – 3’-end polyA tail




*Cap1 structure*




NATURE VOL. 227 AUGUST 15 1970

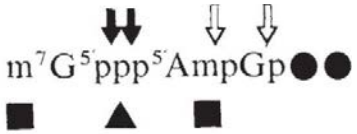
**Adenine-rich Polymer associated with Rabbit Reticulocyte Messenger RNA**

L. LIM E. S. CANELLAKIS

*Nature Vol. 253 January 31 1975*

**A blocked structure at the 5′ terminus of mRNA from cytoplasmic polyhedrosis virus**

YASUHIRO FURUICHI
KIN-ICHIRO MIURA




A proposed structure of the 5' terminal part of CPV mRNA

*Proc. Nat. Acad. Sci. USA*
Vol. 69, No. 6, pp. 1408–1412, June 1972

**Purification of Biologically Active Globin Messenger RNA by Chromatography on Oligothymidylic acid-Cellulose**

HAIM AVIV AND PHILIP LEDER

Purification of mRNA Guanylyltransferase and mRNA(guanine-7-)methyltransferase from Vaccinia Virions

$m^7G(5')pppG^m$- and $m^7G(5')pppA^m$-

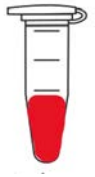
Moss & colleagues

JBC 1975, 250: 9322

Nature 1975, 253: 374
Nature 1975, 255: 28
Nature 1975, 255: 33
Nature 1975, 255: 37

# Functional characterization of mRNA isolates 1969 – 78

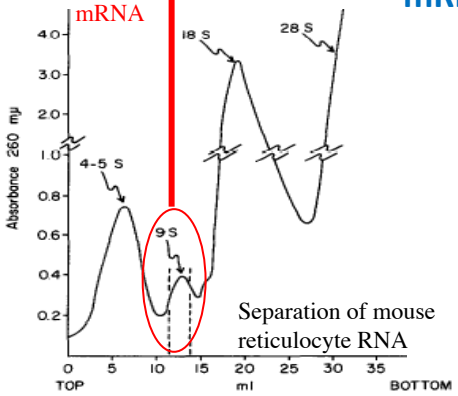
Rodent reticulocyte
(precursor of red blood cell)



Rabbit reticulocyte
cell lysate
BBRC 1969, 37: 204

mRNA translation in a
mammalian cell-free system

Hemoglobin
β-chain
mRNA




Separation of mouse
reticulocyte RNA

**β-Globin**
**mRNA isolation**

**microinjection**
**of mRNA**

**frog oocyte**

mRNA translation in
a living cell

Nature 1971, 233: 177
J Mol Biol 1971, 61: 73

**Liposome**
**formulation**

**Fusion of cells with**
**liposome-mRNA**

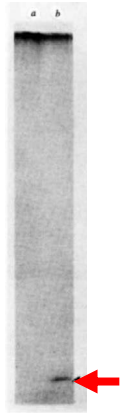
Alec Bangham – liposome 1965

**Murine spleen**
**lymphocytes**

mRNA translation in
primary mammalian cell

Nature 1978, 274: 921
Nature 1978, 274: 923

## Detection of hemoglobin β-chains

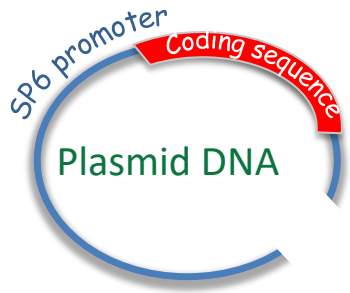


**globin**

**immunoprecipitation**

# 1984 – Synthesizing mRNA in test tube

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



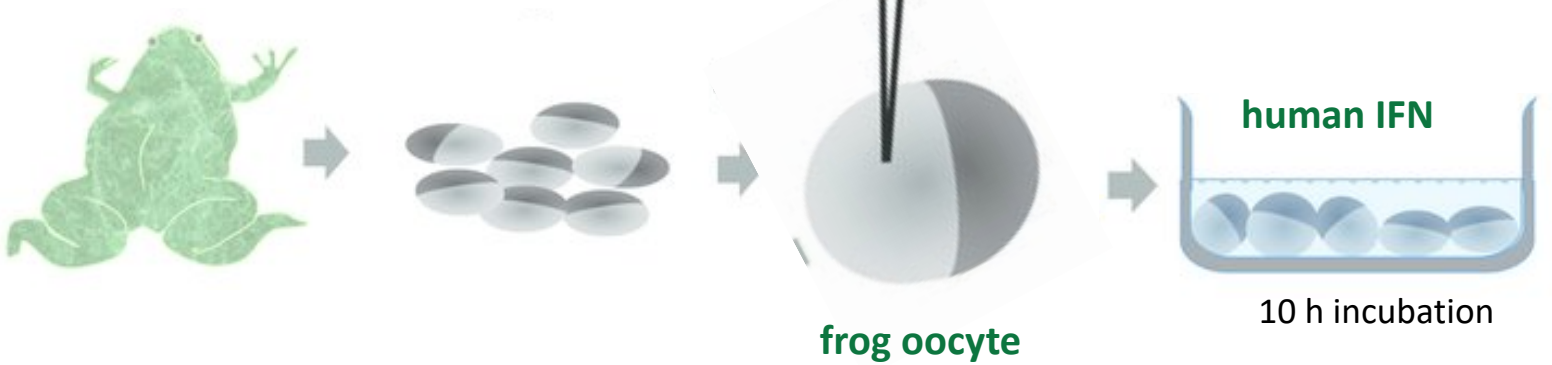

**Transcription/capping**

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

**In vitro-transcribed mRNA (IVT mRNA)**

**Cap structure** — **polyA-tail**


**microinjection of mRNA**

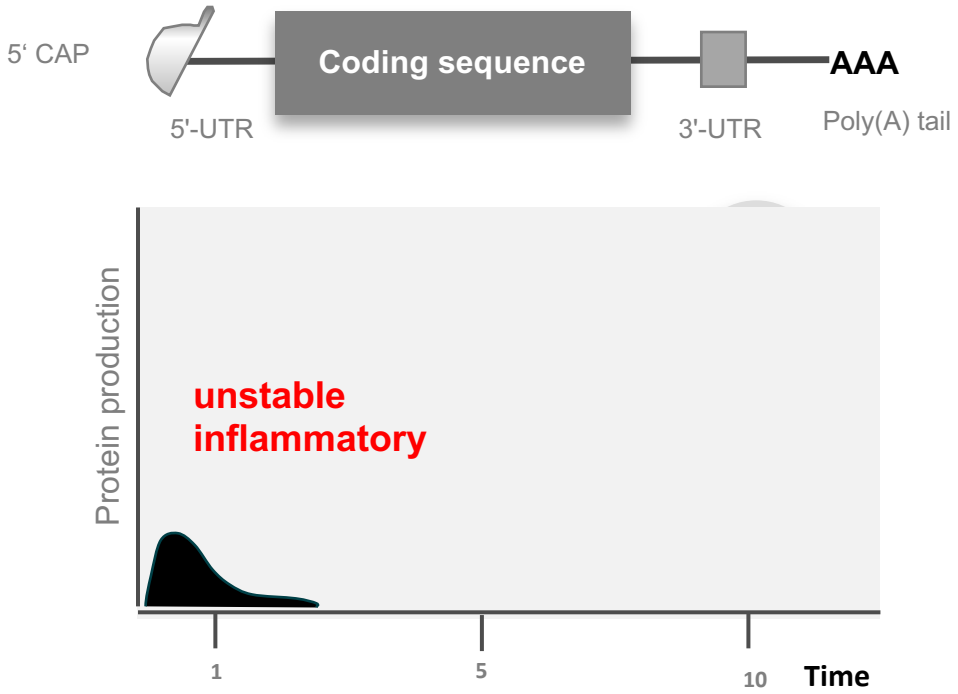



*Nucleic Acids Res. 1984, 12: 7035, 7057*

# Application of in vitro-transcribed mRNA in animals

- **1990** Direct gene transfer into mouse muscle in vivo – Wolff and colleagues
  *Science 1990, 247: 1475*
- **1992** Vasopressin mRNA therapy in rats – Bloom and colleagues
  *Science 1992, 255: 996*
- **1993** NP mRNA for influenza vaccine – Martinon, Meulien and colleagues
  *Eur J Immunol 1993, 23: 1719*
- **1994** NP saRNA for influenza vaccine – Liljeström and colleagues
  *Vaccine 1994, 12: 1510*
- **1995** mRNA for cancer vaccine in mice – Conry, Curiel and colleagues
  *Cancer Res 1995, 55: 1397*
- **1996** mRNA for cancer vaccine human DC – Gilboa and colleagues
  *J Exp Med 1996, 184: 465*

# Challenges for the human use of mRNA in 1990s



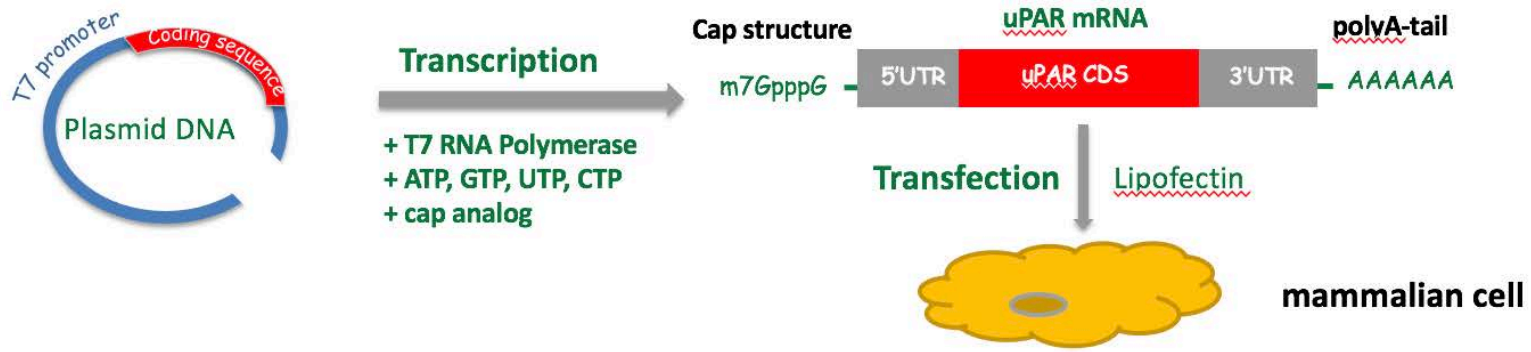

## mRNA

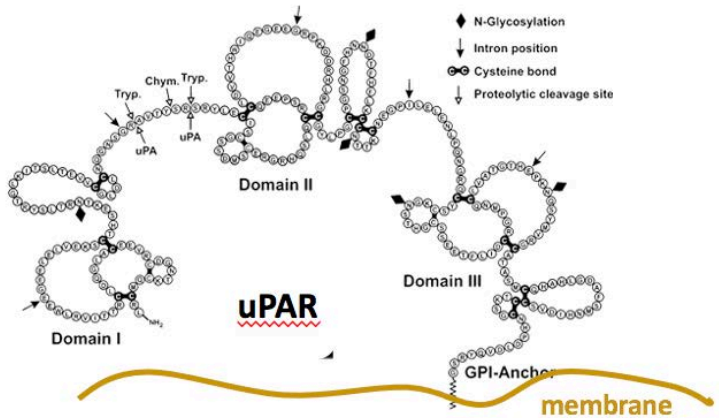
- unstable, degrade
- amount of translated protein is too little
- immunogenic

# Developing mRNA for therapy

Elliot Barnathan

Cardiology - Medical School of University of Pennsylvania




Gene Therapy (1999) 6, 1092–1100

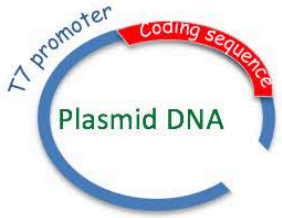
*Overexpression of urokinase receptor in mammalian cells following administration of the in vitro transcribed encoding mRNA*

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

# Developing mRNA for therapy

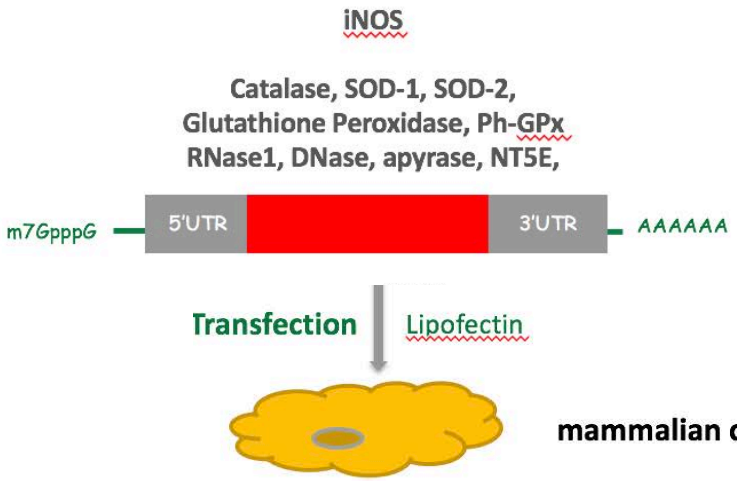
## Neurosurgery - Medical School of University of Pennsylvania

David Langer




**Transcription**

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




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]

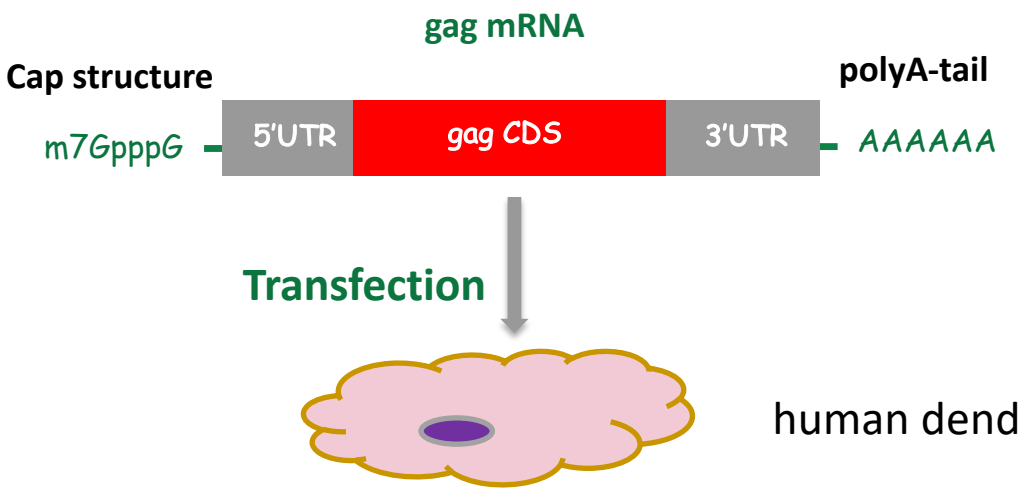
Journal of Neuroscience Methods 105 (2001) 77–86

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

# 1998-2000 - Evaluating gag mRNA in human dendritic cells

Drew Weissman




*J Immunol* 2000; 165:4710-4717

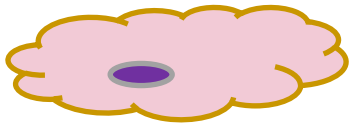
**HIV Gag mRNA Transfection of Dendritic Cells (DC) Delivers Encoded Antigen to MHC Class I and II Molecules, Causes DC Maturation, and Induces a Potent Human In Vitro Primary Immune Response[1]**

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

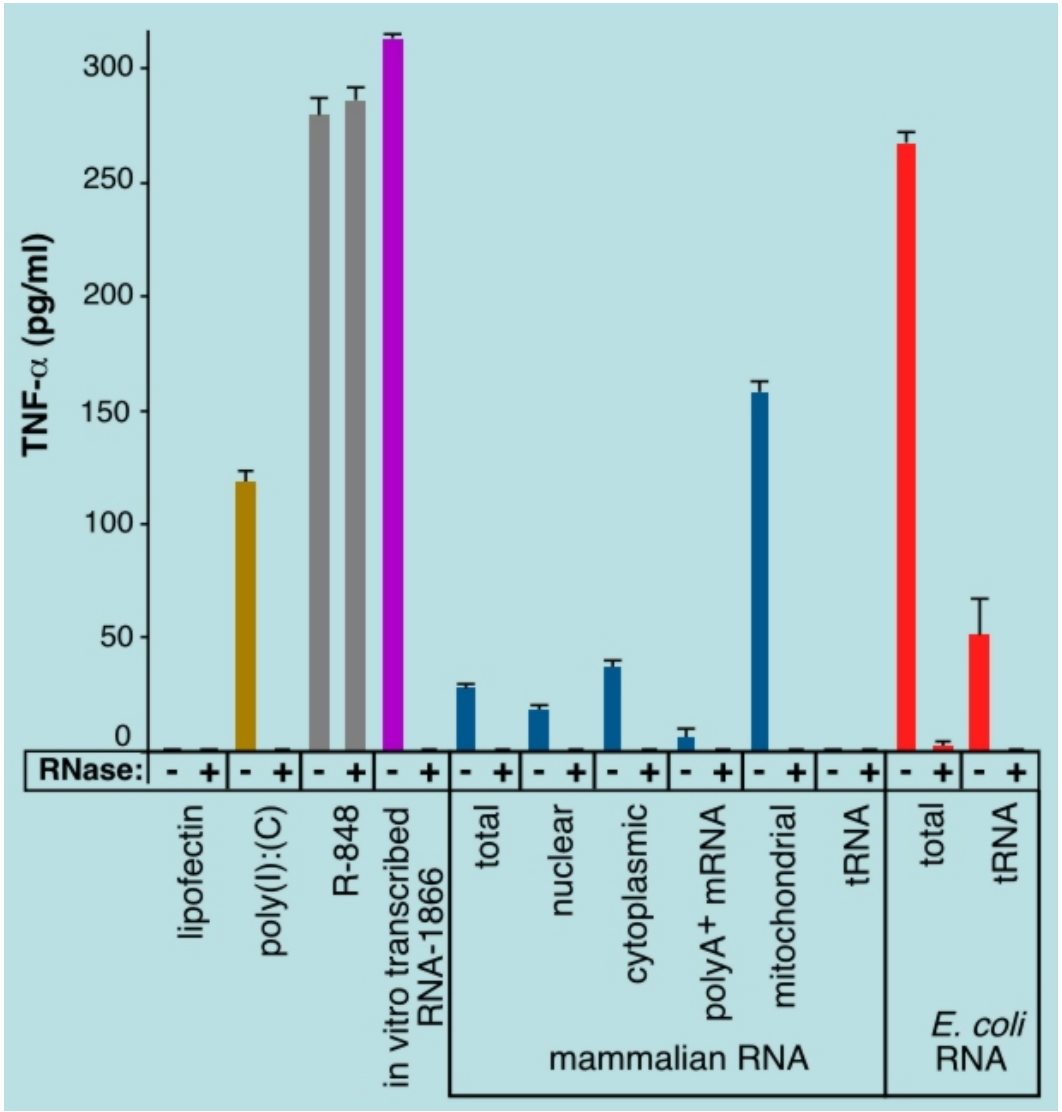
RNA

Transfection



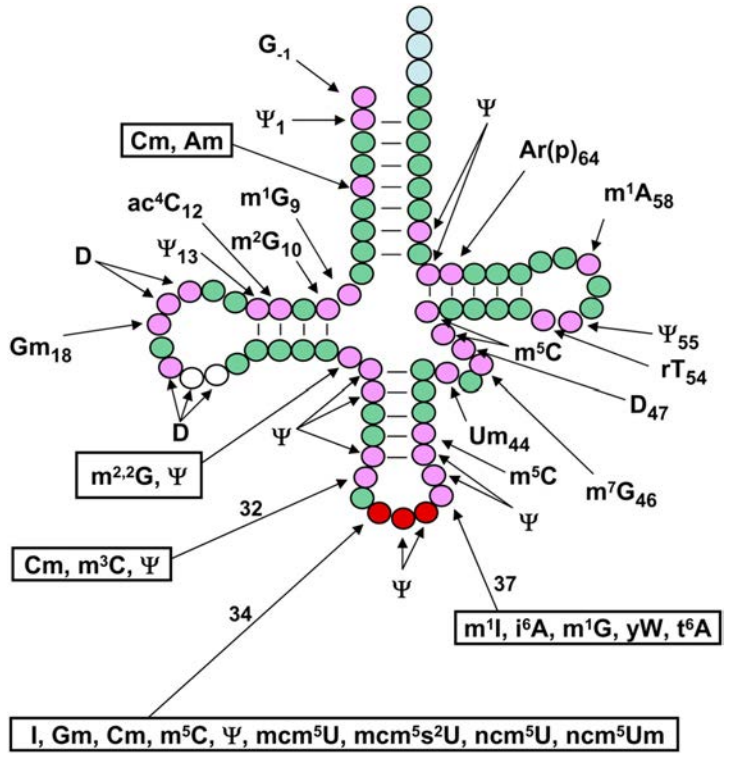
Monocyte-derived human DCs

Inflammatory response
TNF-α




J Immunol 2000, 165: 4710

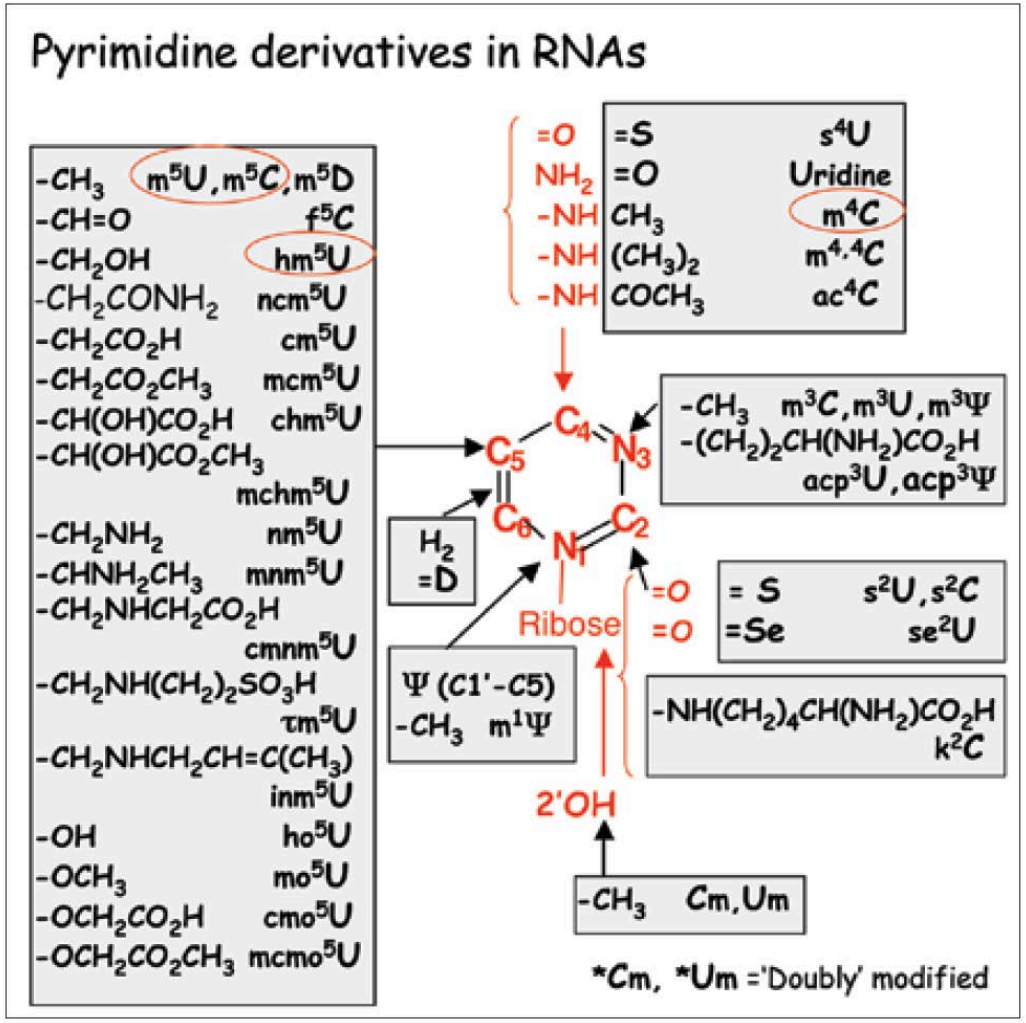
## tRNA

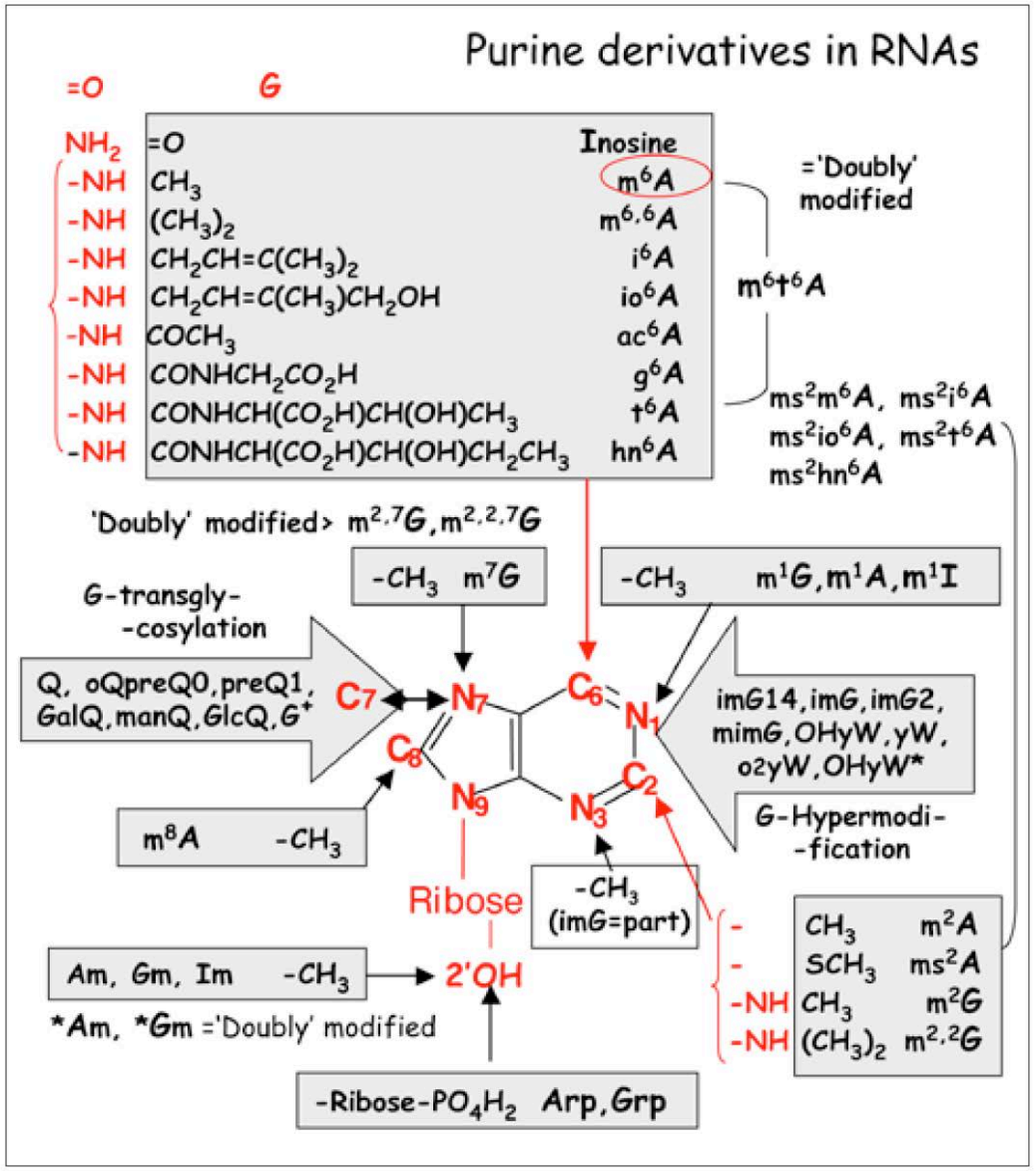



tRNA is enriched in modified nucleosides

Immunity 2005, 23: 165

# 100+ Naturally-occurring modified nucleosides in RNA

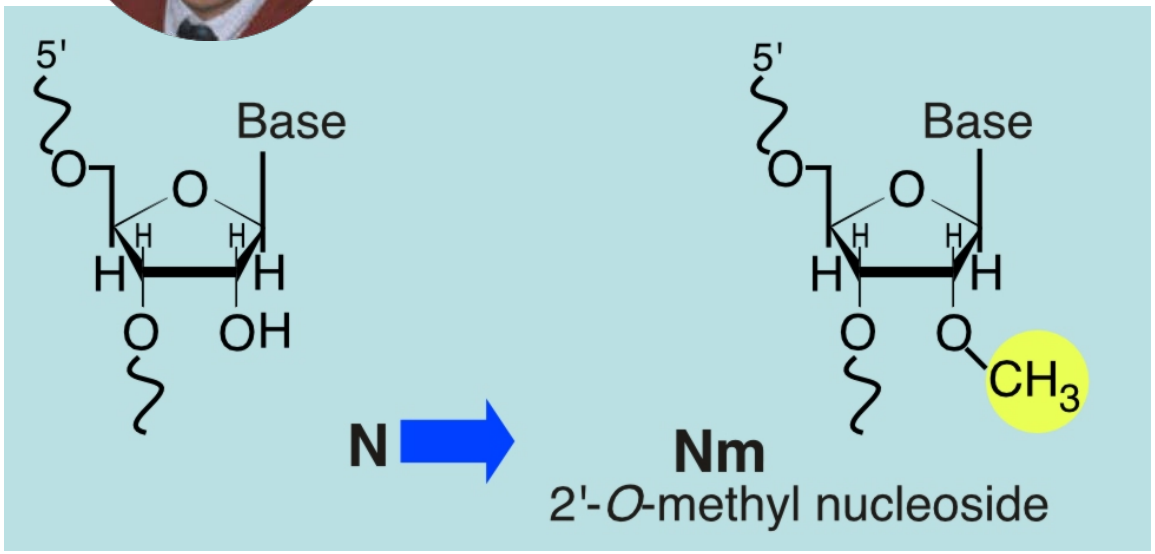


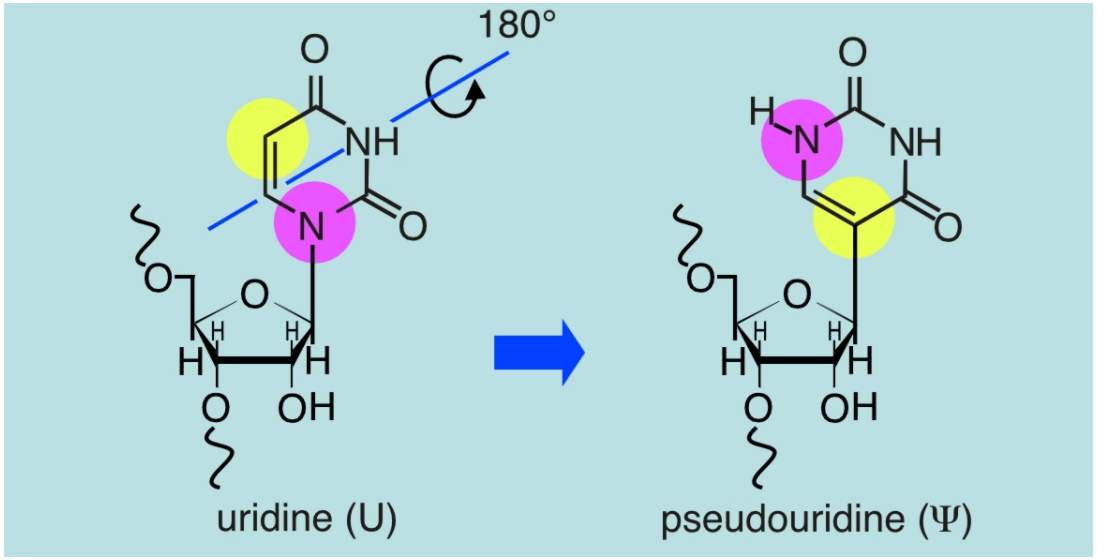




DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution, edited by Henri Grosjean

# 2’-O-methylation and pseudouridylation in RNA

**Tamás Kiss**
U. Toulouse




**2’-O-methylation**

*fibrillarin* (2’-*O*-methyltransferase)
guide RNA (sno/scaRNA)
accessory proteins

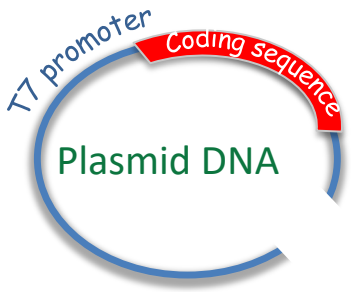
Cell 1996, **85**: 1077

**Pseudouridylation**

*dyskerin* (pseudouridine synthase)
guide RNA (sno/scaRNA)
accessory proteins

Cell 1997, **89**: 799

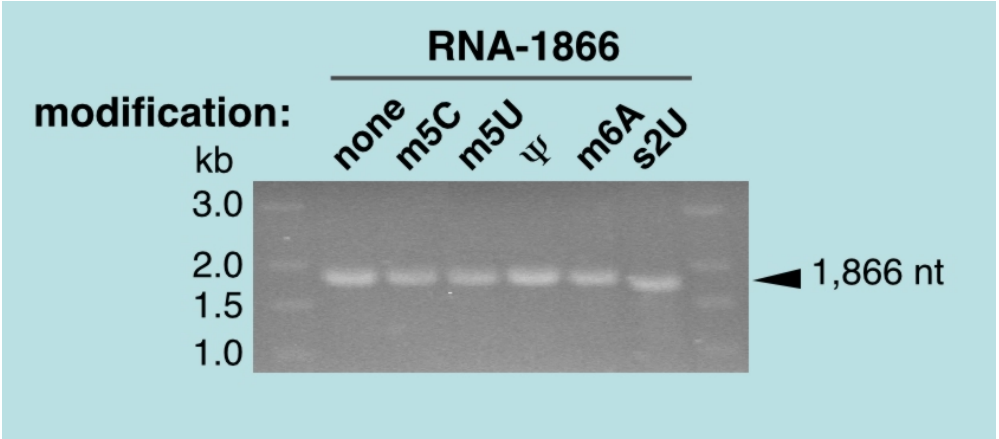
# Incorporation of modified nucleotides into RNA by in vitro transcription




**Transcription**

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

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




*Immunity 2005, 23: 165*

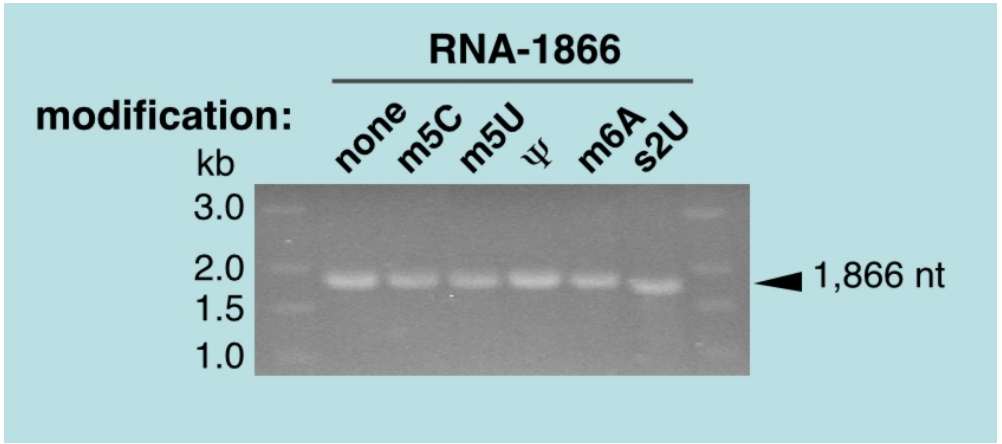
# 2005 – Synthesizing modified mRNA – Measurement of inflammatory response

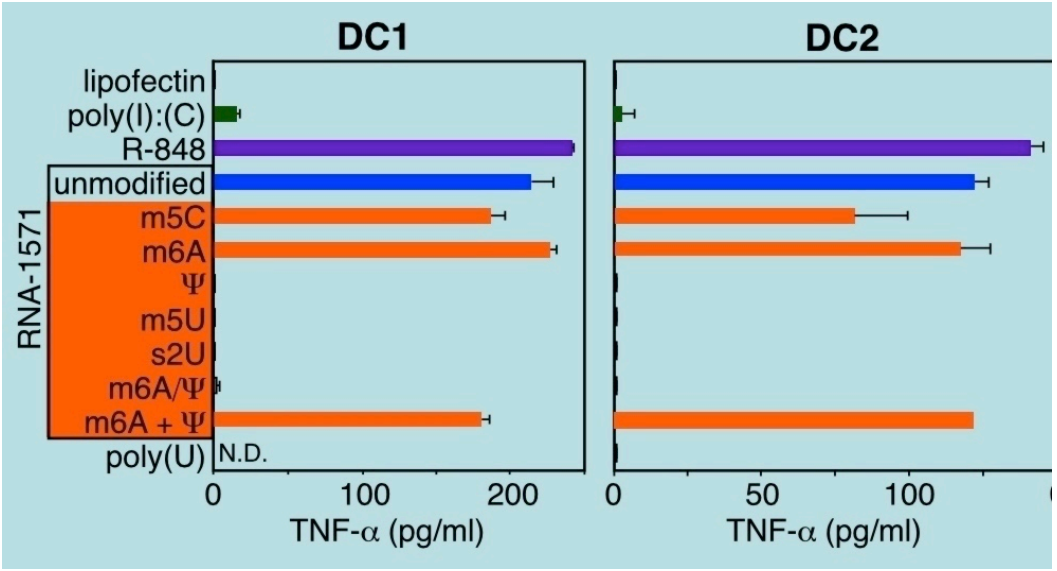
synthetic-RNA

Transfection

Isolated primary human DCs

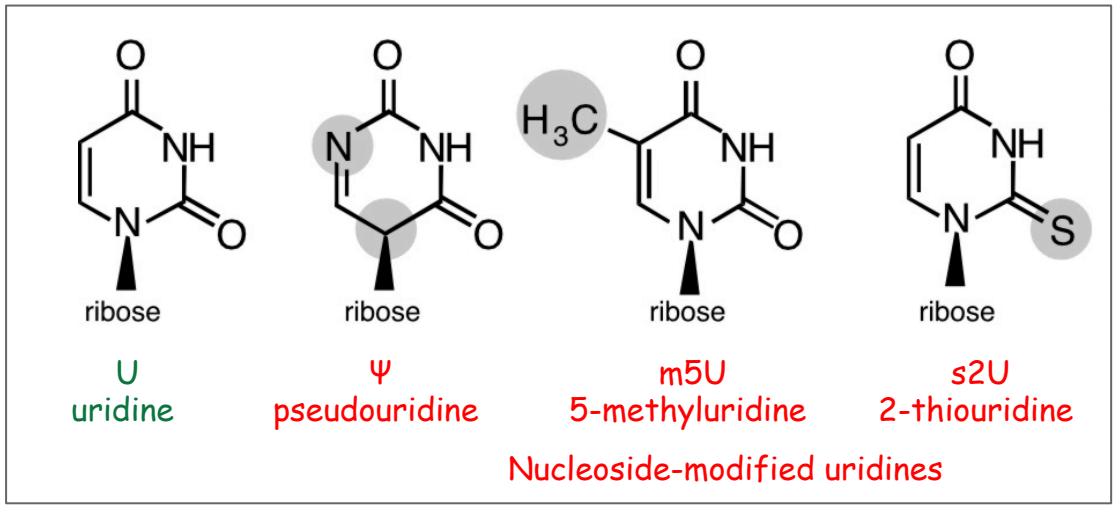
inflammatory response

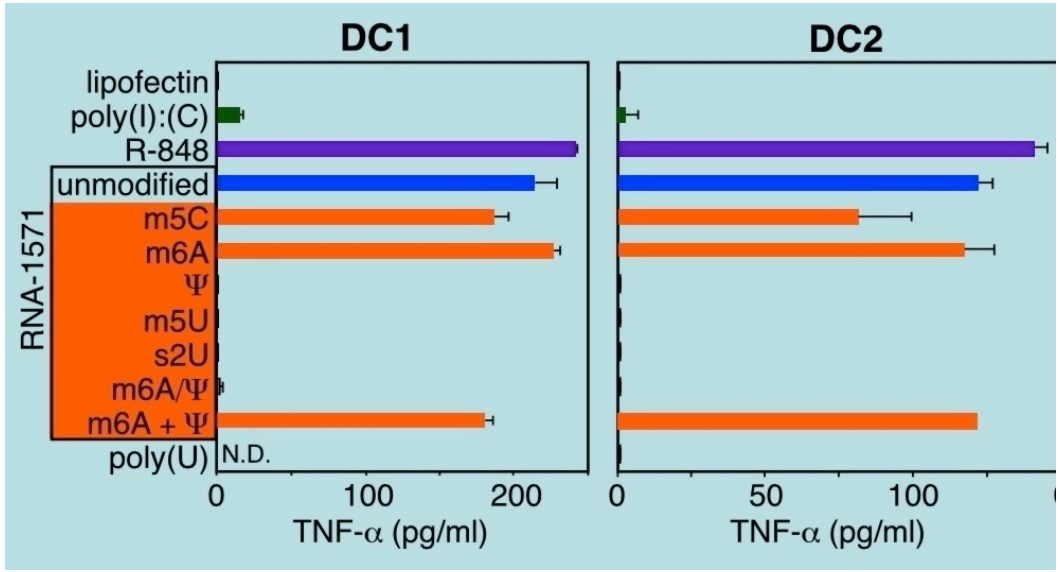


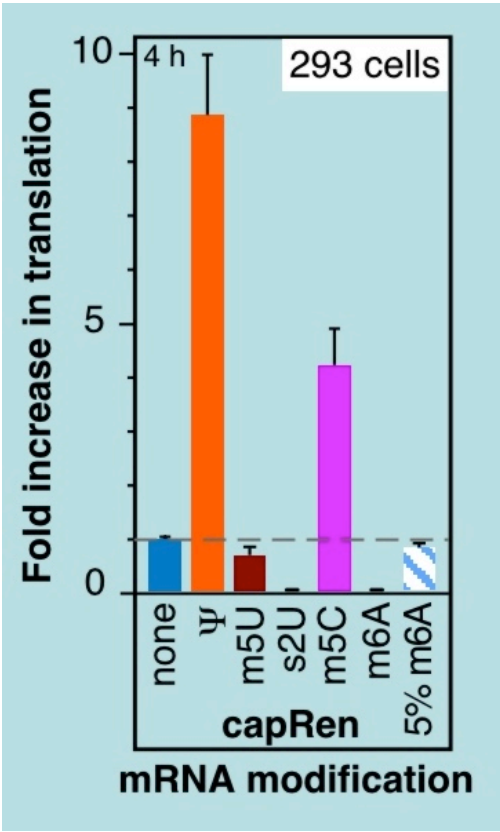

*Immunity 2005, 23: 165*

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




*Immunity 2005, 23: 165*

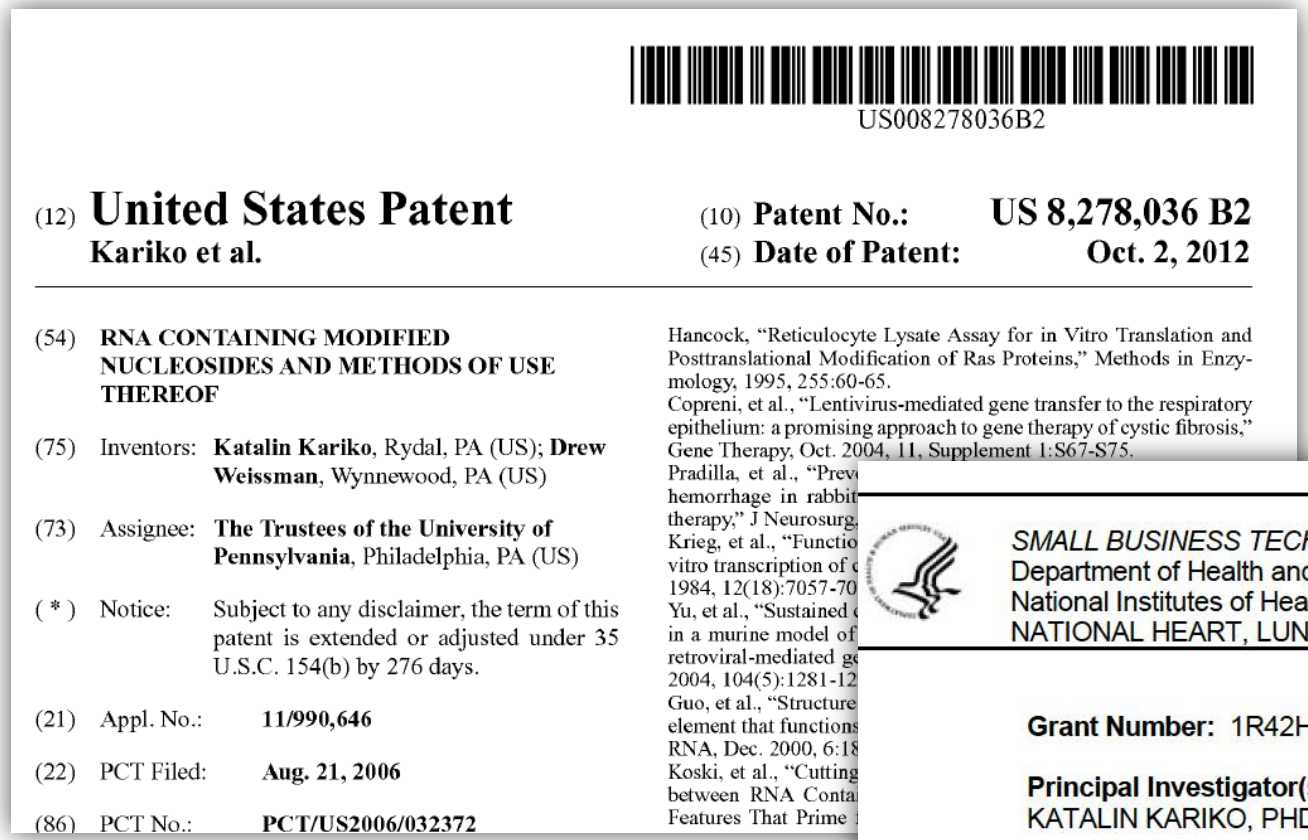



*Mol. Therapy 2008, 16: 1833*

# Patenting the technology – establishing a small biotech – RNARx

Established:
March 13, 2006

RNARx



US008278036B2

(12) **United States Patent**
**Kariko et al.**

(10) **Patent No.: US 8,278,036 B2**
(45) **Date of Patent: Oct. 2, 2012**

(54) **RNA CONTAINING MODIFIED NUCLEOSIDES AND METHODS OF USE THEREOF**

(75) Inventors: **Katalin Kariko**, Rydal, PA (US); **Drew Weissman**, Wynnewood, PA (US)

(73) Assignee: **The Trustees of the University of Pennsylvania**, Philadelphia, PA (US)

( * ) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 276 days.

(21) Appl. No.: **11/990,646**

(22) PCT Filed: **Aug. 21, 2006**

(86) PCT No.: **PCT/US2006/032372**

Hancock, "Reticulocyte Lysate Assay for in Vitro Translation and Posttranslational Modification of Ras Proteins," Methods in Enzymology, 1995, 255:60-65.
Copreni, et al., "Lentivirus-mediated gene transfer to the respiratory epithelium: a promising approach to gene therapy of cystic fibrosis," Gene Therapy, Oct. 2004, 11, Supplement 1:S67-S75.
Pradilla, et al., "Prev
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therapy," J Neurosurg.
Krieg, et al., "Functio
vitro transcription of
1984, 12(18):7057-70
Yu, et al., "Sustained
in a murine model of
retroviral-mediated ge
2004, 104(5):1281-12
Guo, et al., "Structure
element that functions
RNA, Dec. 2000, 6:18
Koski, et al., "Cutting
between RNA Conta
Features That Prime

Notice of Award

*SMALL BUSINESS TECHNOLOGY TRANSFER PROG* **Issue Date:** 05/28/2007
Department of Health and Human Services
National Institutes of Health
NATIONAL HEART, LUNG, AND BLOOD INSTITUTE

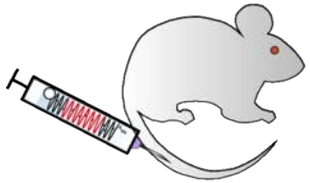
**Grant Number:** 1R42HL087688-01

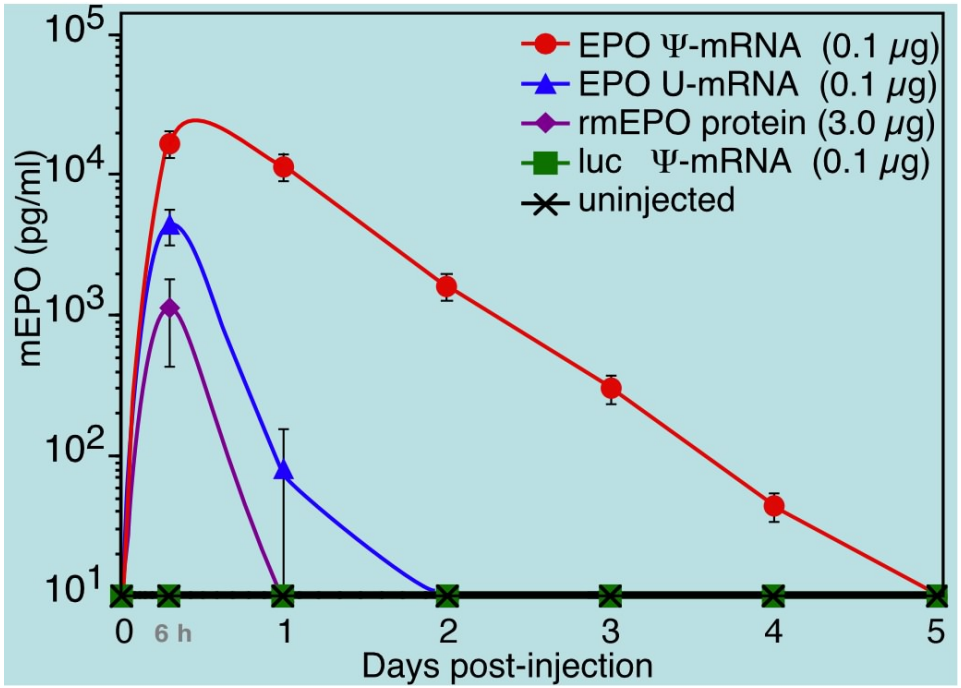
**Principal Investigator(s):**
KATALIN KARIKO, PHD

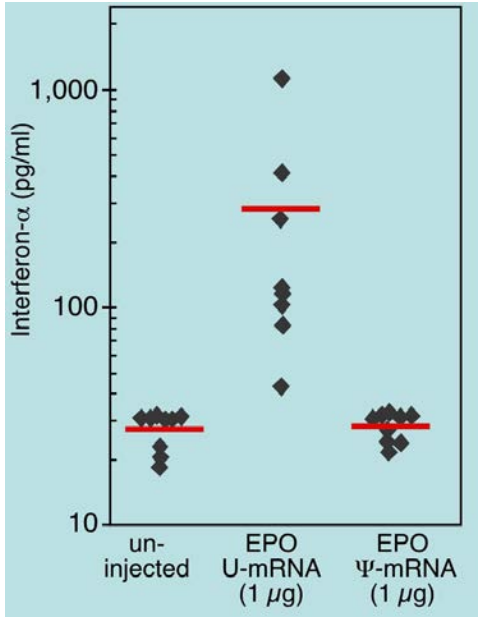
**Project Title:** Erythropoietin-encoding mRNA for treatment of anemia

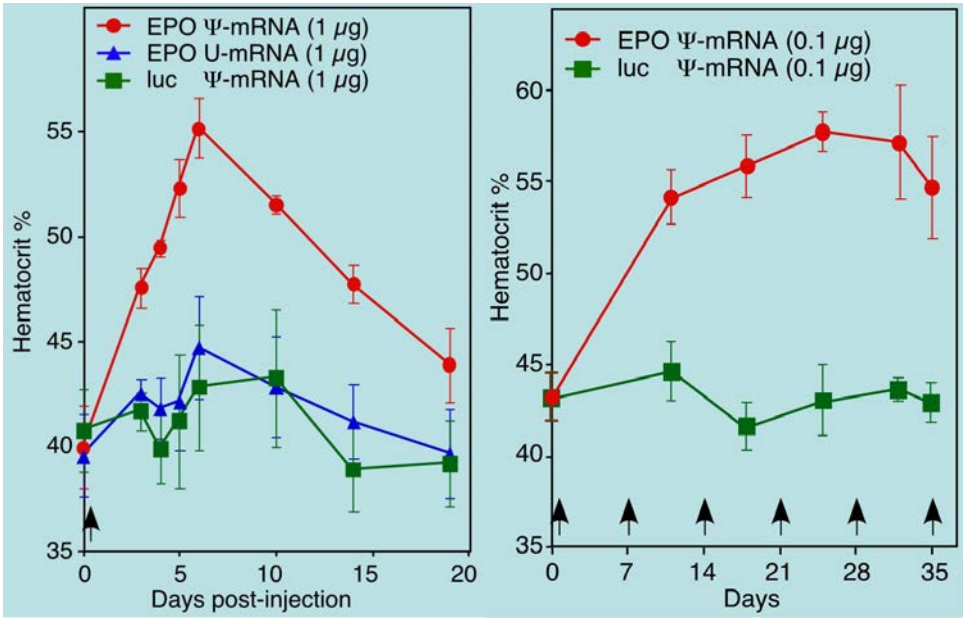
KATALIN KARIKO
CEO

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





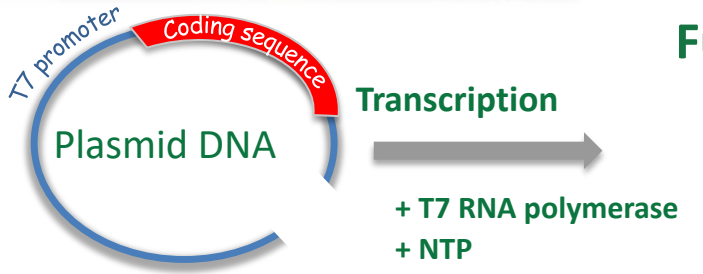




*Mol. Therapy 2012, 20: 948*

# 2013 BioNTech

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

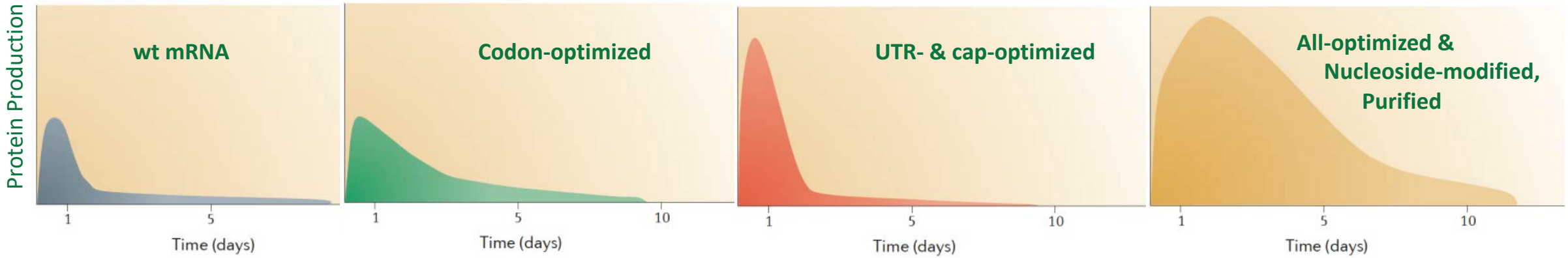



**Transcription**

**+ T7 RNA polymerase**
**+ NTP**
**+ cap1 analog**

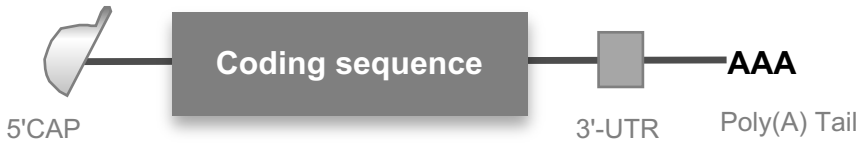
## Further optimizing IVT mRNA

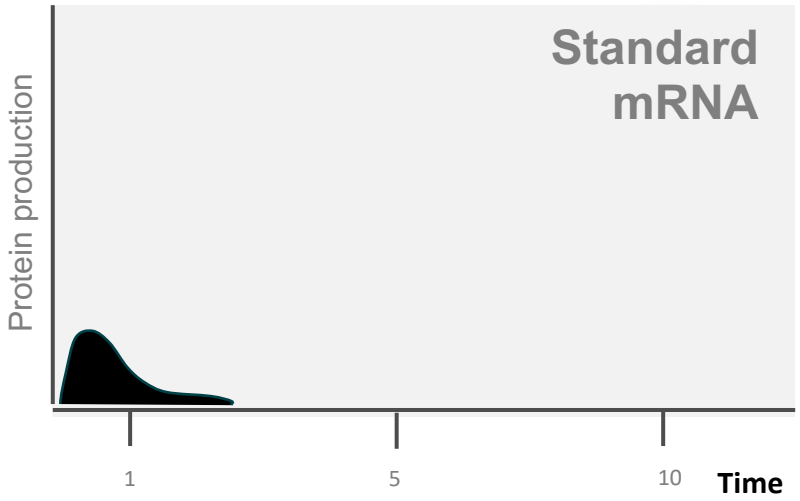

In vitro-transcribed mRNA



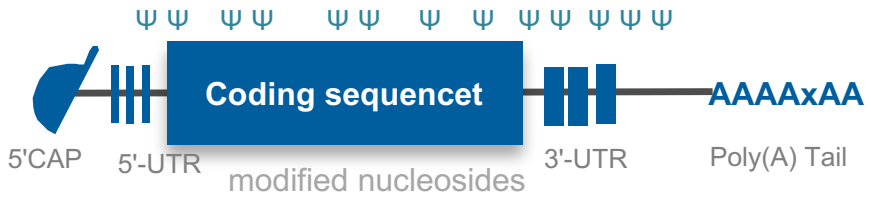
# Optimizing mRNA performance by multiple modifications

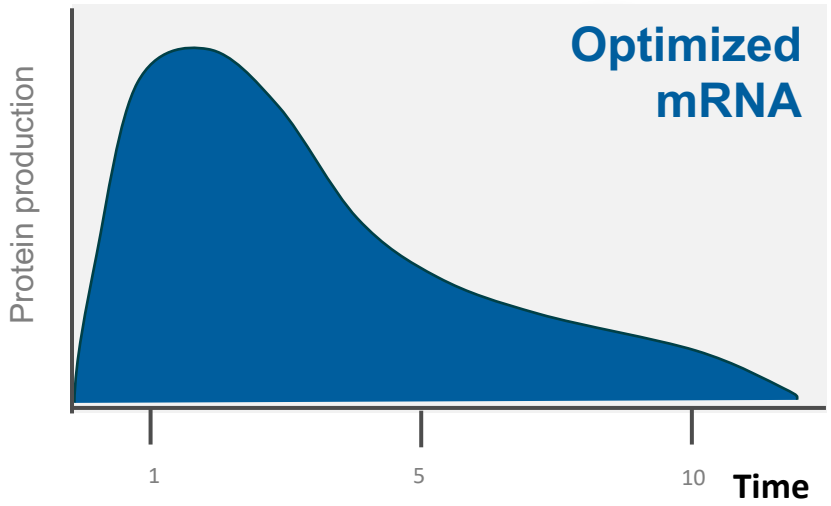
The path to the development of a new class of active substances




**Modification of the mRNA structural elements**




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

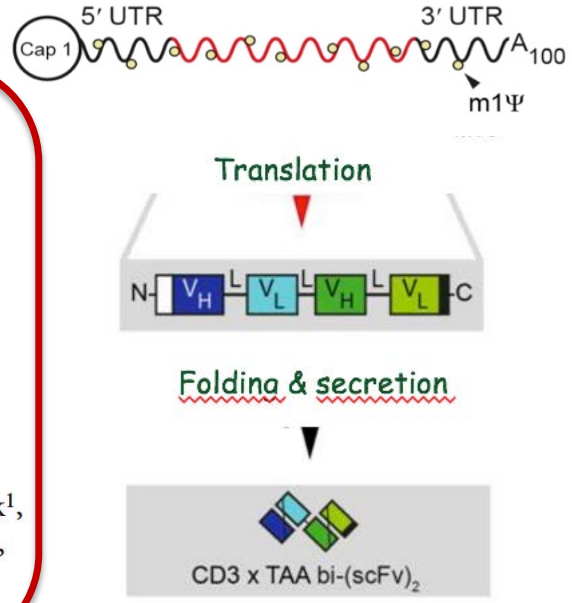
**Sahin U, Karikó K, Türeci Ö**. (2014) mRNA based therapeutics - developing a new class of drugs, *Nat Rev Drug Disc* 13: 759-780.
Karikó, K et al. (2005) Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* 23: 165-175;
Holtkamp S et al. (2006) Modification of antigen-encoding RNA increases stability, translational efficacy, and T-cell stimulatory capacity of dendritic cells. *Blood* 108: 4009-4017.

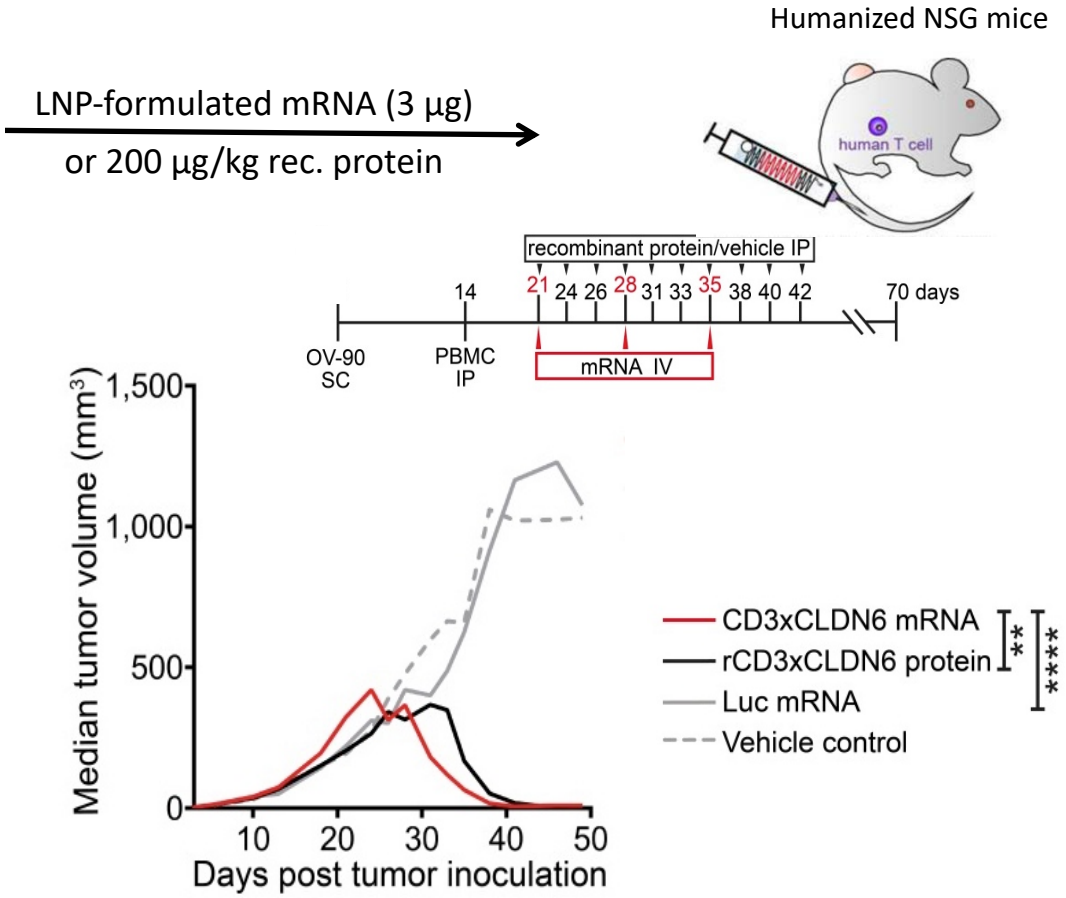
# Therapeutic efficacy of CD3xCLDN6 RiboMAB-encoding mRNA

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]




- 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)

ClinicalTrials.gov Identifier: NCT05262530

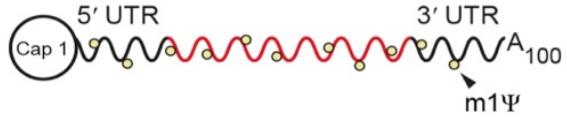
# Intratumor injection of mRNA for cancer treatment

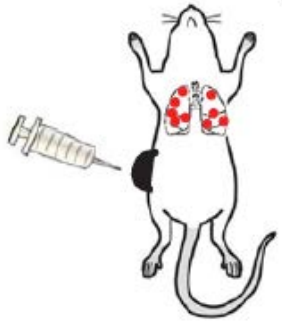
Science Translational Medicine

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

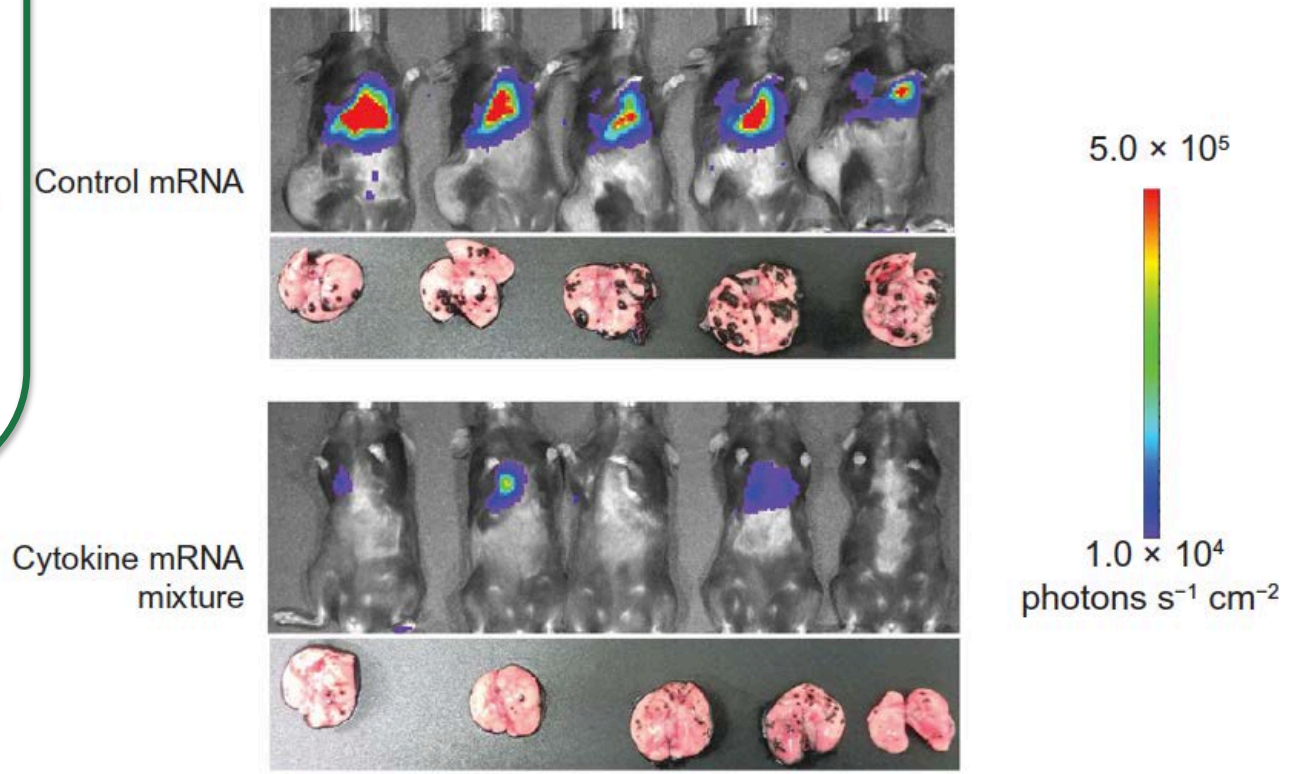
Christian Hotz[1†], Timothy R. Wagenaar[2*†], Friederike Gieseke[1], Dinesh S. Bangari[2], Michelle Callahan[2], Hui Cao[2], Jan Diekmann[1], Mustafa Diken[1,3], Christian Grunwitz[1], Andy Hebert[2], Karl Hsu[2], Marie Bernardo[2], Katalin Karikó[1], Sebastian Kreiter[1,3], Andreas N. Kuhn[1], Mikhail 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)



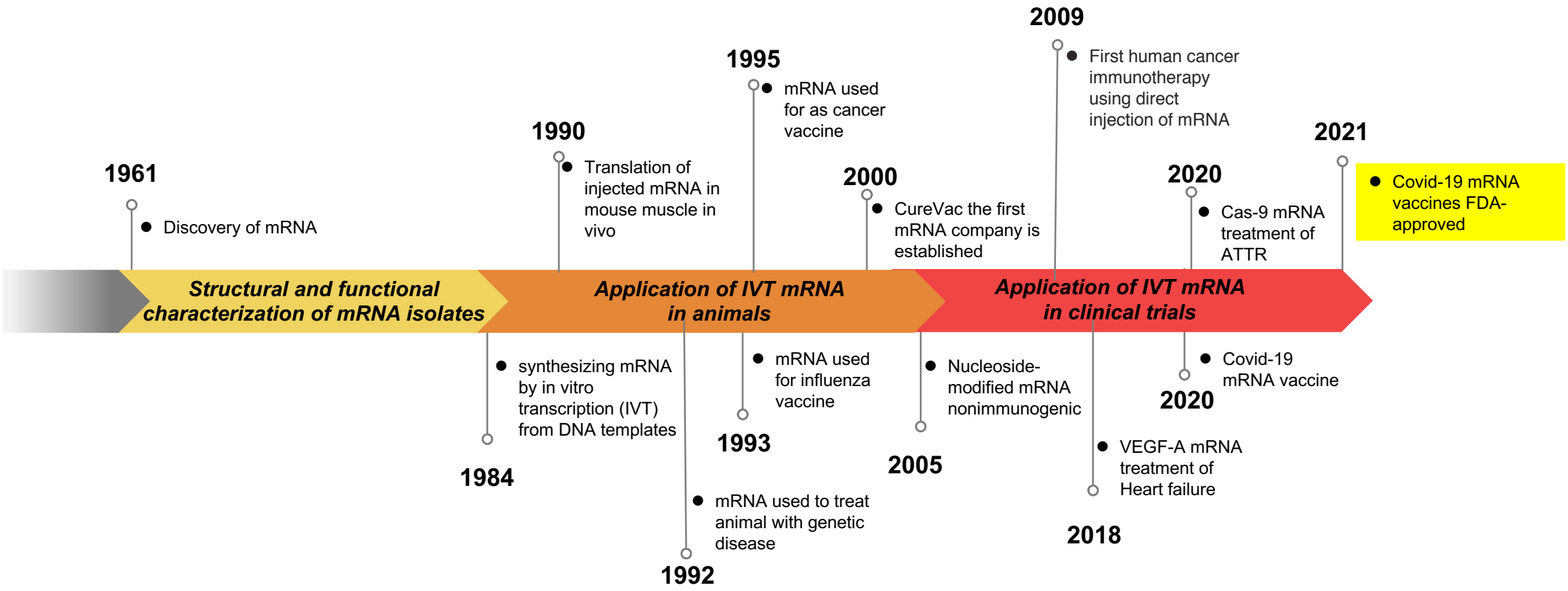



interleukin-12 single chain mRNA
interferon-alpha mRNA
GM-CSF mRNA
IL-15 sushi mRNA




ClinicalTrials.gov Identifier: NCT03871348

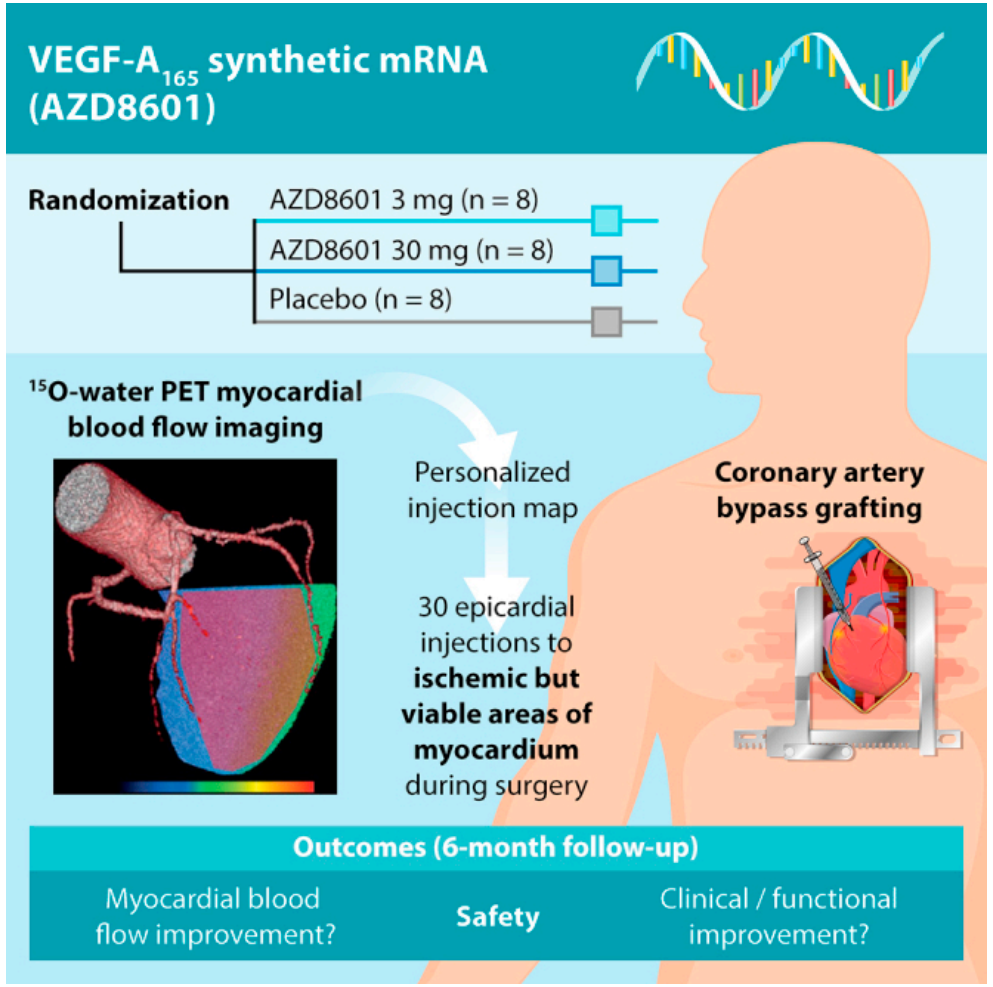
# Milestones of mRNA development for therapy



# Timeline: development of mRNA vaccines against SARS-CoV-2


Barbier et al. *Nature Biotechnology* 2022

# VEGF-A mRNA treatment of heart failure Phase-2 clinical trial




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

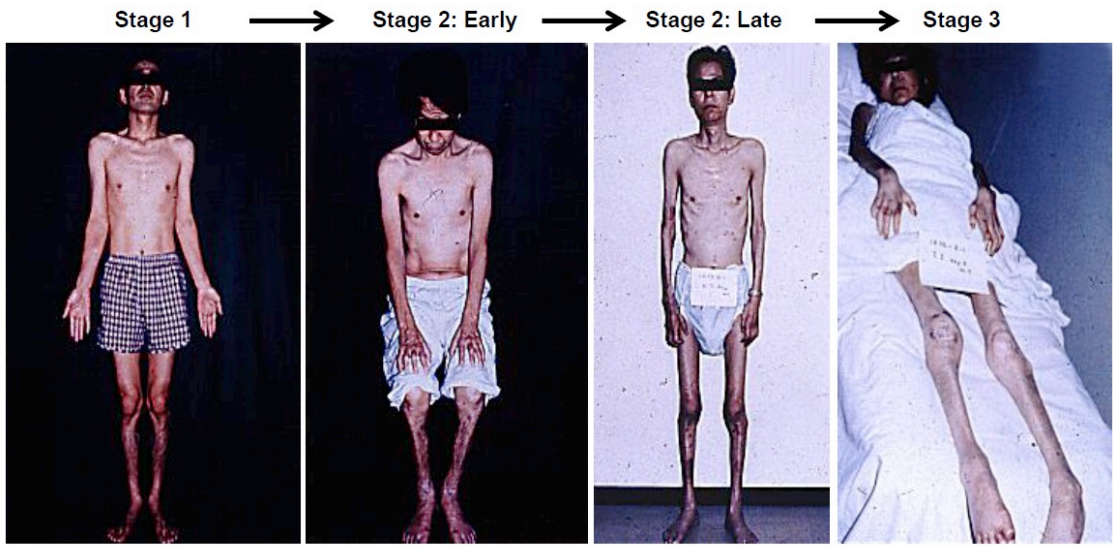
https://www.3sat.de/wissen/wissenschaftsdoku/220203-sendung-wido-102.html

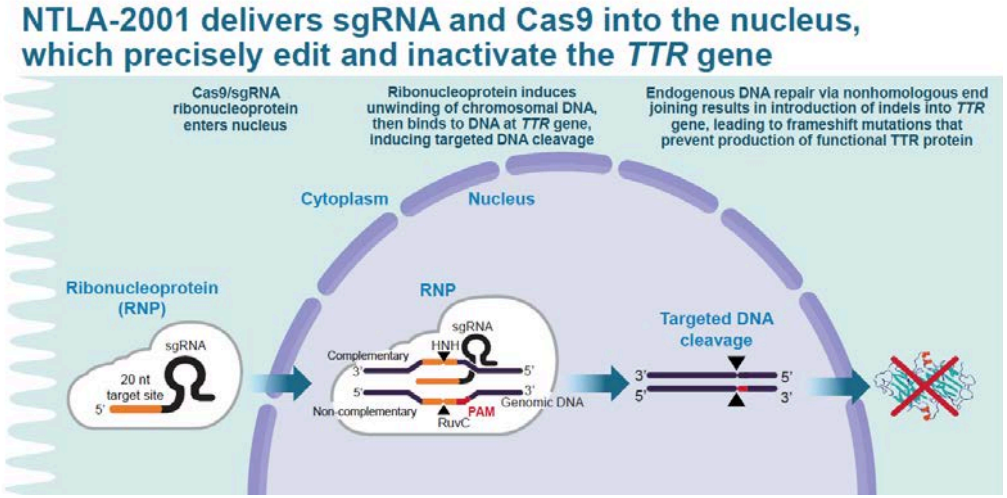
January 2022

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

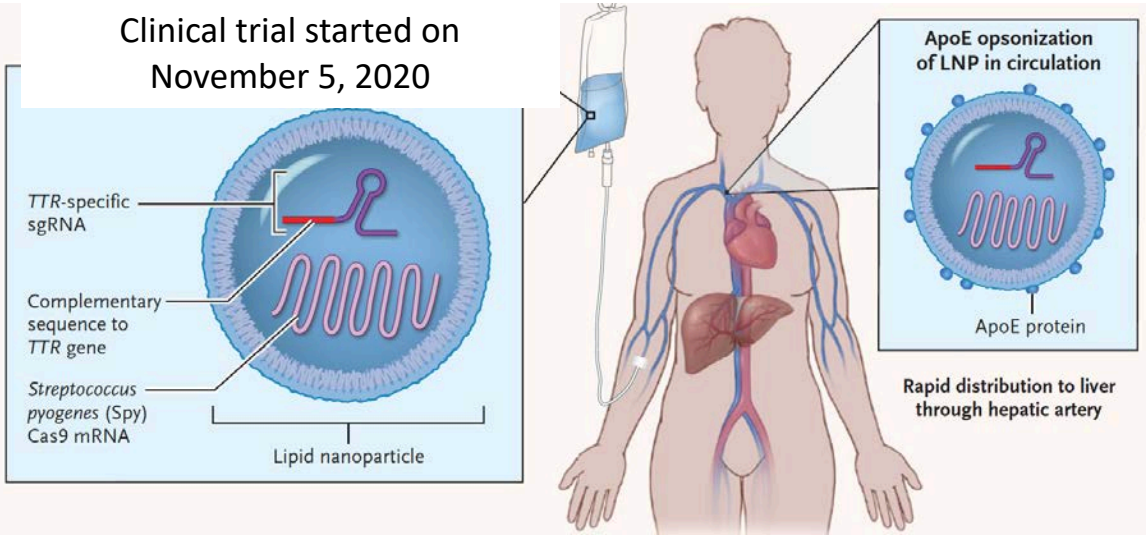
ClinicalTrials.gov Identifier: NCT03370887

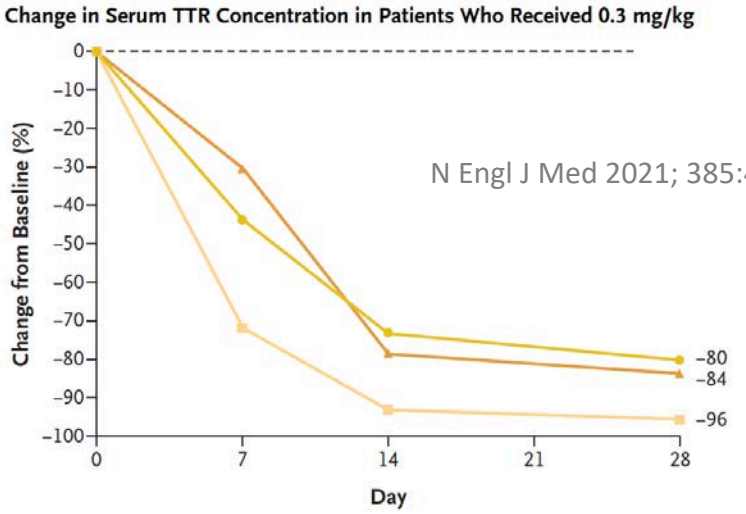
# CAS-9 mRNA for treatment of patients suffering from ATTR – by Intellia




Clinical trial started on November 5, 2020

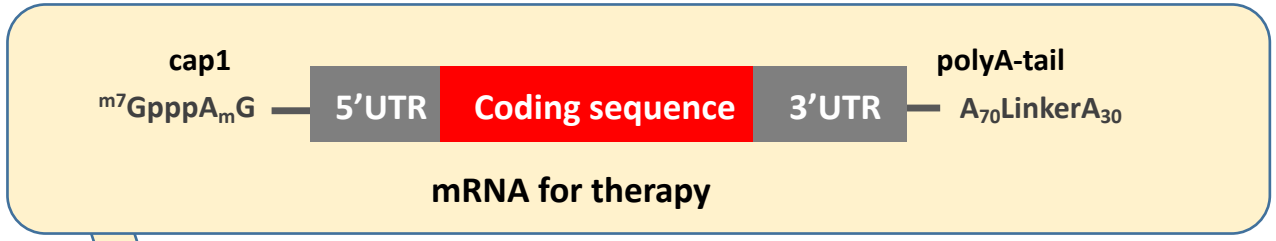


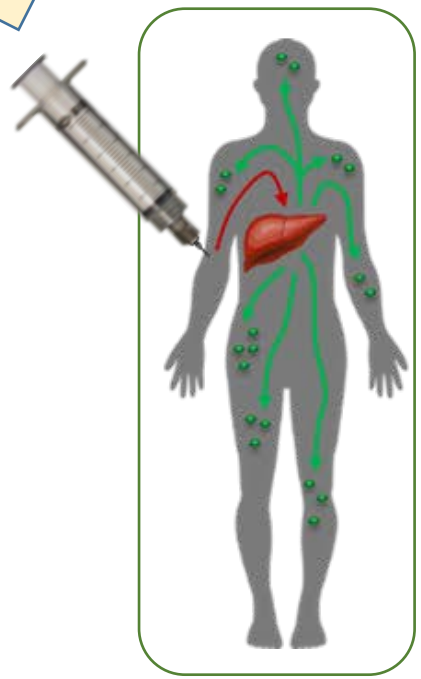




N Engl J Med 2021; 385:493-502

ClinicalTrials.gov Identifier: NCT04601051

# 2023 and beyond - mRNA is a new class of medicine



## mRNA in clinical trials to prevent or treat

- **infectious disease**
  - **RSV, Flu, CMV,** HIV, ZKV, HSV, EBV, HMPV, Nipah, TB, malaria
- **cancer**
  - vaccines, antibodies, CAR-T cells, intratumor injection of cytokine mRNAs
- **acute diseases**
  - VEGFA, relaxin heart falilure, VEGFA wound healing
- **genetic diseases**
  - OTCD, Propionic acidemia, methylmalonic acidemia, glycogen storage disease, genome editing (Cas9 mRNA), cystic fibrosis, sickle cell anemia