BIONTECH

http://www.biontech.de

Immunogenicity of RNA therapeutics

Katalin Karikó, PhD
BioNTech RNA Pharmaceuticals
Mainz, Germany

7th EIP Congress, Lisbon
February 25, 2015

katalin.kariko@biontech.de

RNA therapeutics in clinical trials

BIONTECH

- RNA is not in clinical practice
- RNA is tested in clinical trials for 40+ years

Synthetic RNA

- Long double-stranded RNA (dsRNA)
  polyI:C, polyA:U, polyI:C_{12}U (Ampligen),
  polyICLC (polyI:C poly-L-lysine)

- Short RNA
  siRNA*, aptamer*, microRNA, ribozyme, isRNA,

- In vitro-transcribed RNA

Isolated RNA

- Autologous tumor RNA*

* in phase III clinical trial

Synthesis of RNA therapeutics

Chemically synthesized RNA

- Short RNA: siRNA, aptamer, microRNA, ribozyme, isRNA

Enzymatically synthesized RNA

- Polymerization by polynucleotide phosphorylase
  long dsRNA homopolymers: polyI;C, polyA:U, polyI:C_{12}U

- Phage RNA polymerase (e.g. T7RNAPol)
  in vitro-transcribed mRNA encoding
  viral and cancer antigens

Immunogenicity of RNA therapeutics

BIONTECH

Immunogenicity: therapeutic objective 😊

vaccine adjuvant activities of dsRNA, isRNA

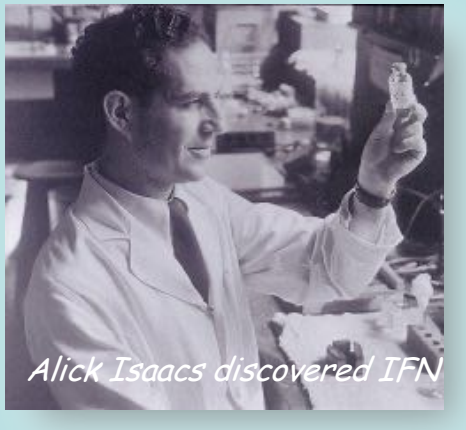
Immunogenicity: added benefit 😌

Adjuvant activity of in vitro-transcribed RNA – encoding cancer and viral antigens

Immunogenicity: harmful 😟

In vitro-transcribed RNA – encoding therapeutic proteins; allergen or antigen for inducing tolerance, siRNA; aptamer

Interferon induction by RNA - a short history


Alick Isaacs discovered IFN

1957 Interferon - inhibits viral replication

Proc R Soc Lond B Biol Sci. (1957) 147:258-67

1963 RNA induce interferon

The Lancet (1963) 282: 113-116

1967 dsRNA, polyI:C, polyA:U induce interferon

Proc Natl Acad Sci USA (1967) 58: 782-789,*1004-1010,* 1719-1722, 2102-2108

1976-87 Clinical trial with polyI:C, polyICLC, polyA:U, polyI:C_{12}U (Ampligen) to treat cancer, AIDS

Natl Cancer Inst (1976) 57:599-602; Cancer Treat Rep (1978) 62: 1907-12
Lancet (1980) Jul 26;2(8187):161-4; J Biol Res Mod (1985) 4: 669-75
Lancet (1987) Jun 6; 1(8545):1286-92

Therapeutic dsRNA was ineffective or very toxic

Mechanism of action was unknown until discovery of RNA sensors

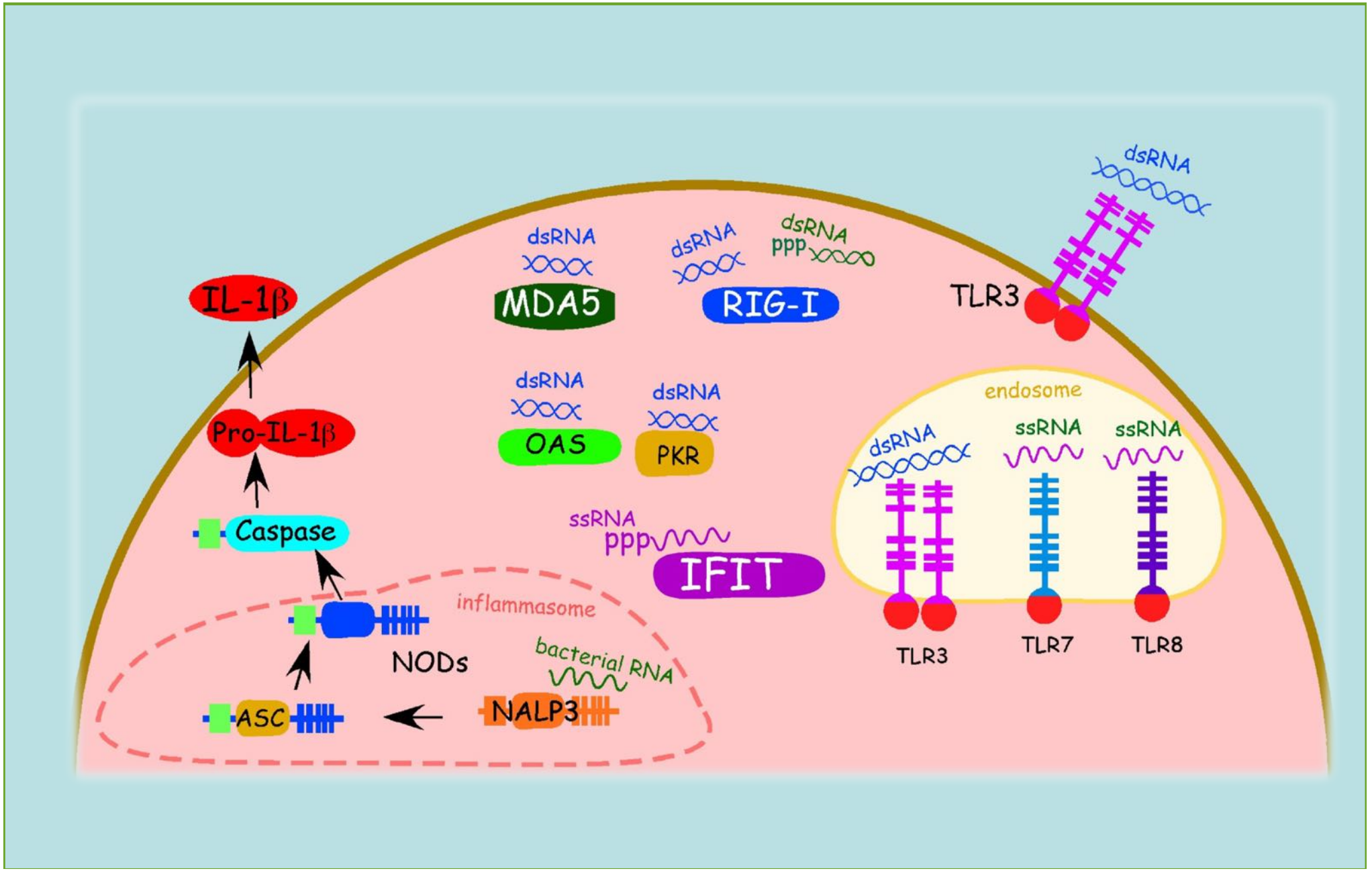
2001	**TLR3**	*Nature* 413, 732-8 (2001)
2004	**TLR7**	*Science* 303, 1529-1531 (2004)
	TLR8	*Science* 303, 1526-1529 (2004)
	RIG-I	*Nat Immunol 5, 730-737 (2004)*
2005	**MDA5**	*Nat Immunol* 6, 981-988 (2005)

RNA sensors and their activators

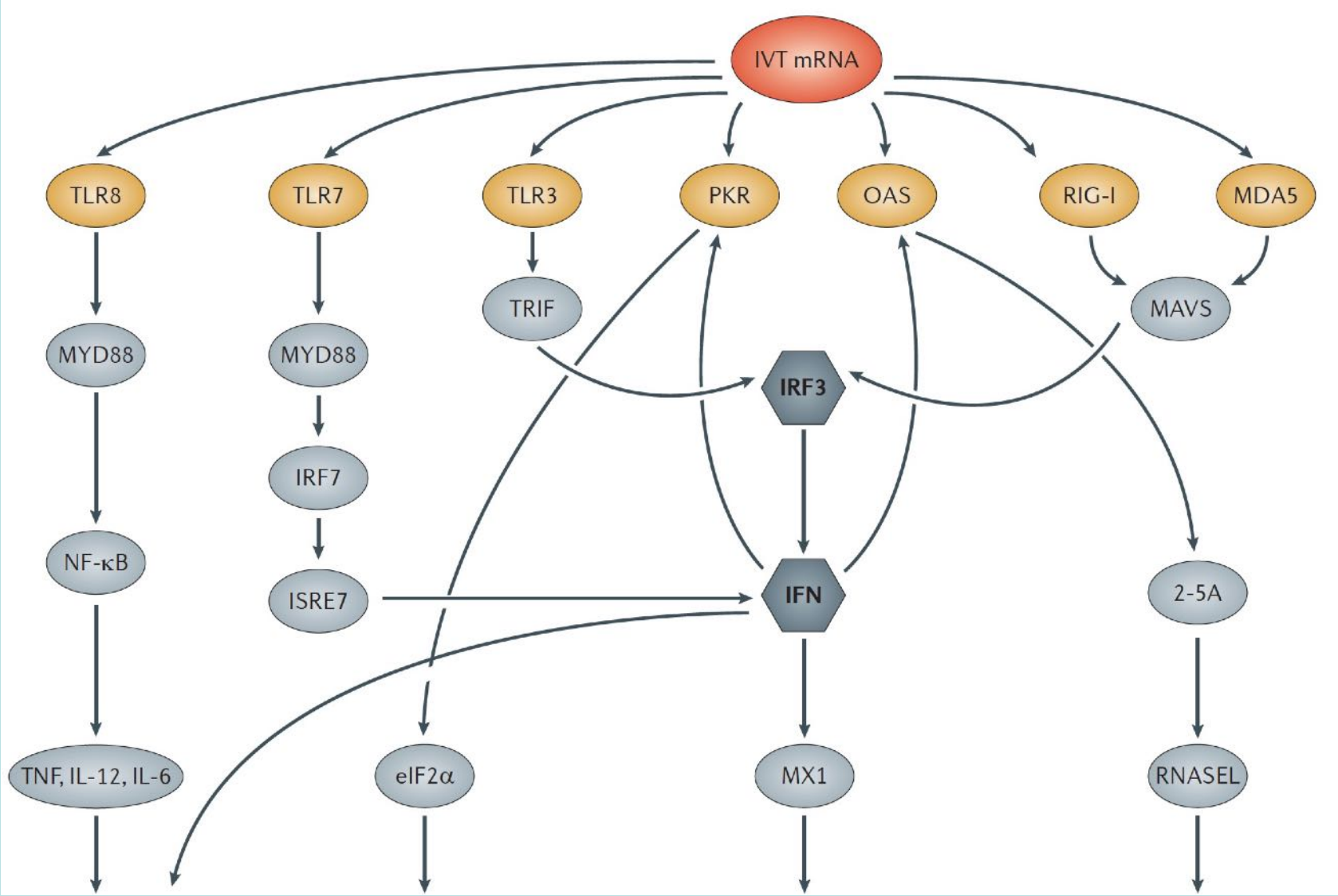
Function	RNA sensors	Activator RNA	Function
Regulator			Inflammatory, antimicrobial, antitumor
	TLR3	dsRNA	Inflammatory cytokines, interferon-β
	TLR7	ssRNA, polyU, bacterial RNA	Interferon-α production
	TLR8	GU-rich ssRNA	Inflammatory cytokines and interferon production
	RIG-I	ppp(ds)RNA	Inflammatory cytokines and interferon-β production
	MDA5	dsRNA	Interferon production
Effector			antimicrobial
	PKR	dsRNA, pppRNA	Protein synthesis inhibition Cytokines production
	OAS (RNaseL)	dsRNA	Antiviral: degrade ssRNA
	NALP3	bacterial RNA	IL-1β production
	IFIT1	pppRNA	Protein synthesis inhibition

Current Opinion in Drug Discovery & Development 2007, 10:523

Subcellular location of the RNA sensors



Inflammatory response to in vitro-transcribed (IVT) mRNA



Immunogenicity of IVT mRNA

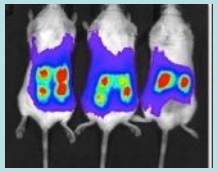
Type of immune response depends on

Particle size of formulated RNA (Blood. 2010;115: 4533-4541)

naked RNA → IFN
nanoparticle → IFN-α
microparticle (lipoplexes) → TNF-α

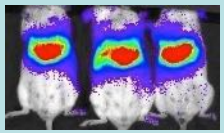
Delivery route

intradermal



skin

intravenous

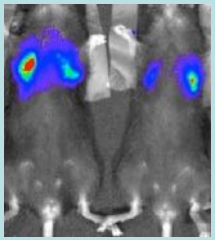


liver

spleen

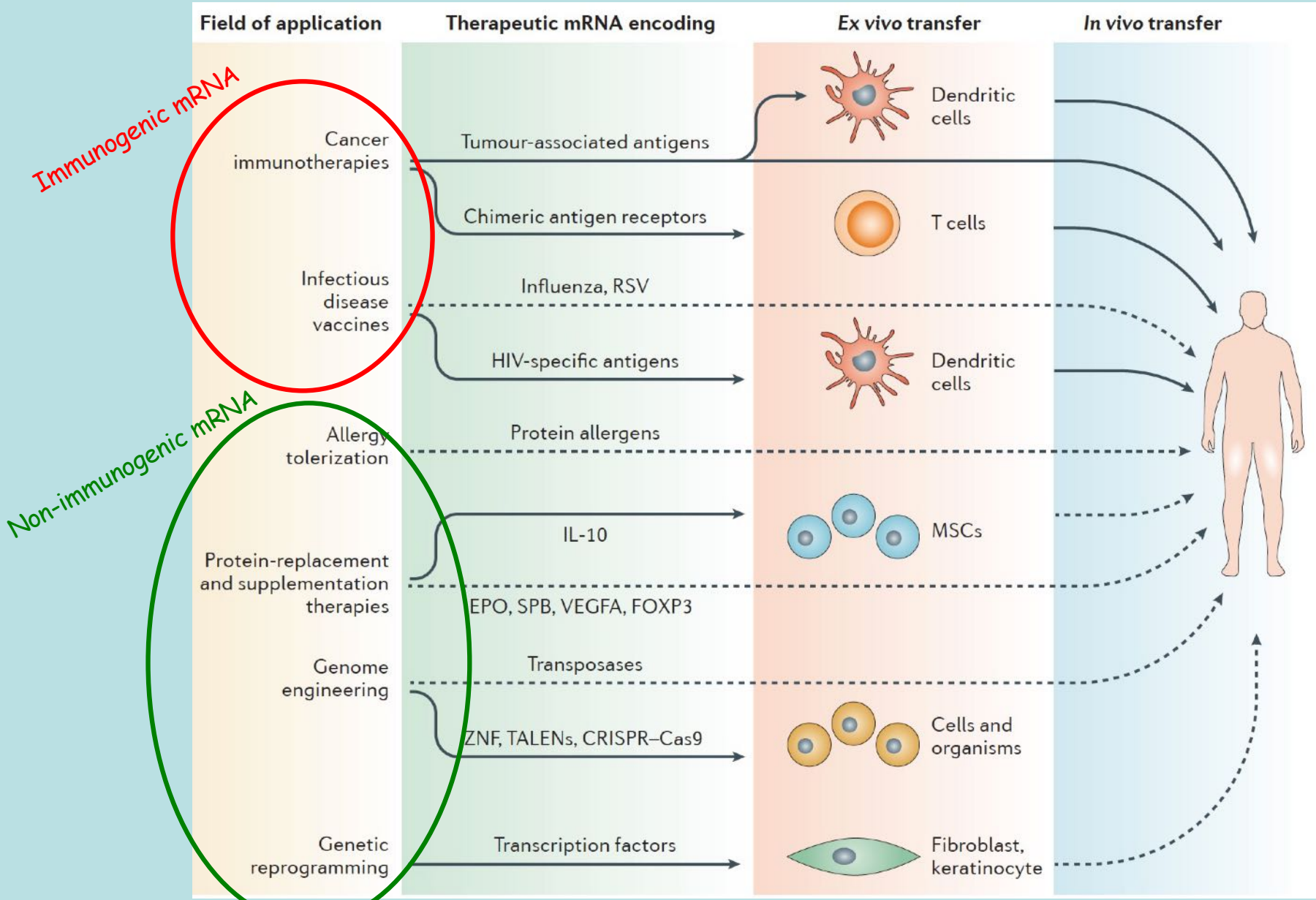
formulation-dependent delivery of RNA

intratracheal



lung

mRNA-based therapeutics – developing a new class of drugs

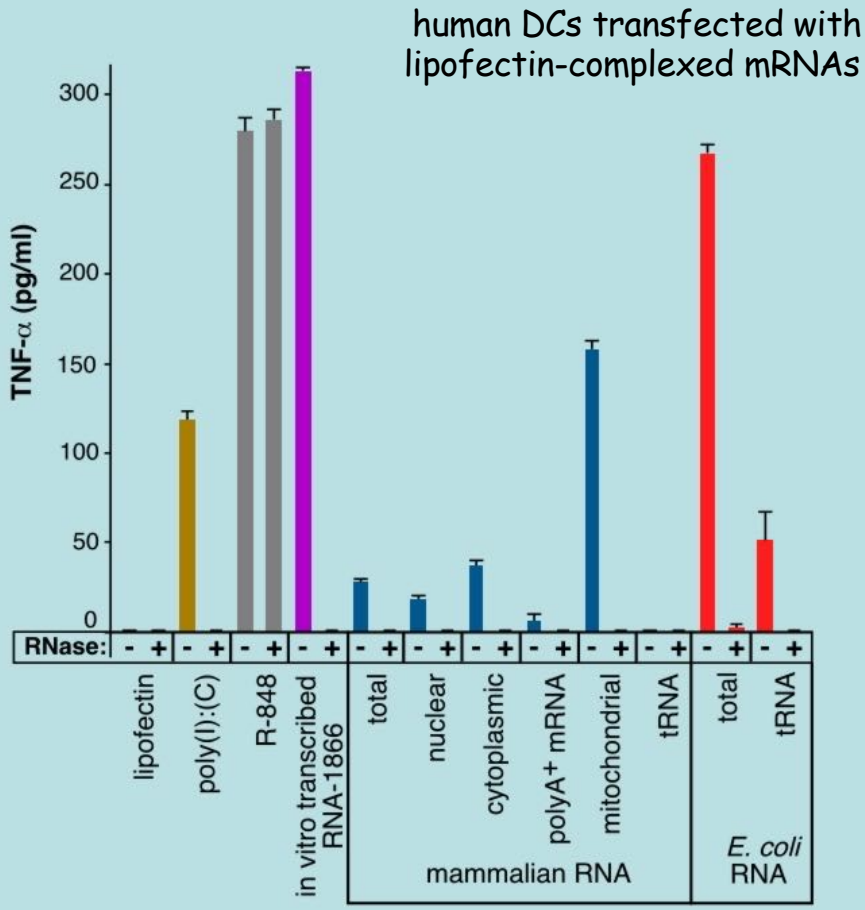


Nature Reviews Drug Discovery 2014, 13: 759

How to avoid activation of RNA sensors?

Generating non-immunogenic RNA

Natural RNAs are not equally potent activators of dendritic cells (DCs)

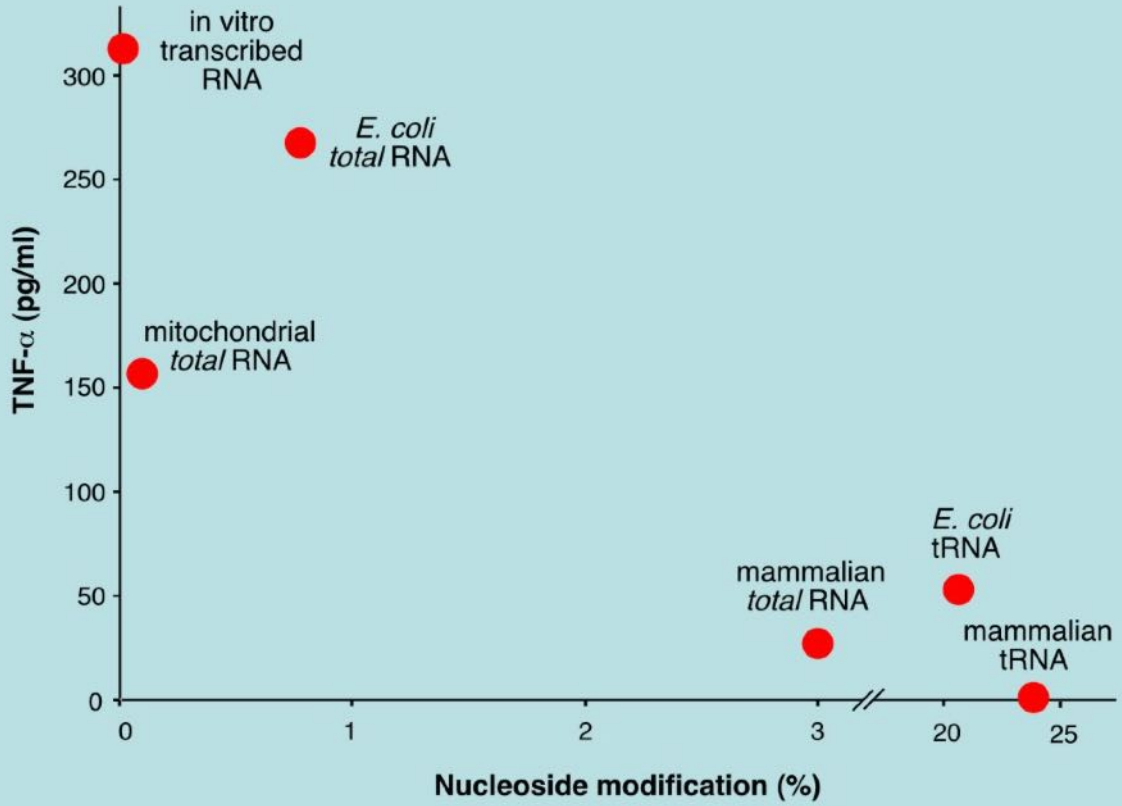


mRNA, tRNA is less immunogenic than
IVT RNA, bacterial RNA and mitochondrial RNA

Monocyte-derived DC
(GM-CSF + IL-4)

Immunity 2005, 23: 165

Natural RNAs are not equally potent activators of dendritic cells (DCs)



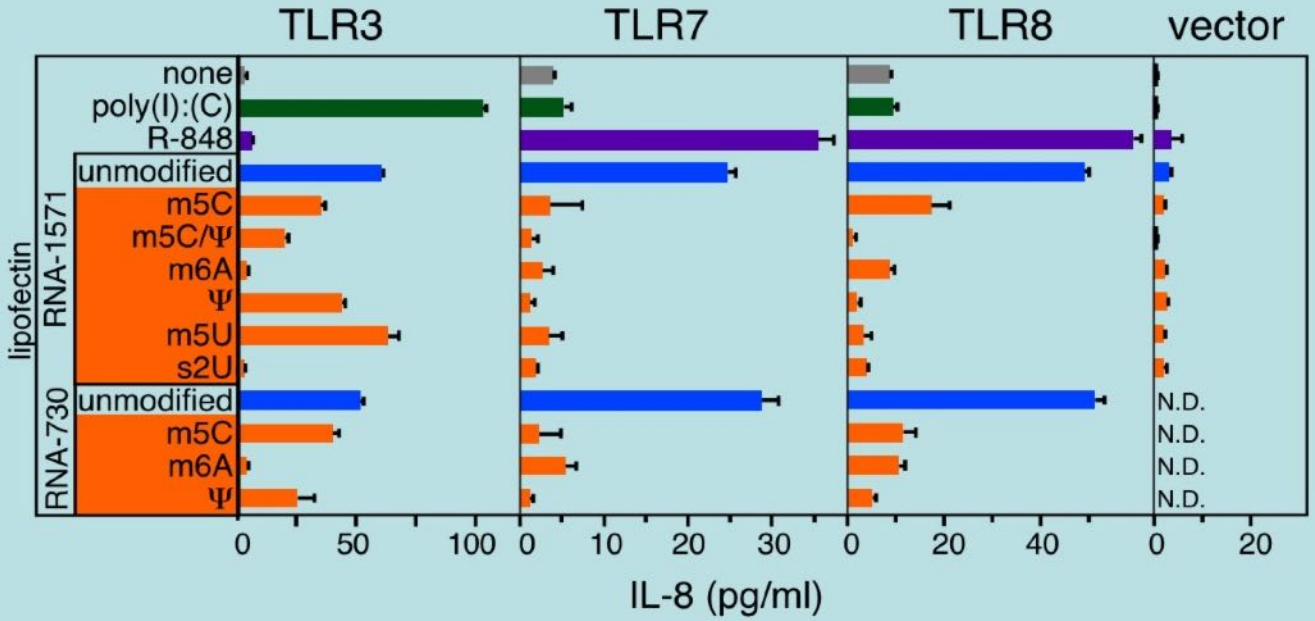
Inverse correlation:

- high level of modified nucleoside
- low level of immunogenicity of RNA

Current Opinion in Drug Discovery & Development 2007, 10:523

Nucleoside-modifications in IVT RNA suppress its immunogenicity

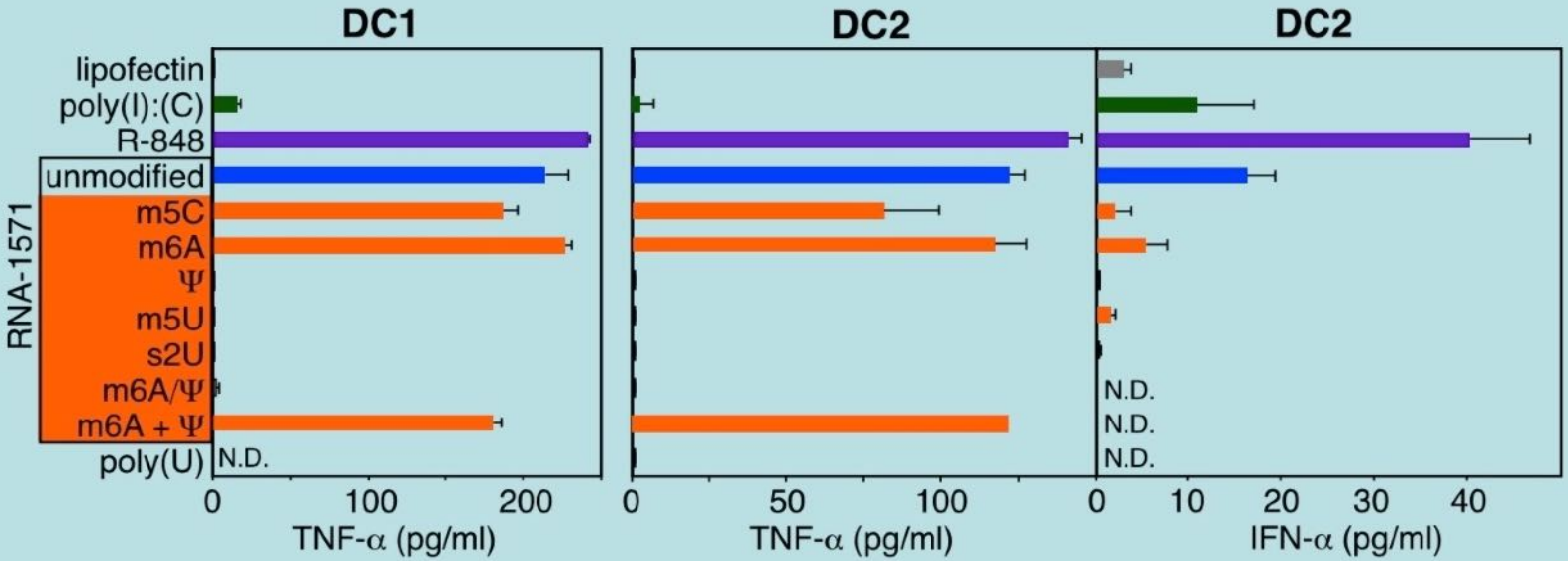
mRNAs containing modified nucleosides replacing 100% of the corresponding unmodified nucleosides are transcribed in vitro and tested on stable-transformed HEK-293 cells expressing TLR3, TLR7 and TLR8

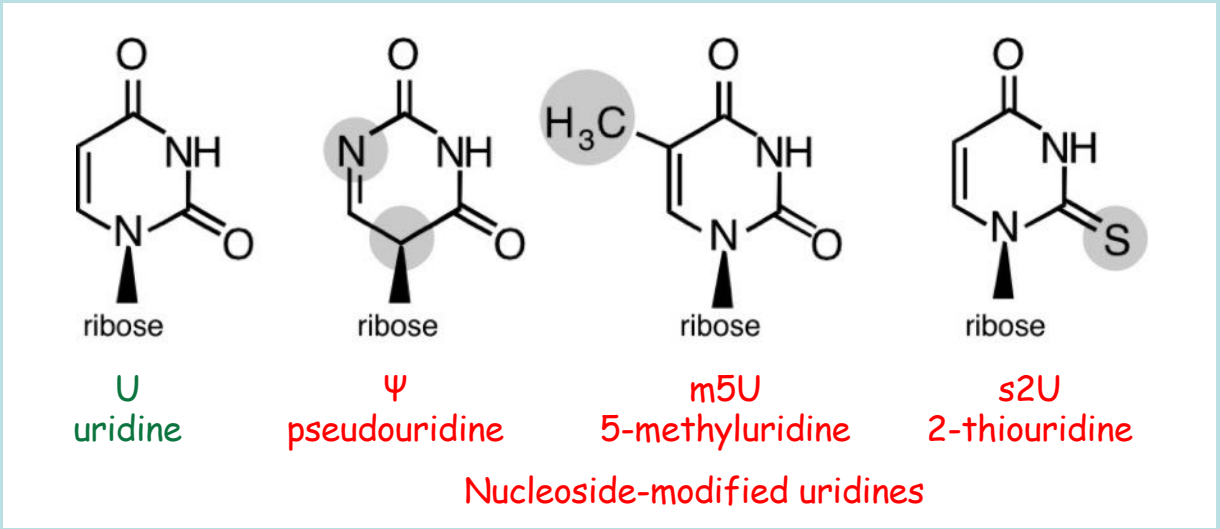


Modified nucleosides in RNA: reduce activation of RNA sensors

TLR3, TLR7, TLR8	Immunity 2005, 23: 165
RIG-I	Science 2006, 314: 994
PKR	RNA 2008, 14: 1201

Primary human DCs do not respond to RNA containing modified uridine

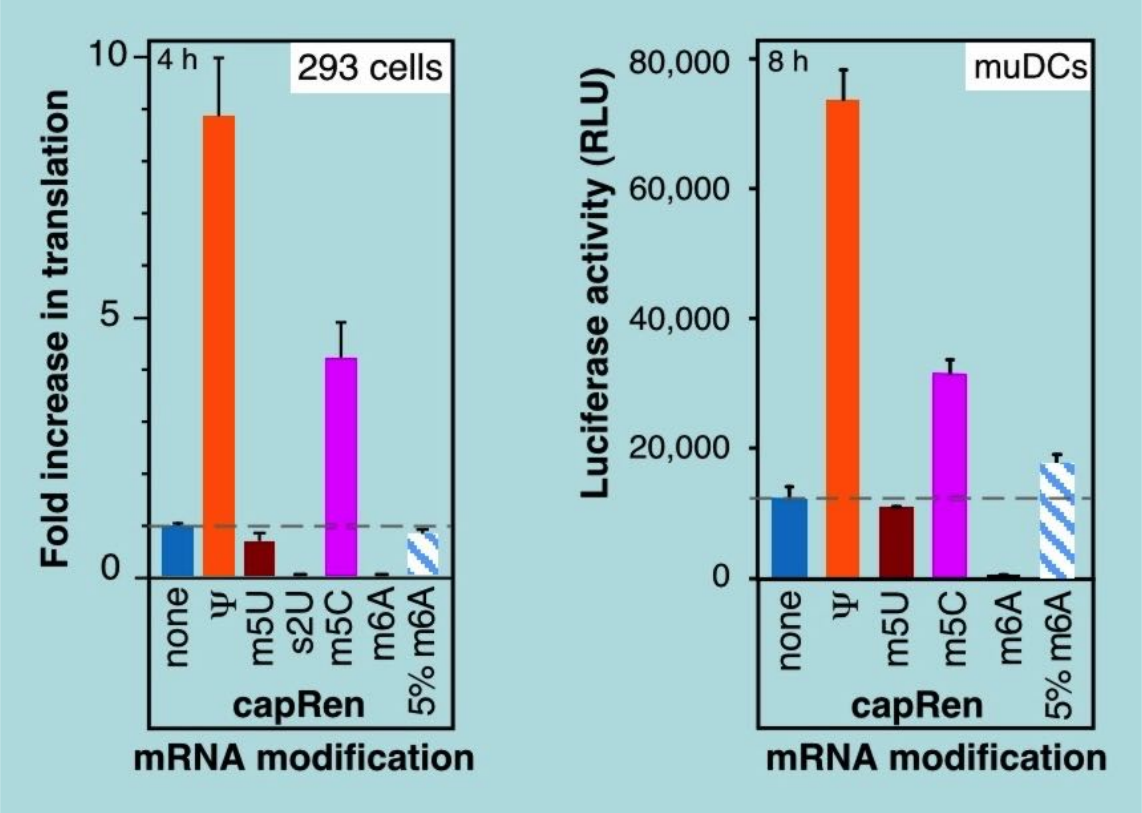




N.D.: not determined

Immunity 2005, 23: 165

Superior translation of lipofectin-delivered Ψ-modified mRNAs in cultured cells

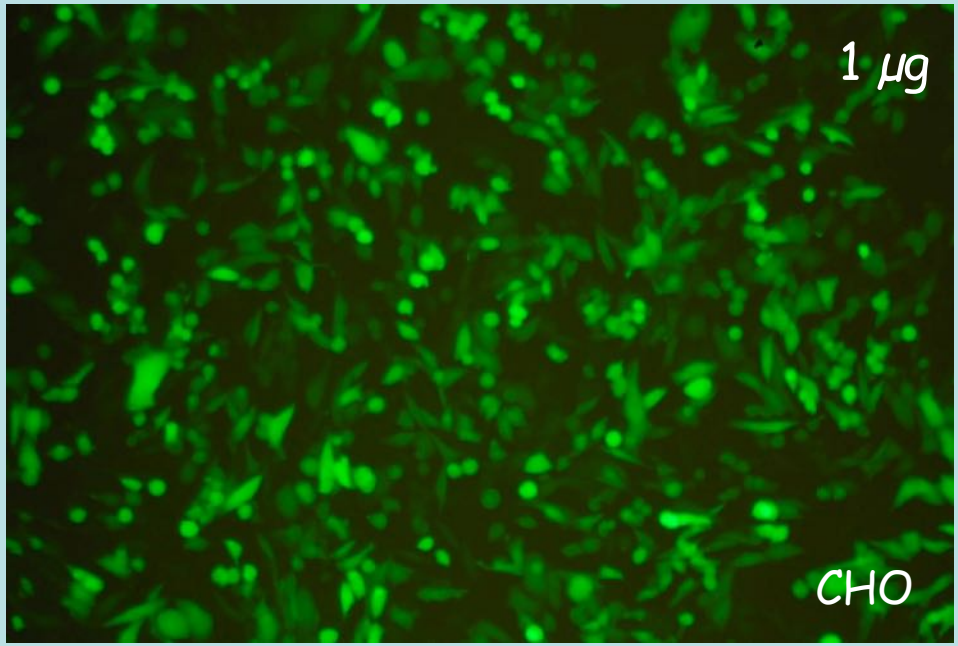


Translation of modified uridine-containing vs uridine-containing IVT mRNA

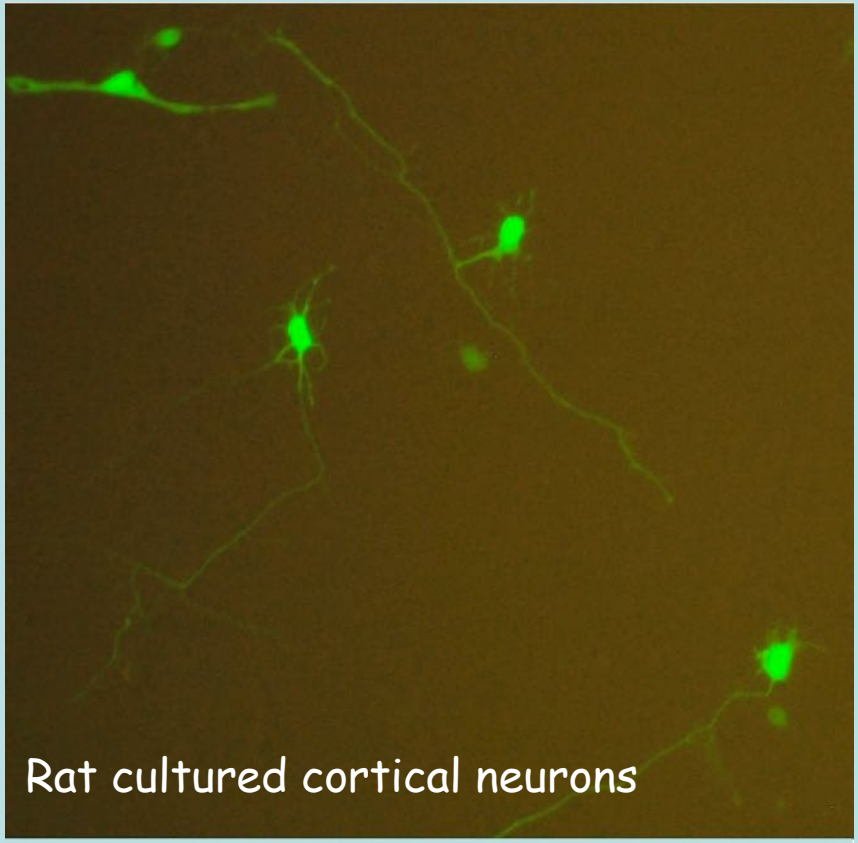
- Ψ-mRNA ⬆ very high level
- m5U mRNA = same as U mRNA
- s2U mRNA Ø no translation

Mol Ther 2008, 16: 1833

Expression of GFP in cultured cells following transfection with eGFP-encoding Ψ-mRNA



cap1-globin-eGFP-A_n

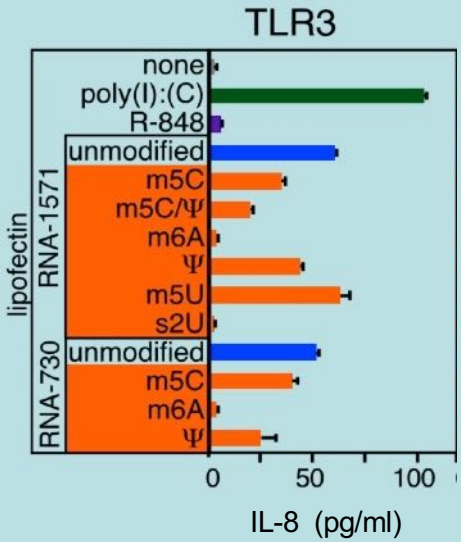


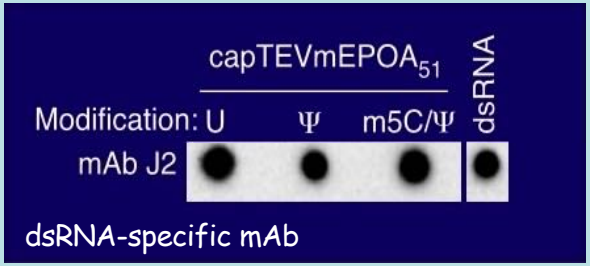
Ψ-modified mRNAs translate very efficiently in different call types, including neurons

Mol Ther 2008, 16: 1833

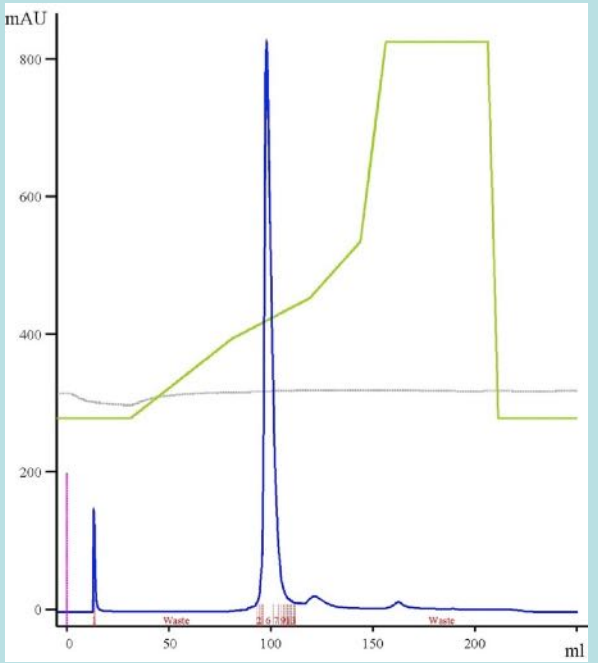
dsRNA contaminants can be removed from IVT mRNA by HPLC purification

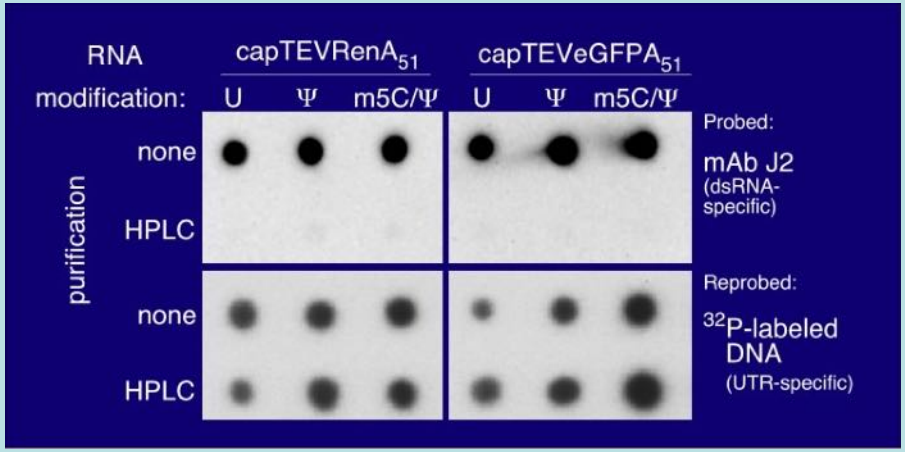
dsRNA contaminants in IVT mRNA activate TLR3





HPLC purification of IVT mRNA

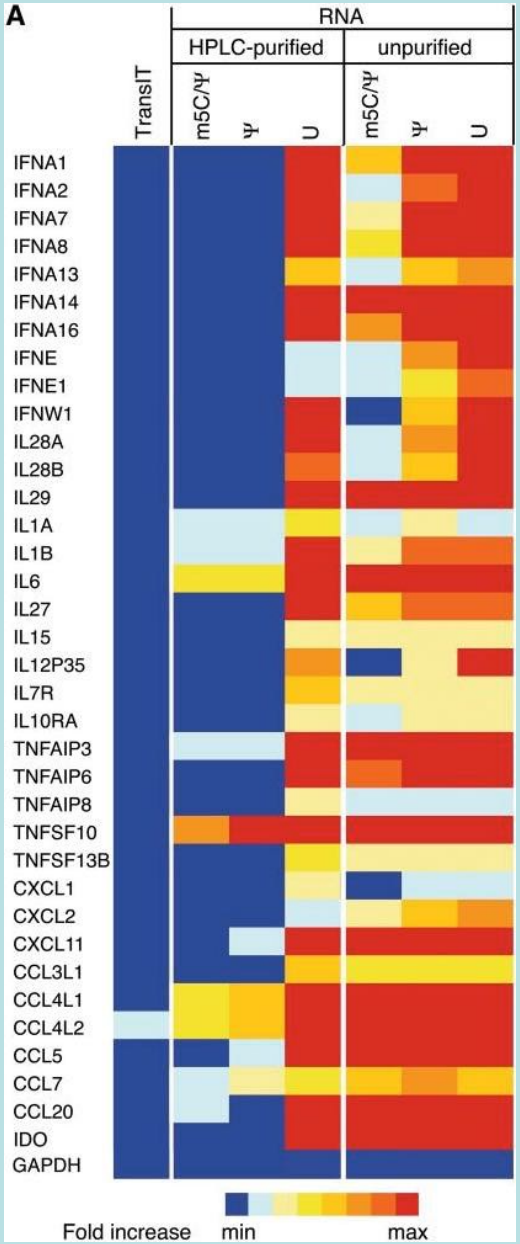




IVT mRNA is free of dsRNA contaminant

Nucleic Acids Res. 2011,39:e142

HPLC-purified, Ψ- and Ψ/m5C-containing mRNA is not immunogenic

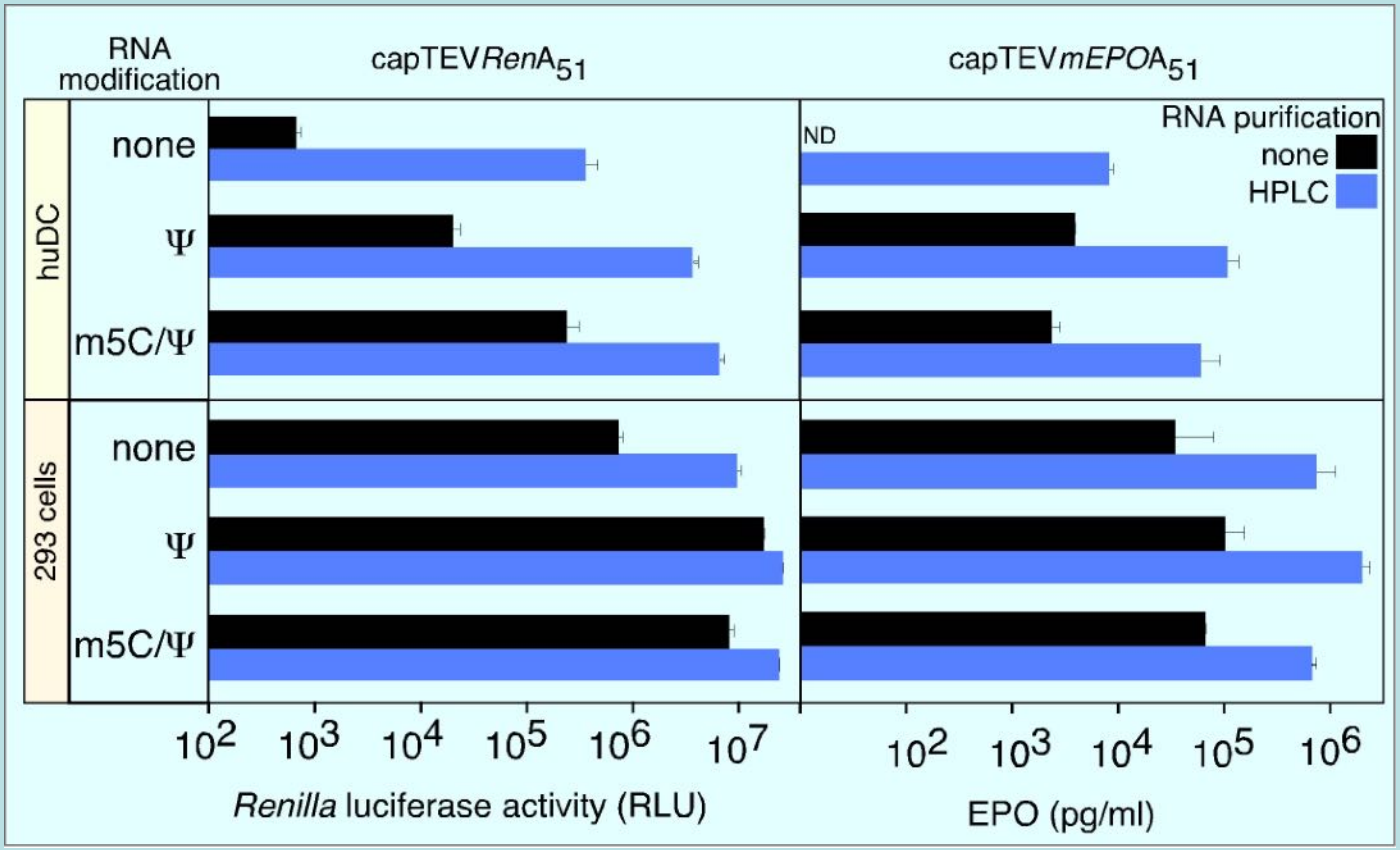


HPLC-purification eliminates immunogenicity of , Ψ- and Ψ/m5C-containing mRNA, but U-containing RNA remains immunogenic

hu MDDCs
at 6 h posttransfection

Nucleic Acids Res. 2011,39:e142

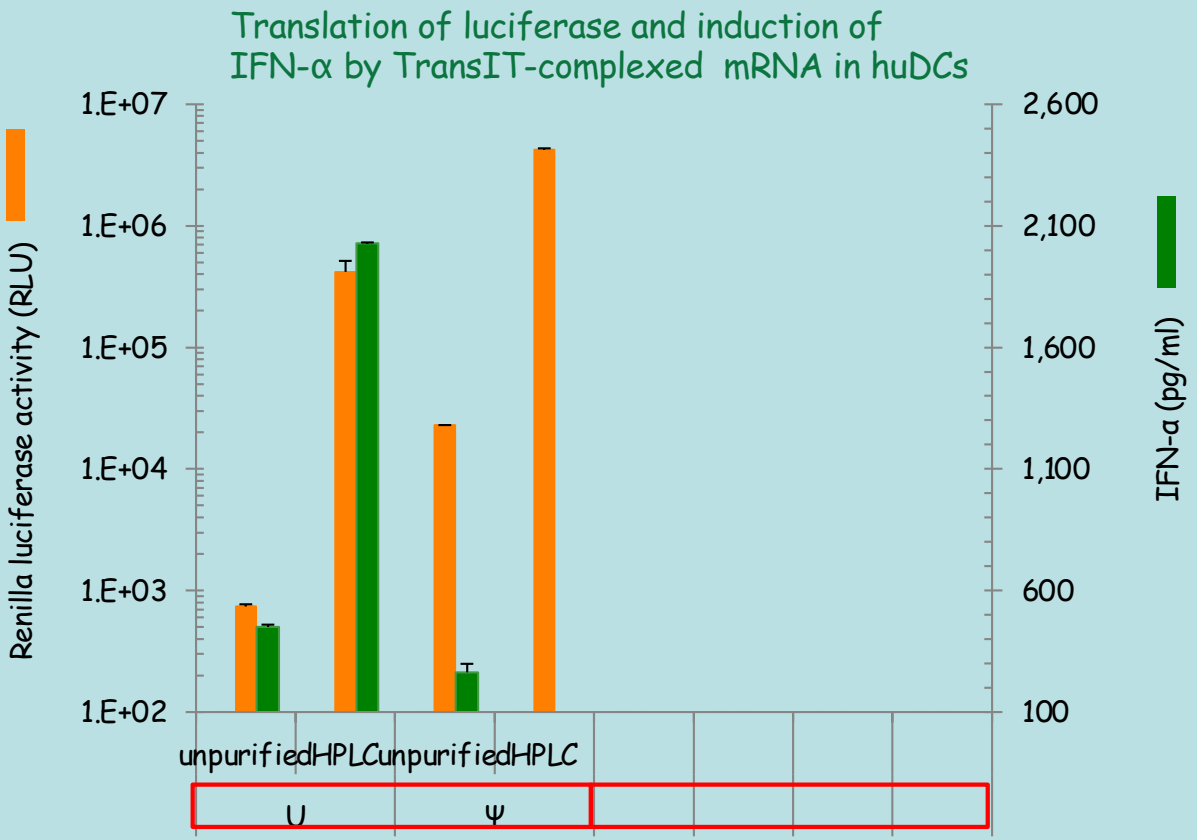
High level translation of HPLC-purified mRNA in huDCs and 293 cells



hu MDDCs
at 6 h posttransfection

Nucleic Acids Res. 2011,39:e142

Performance of IVT mRNA is greatly enhanced by HPLC purification huDCs

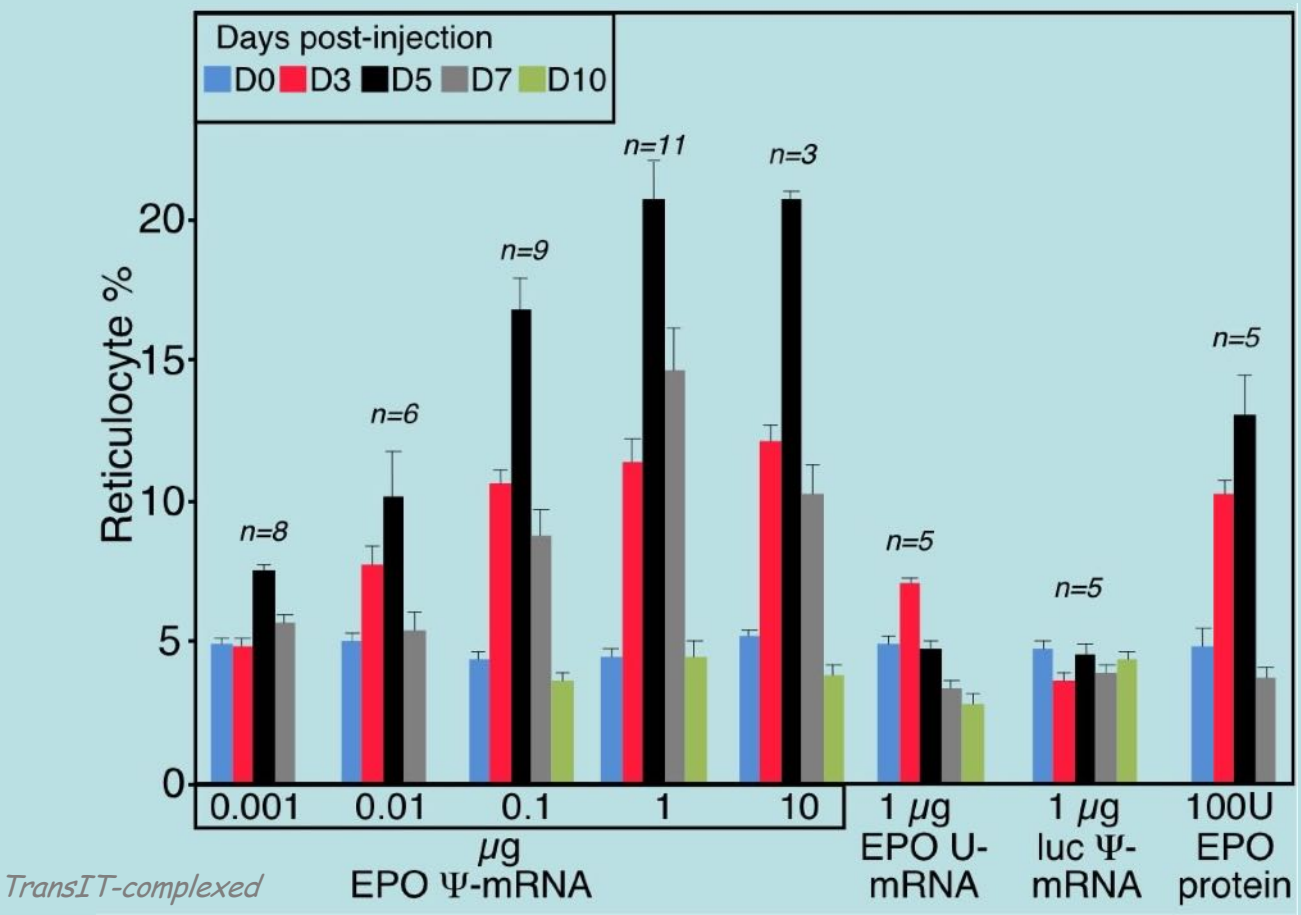


Importance of mRNA purification by HPLC:

- antigen-encoding U-containing mRNA: antigen level ⬆, immunogenicity ⬆
- therapeutic protein-encoding Ψ-containing mRNA: protein level ⬆, immunogenicity Ø

Unpublished data, E: 12 9 2010

HPLC-purified mEPO mRNA delivery by i.p. increases reticulocyte counts in mice



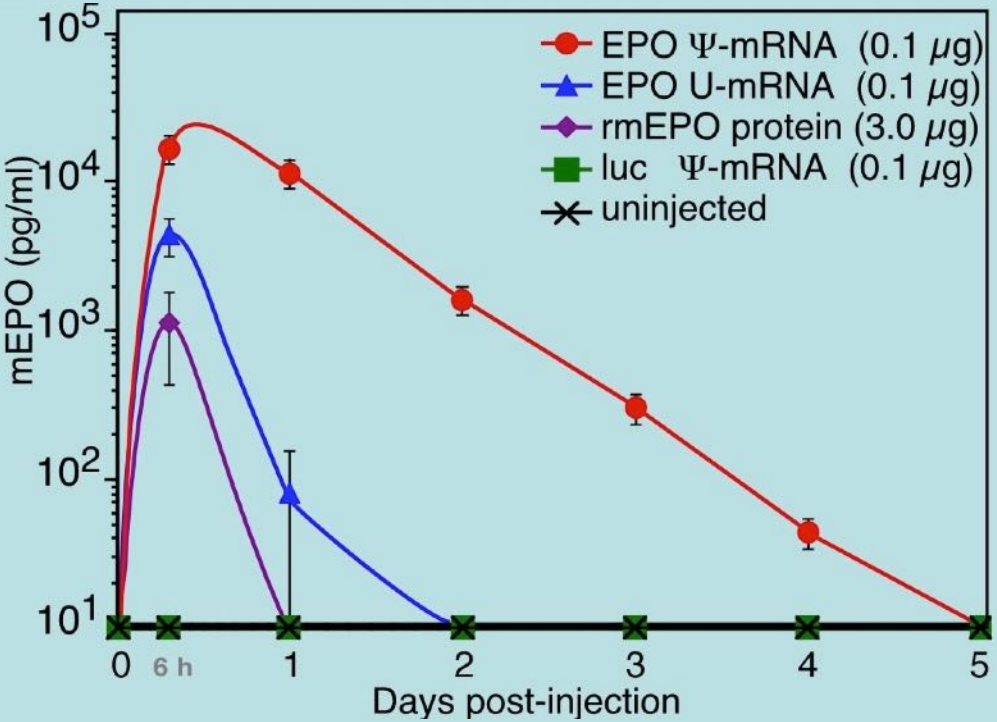
Increase of the reticulocyte levels

- 10 ng Ψ-containing EPO mRNA is more potent than
- 1000 ng U-containing EPO mRNA

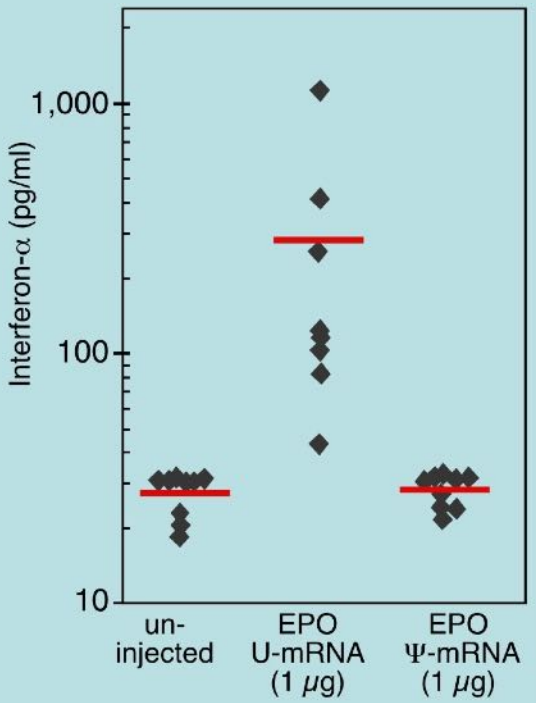
Mol. Therapy 2012, 20: 948

EPO levels in plasma of mice following mEPO mRNA delivery by i.p.

TransIT-complexed



Induction of IFN-a following mEPO mRNA delivery into mice by i.p.

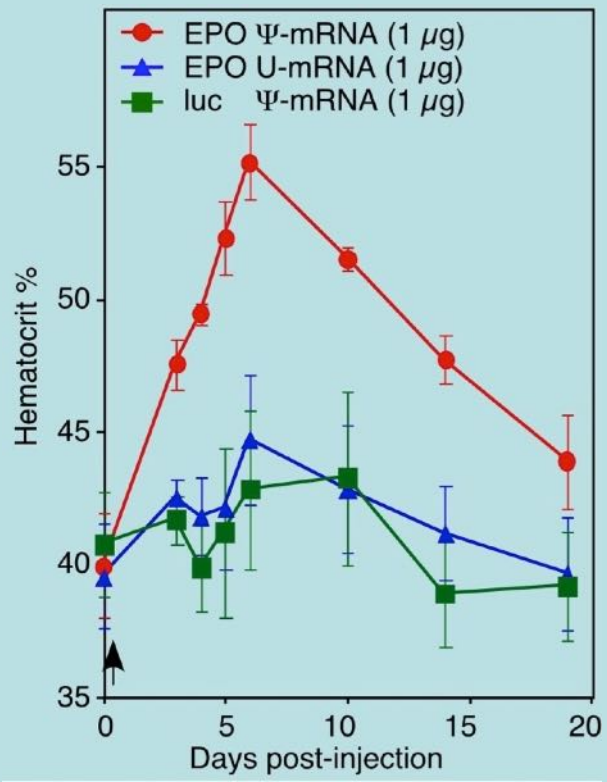


Purified, Ψ-modified EPO mRNA translation for long duration due to

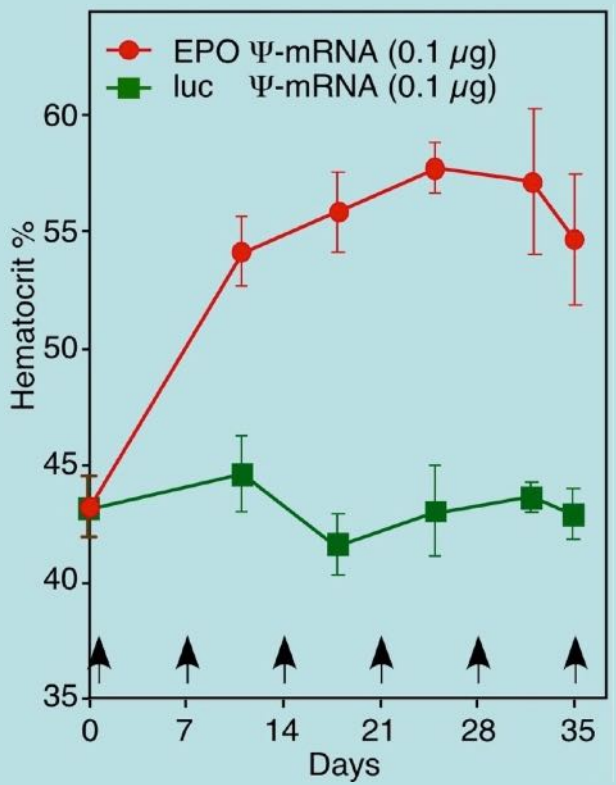
- diminishing PKR activation — Nucleic Acids Res. 38: 5884 (2010)
- increase resistance to cleavage by RNase L. — Nuc. Acids Res. 39, 9329 (2011).
- lack of IFN-a induction — Mol.Therapy, 20: 948 (2012)

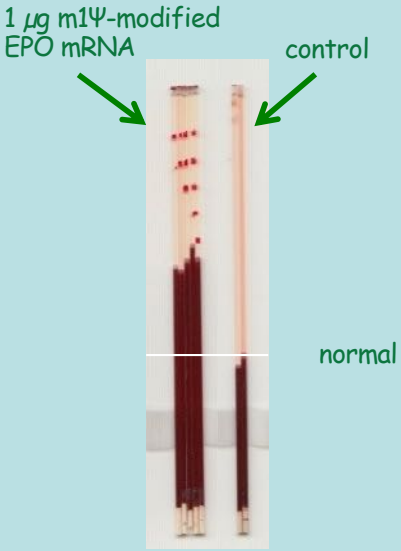
Mol.Therapy 2012, 20: 948

Hematocrit levels following mEPO mRNA delivery into mice by i.p.



TransIT-complexed





Hematocrit capillary tubes at day 8 post EPO mRNA injection

Low dose of non-immunogenic EPO mRNA (purified, Ψ-modified) has therapeutic effect

Mol. Therapy 2012, 20: 948

Immunogenicity of RNA therapeutics

BIONTECH

Conclusion

- Purified, uridine-containing IVT mRNA encoding cancer or viral antigen is ideal for vaccination by ensuring high antigen levels and providing adjuvant activity by inducing cytokines
- Purified, pseudouridine-containing IVT mRNA encoding therapeutic proteins can provide medical solution for diseases that can be cured by extracellular or intracellular protein supplementation

Acknowledgements

University of Pennsylvania:

Bart Anderson PhD

Drew Weissman MD, PhD

Hiromi Muramatsu PhD

http://www.biontech.de

BioNTech RNA Pharmaceuticals
Mainz, Germany