VACTRAIN

Viral vs non-viral vectors for anticancer gene therapy

Dzmitry Shcharbin [1,2] & **Maria Bryszewska** [2]

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2 - Department of General biophysics, University of Lodz, Lodz, Poland.

This work was supported by project VACTRAIN within HORIZON 2020 and by project NANOGENE within MSC IRSES of 7th EU FP, co-financed by the Polish Ministry of Science and Higher Education.

Revolution 2015 in Gene Therapy

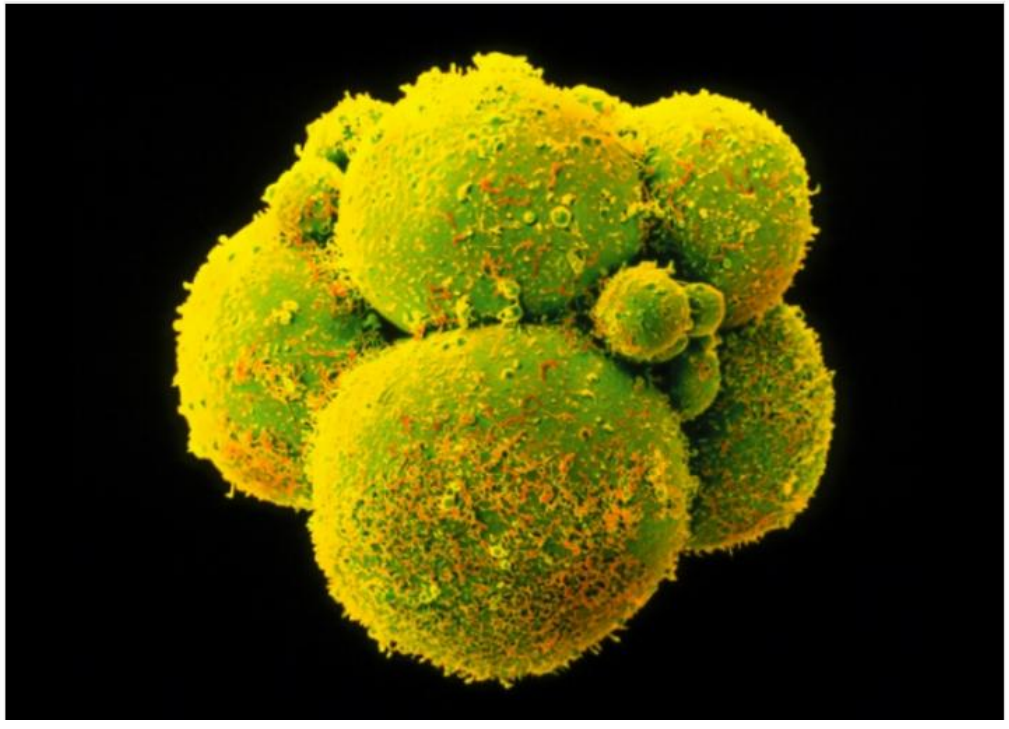
NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

David Cyranoski & Sara Reardon

22 April 2015



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Nobel Prizes 2015

Anti-parasite drugs sweep Nobel Prize in medicine 2015

Chinese pharmacologist Youyou Tu developed key anti-malarial drug artemisinin.

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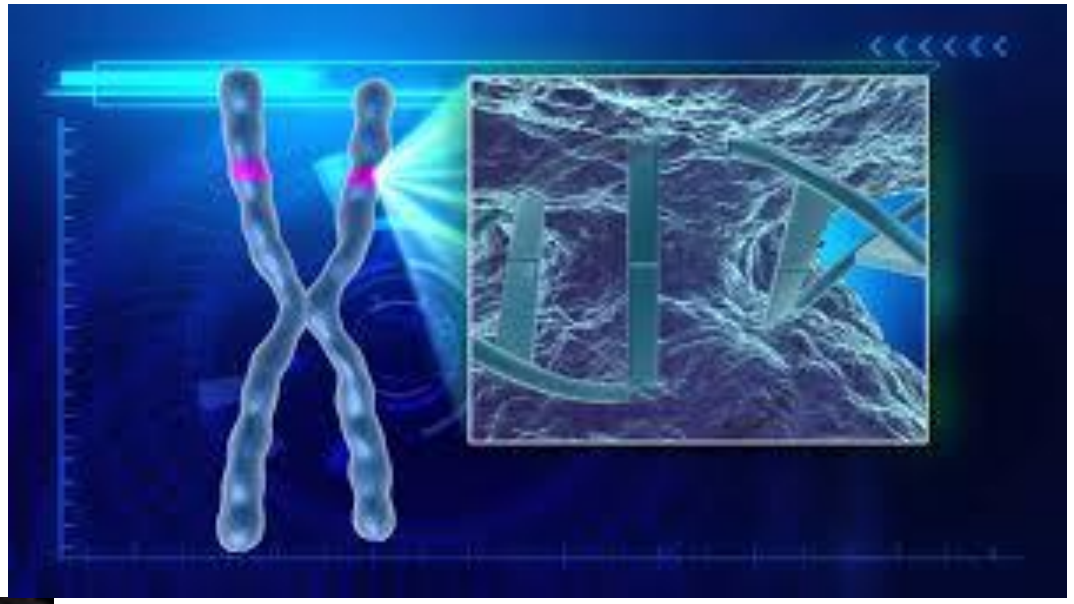
1. **Gene-editing record smashed in pigs**
   Nature | 06 October 2015
2. **South Korean economist to lead climate-science panel**
   Nature | 06 October 2015
3. **Strong placebo response thwarts painkiller trials**
   Nature | 06 October 2015

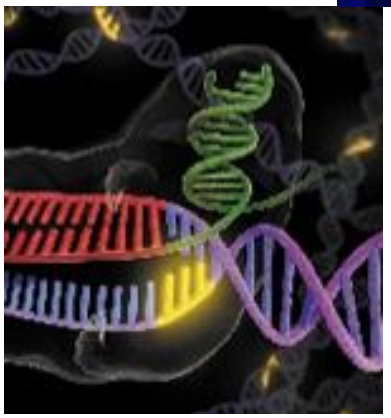
Nobel Prizes 2015

Morphing neutrinos win physics Nobel

Demonstration by Takaaki Kajita and Arthur McDonald that neutrinos oscillate between



Embryo with fatal blood disorder *(β-thalassaemia)*



Cutting the bad gene by CRISPR/Cas9

NATURE | NEWS

Chinese scientists genetically modify human embryos

Rumours of germline modification prove true — and look set to reignite an ethical debate.

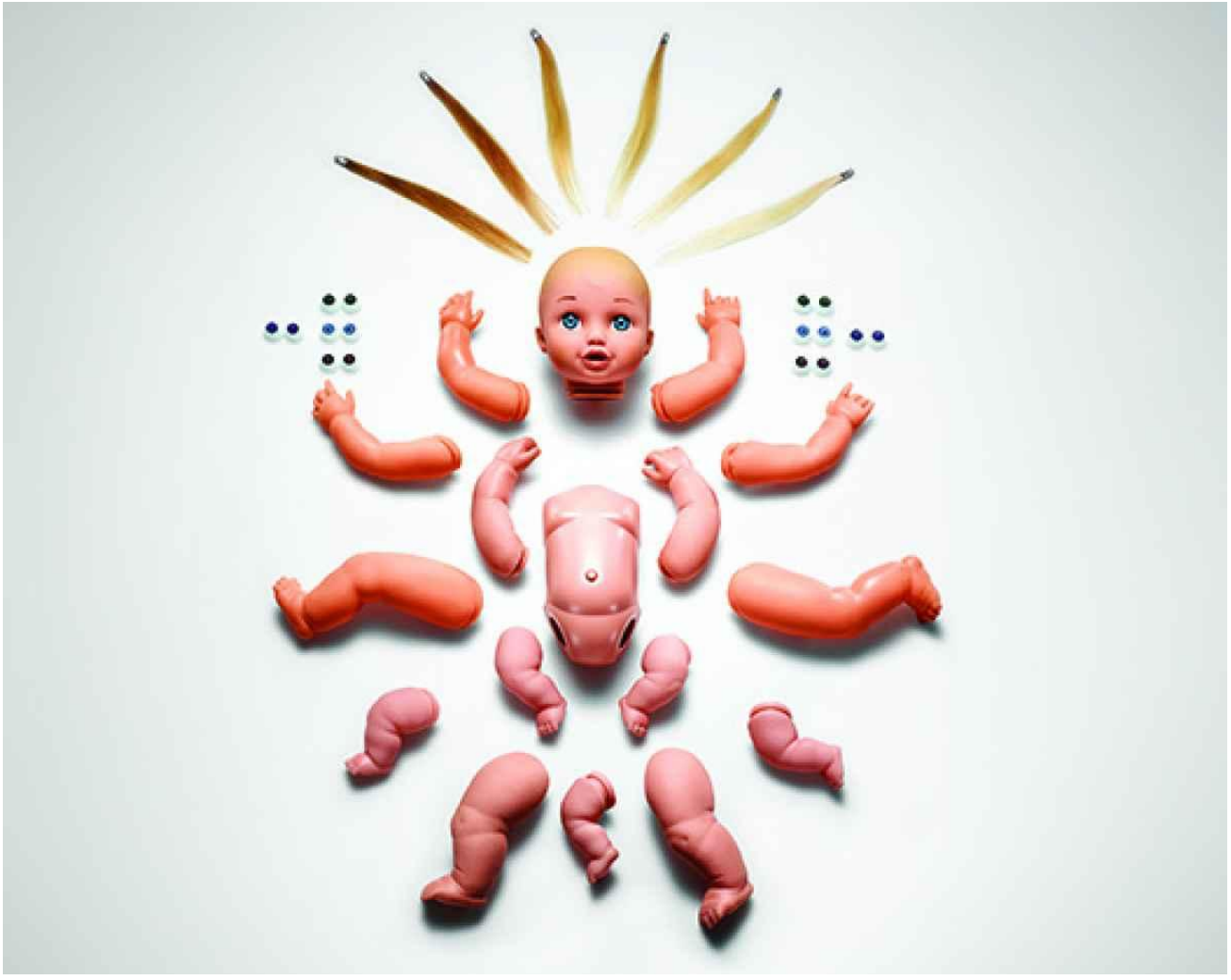
David Cyranoski & Sara Reardon

22 April 2015

The enzyme complex CRISPR/Cas9 (C-Cas9), which binds and splices DNA at specific locations. The complex can be programmed to target a problematic gene, which is then replaced or repaired by another molecule introduced at the same time.

What did they obtain?

Meta-Homo Sapiens = Artificial H. Sapiens



The Human Genome Project (HGP) is often referred to as "Brave New World" 1990 – 2003

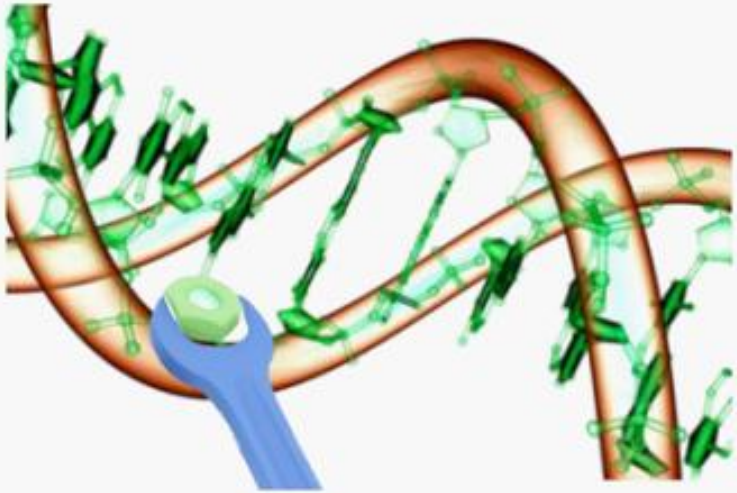
+

The Human Proteome Project (HPP) 2008 – ongoing

Gene Therapy

Construction of human beings

Delivery of genes



Gene silencing

DNA

mRNA

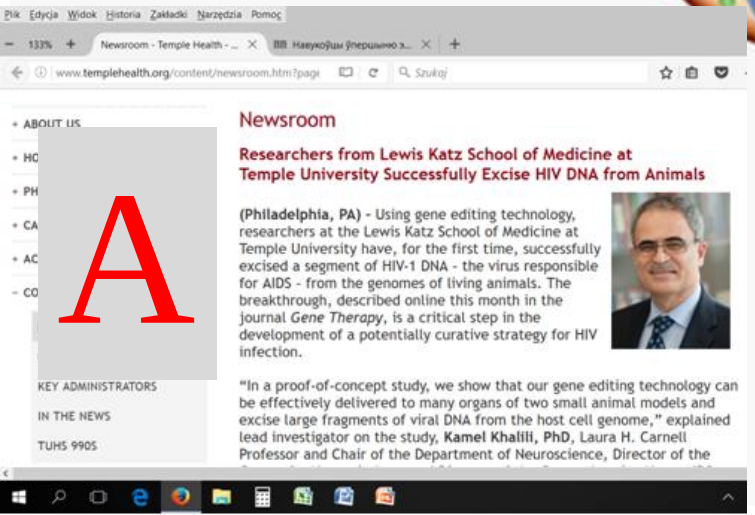
Protein

Anti-cancer Gene Therapy

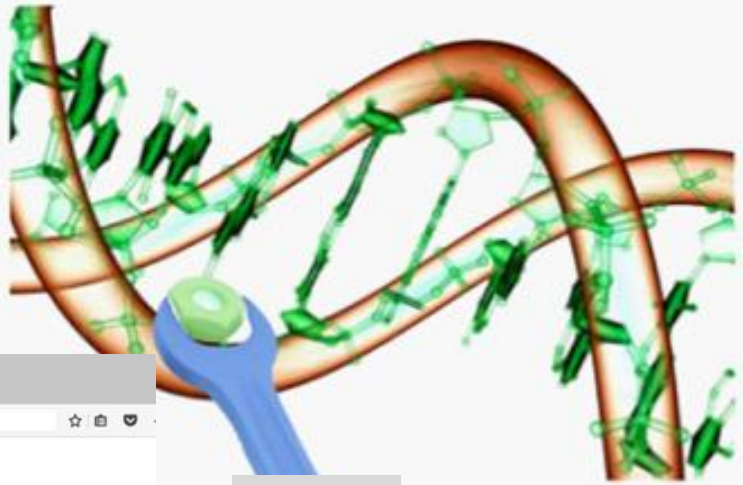
Construction of human beings

???

A



Delivery of genes



B

Gene silencing

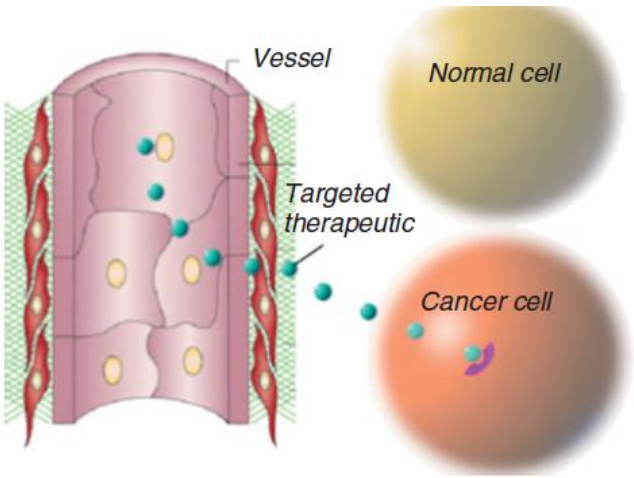
DNA

mRNA

Protein

C

How to cite this article:
WIREs Nanomed Nanobiotechnol 2013. doi: 10.1002/wnan.1226



B. Delivery of genes to kill cancer cells

Viral systems *versus* Non-viral systems

[Simply, the best !!!]

1) More efficient transfection,
2) Less toxic,
3) More selective,
4) Effects *in vivo* are more pronounced.

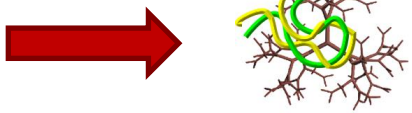
1) Risk of insertional mutagenesis,
2) Can be immunogenic.

1) Less efficient transfection,
2) Still more toxic,
3) Still less selective
4) Effects *in vivo* are less pronounced.

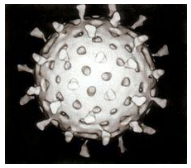
1) No risk of insert. mutagenesis,
2) Non-immunogenic.

Canada - Poland - Belarus - France

Gene encoding medical protein
GDNF (*Recovery of injured nerve)*

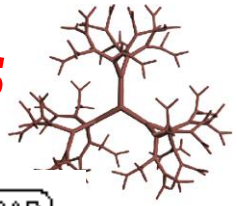


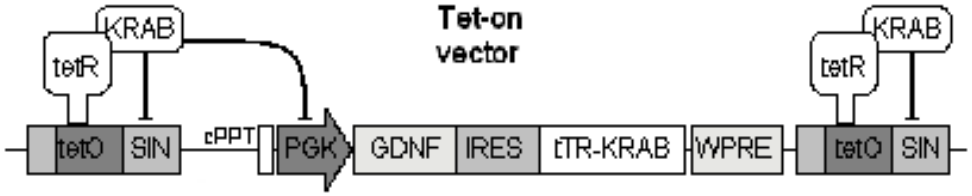
Lentivirus



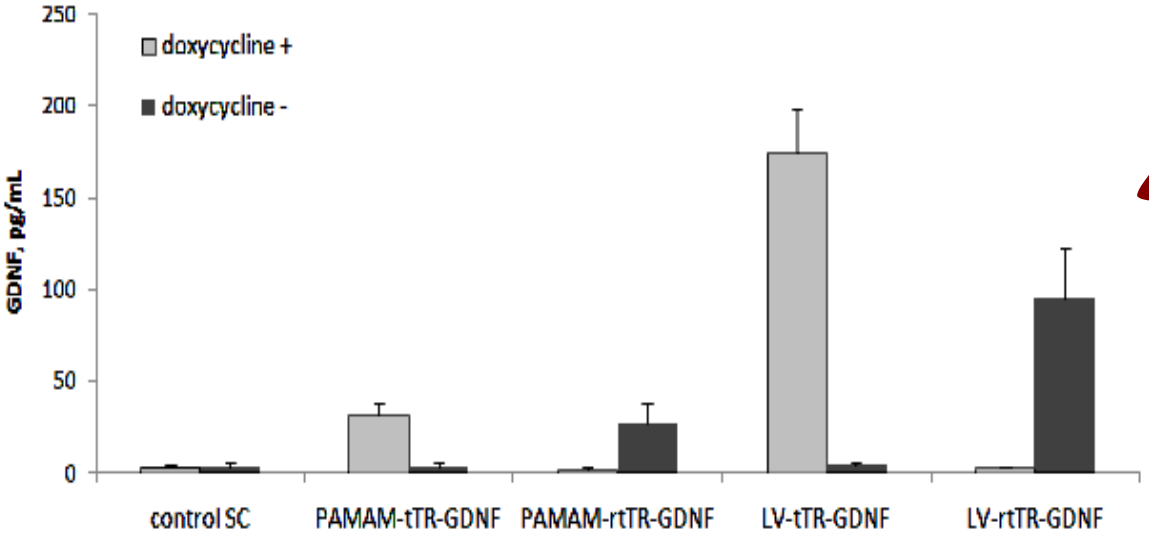
PAMAM
dendrimer G4

versus

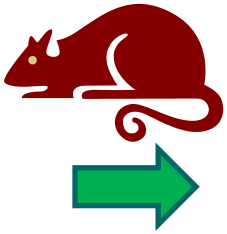


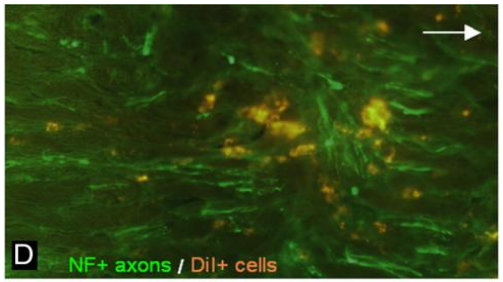


Efficiency *in vitro*



Rat (*in vivo*)





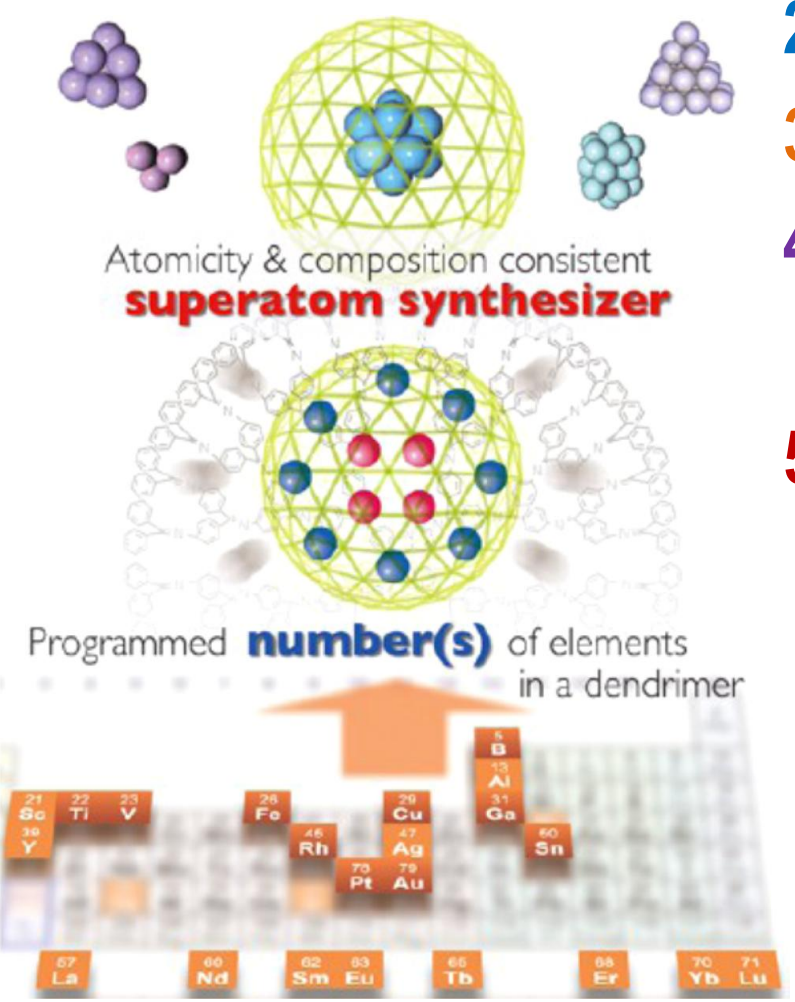
GDNF SC dox+/+

Recovery of injured nerve

A. Shakhbazau, C. Mohanty, D. Shcharbin, M. Bryszewska, A.-M. Caminade, J.-P. Majoral, J. Alant, R. Midha. Doxycycline-regulated GDNF expression promotes axonal regeneration and functional recovery in transected peripheral nerve // *J. Control. Release, 2013, Vol. 172, P. 841-851.*

But why we are developing non-viral systems ?

They are like constructor details, so

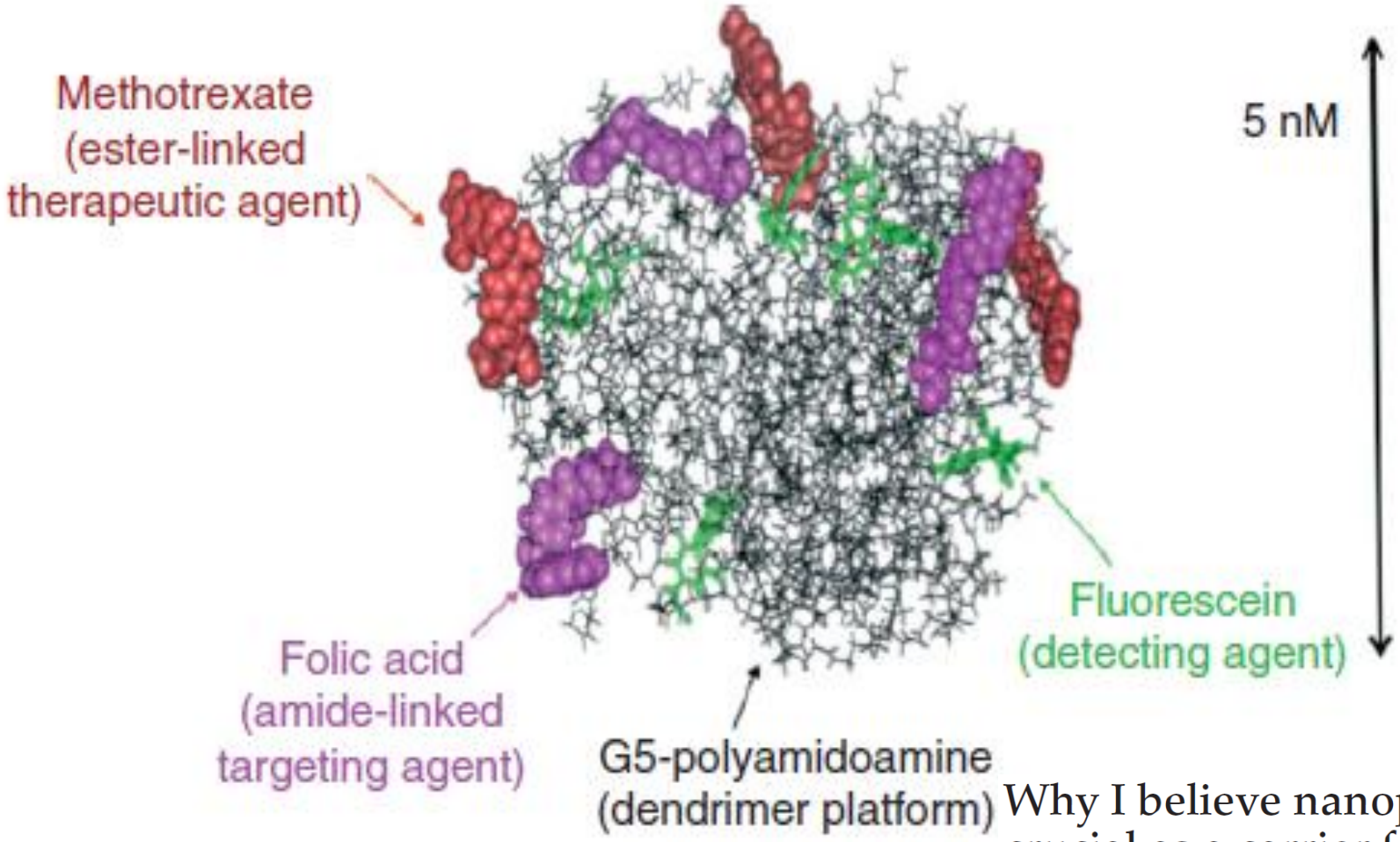


1) We can tune their efficiency,
2) We can make them bio-available,
3) We can make them self-destroying,
4) We can create complex constructions,
5) We can modify the routes of administration.

A Systematic Framework and Nanoperiodic Concept for Unifying Nanoscience: Hard/Soft Nanoelements, Superatoms, Meta-Atoms, New Emerging Properties, Periodic Property Patterns, and Predictive Mendeleev-like Nanoperiodic Tables
Donald A. Tomalia, Shiv N. Khanna,
DOI: 10.1021/acs.chemrev.5b00367. Chem. Rev. XXXX, XXX, XXX−XXX

Examples: Anticancer nano-conjugate

4) We can create complex constructions

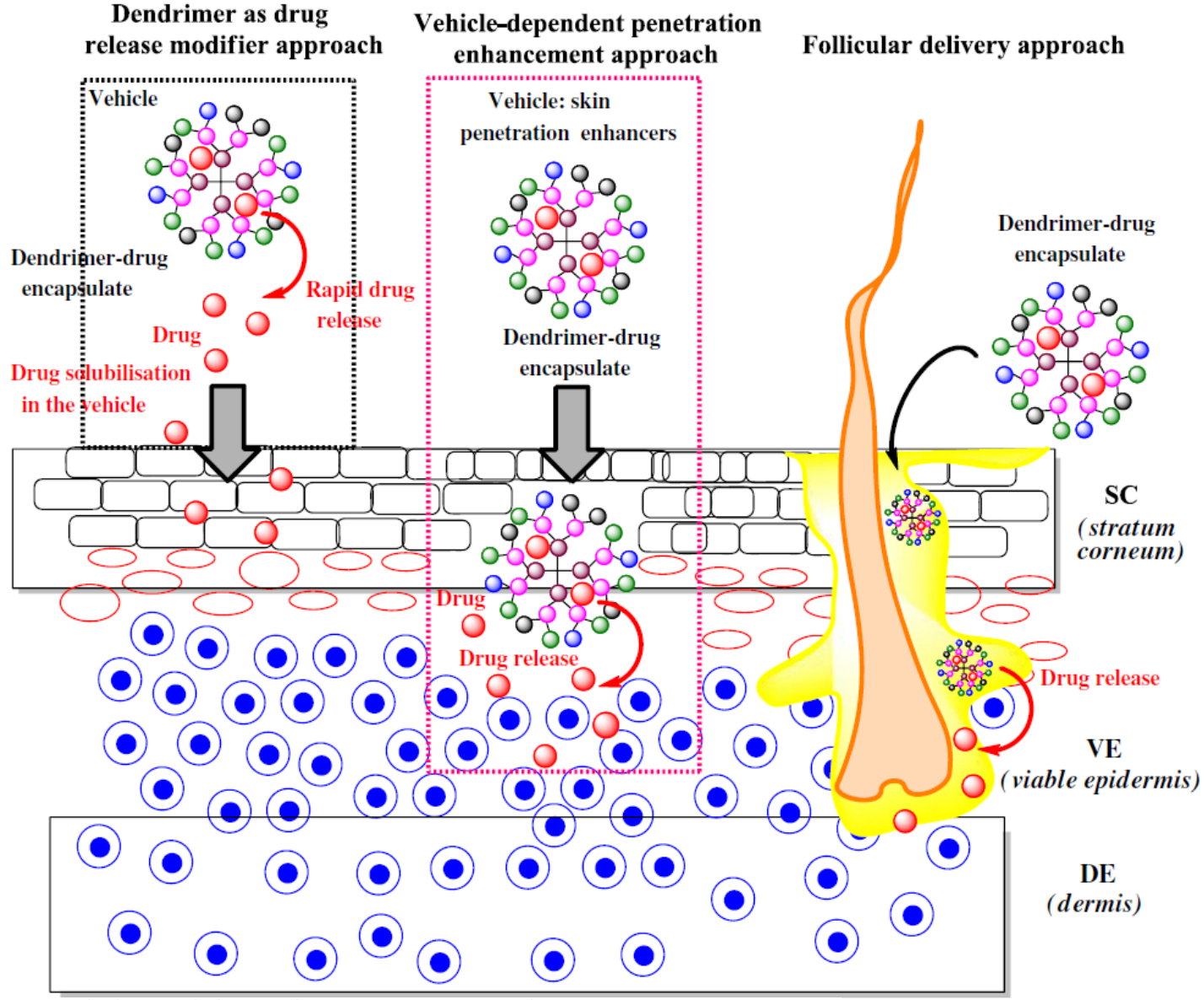


Why I believe nanoparticles are crucial as a carrier for targeted drug delivery

James R. Baker, Jr*

How to cite this article:
WIREs Nanomed Nanobiotechnol 2013. doi: 10.1002/wnan.1226

5) We can modify the routes of administration



Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: A concise overview☆

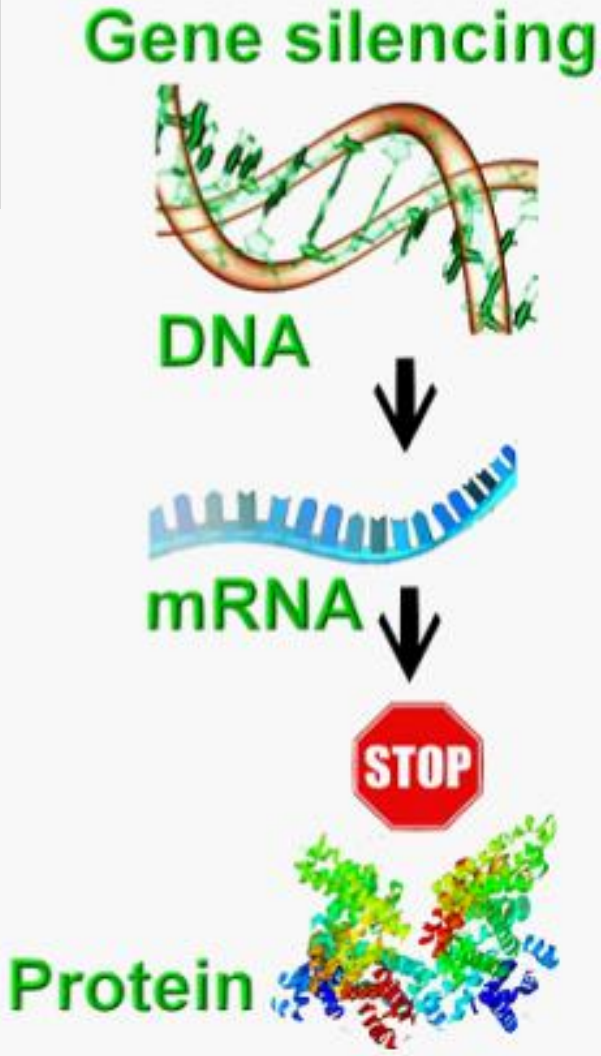
Advanced Drug Delivery Reviews 65 (2013) 1316–1330

Serge Mignani [a,*], Saïd El Kazzouli [b], Mosto Bousmina [c], Jean-Pierre Majoral [d,*]

Anti-cancer Gene Therapy

C

Small RNAs



The Nobel Prize in Physiology or Medicine 2006

Photo: L. Cicero

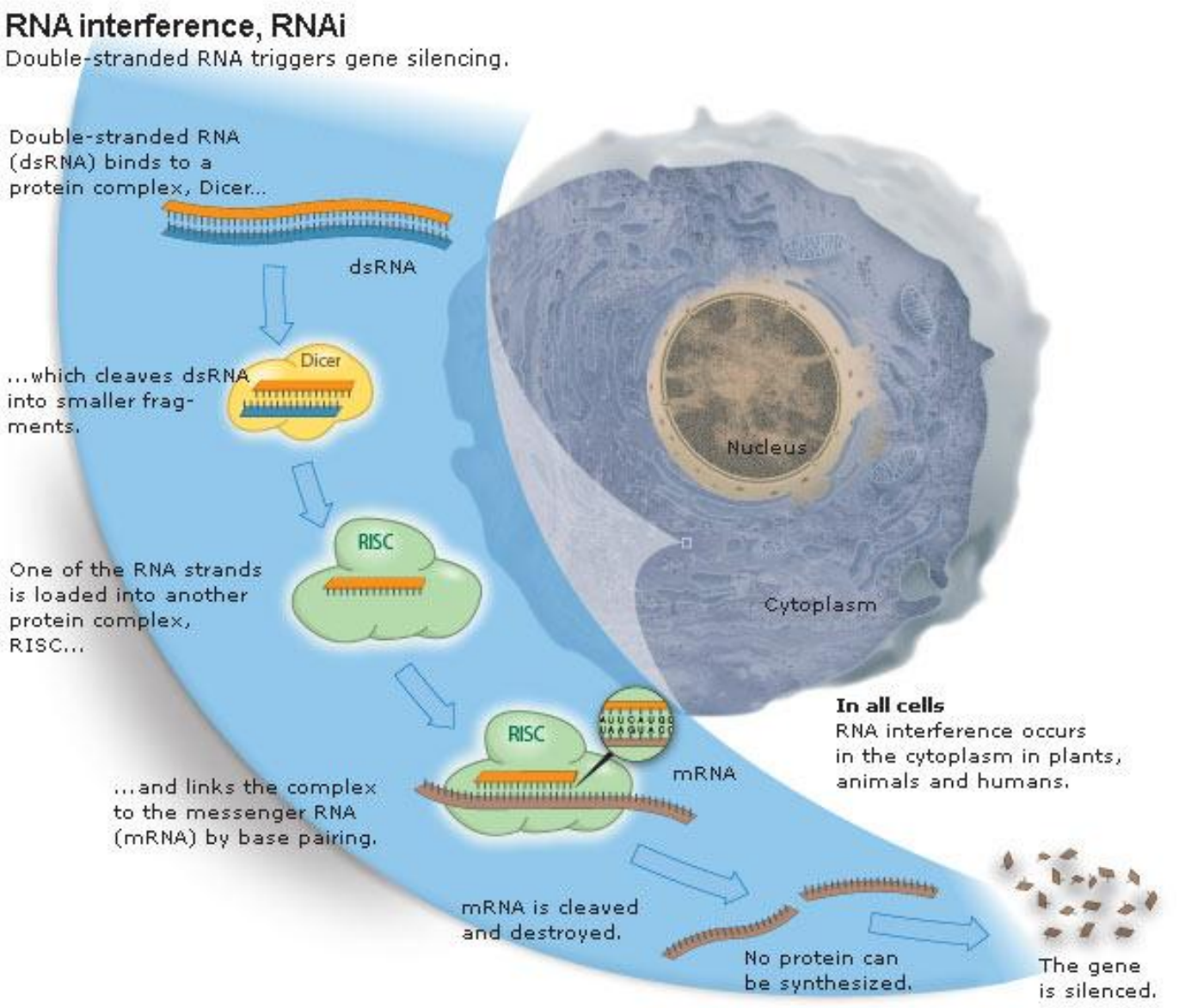
Andrew Z. Fire

Prize share: 1/2

Photo: J. Mottern

Craig C. Mello

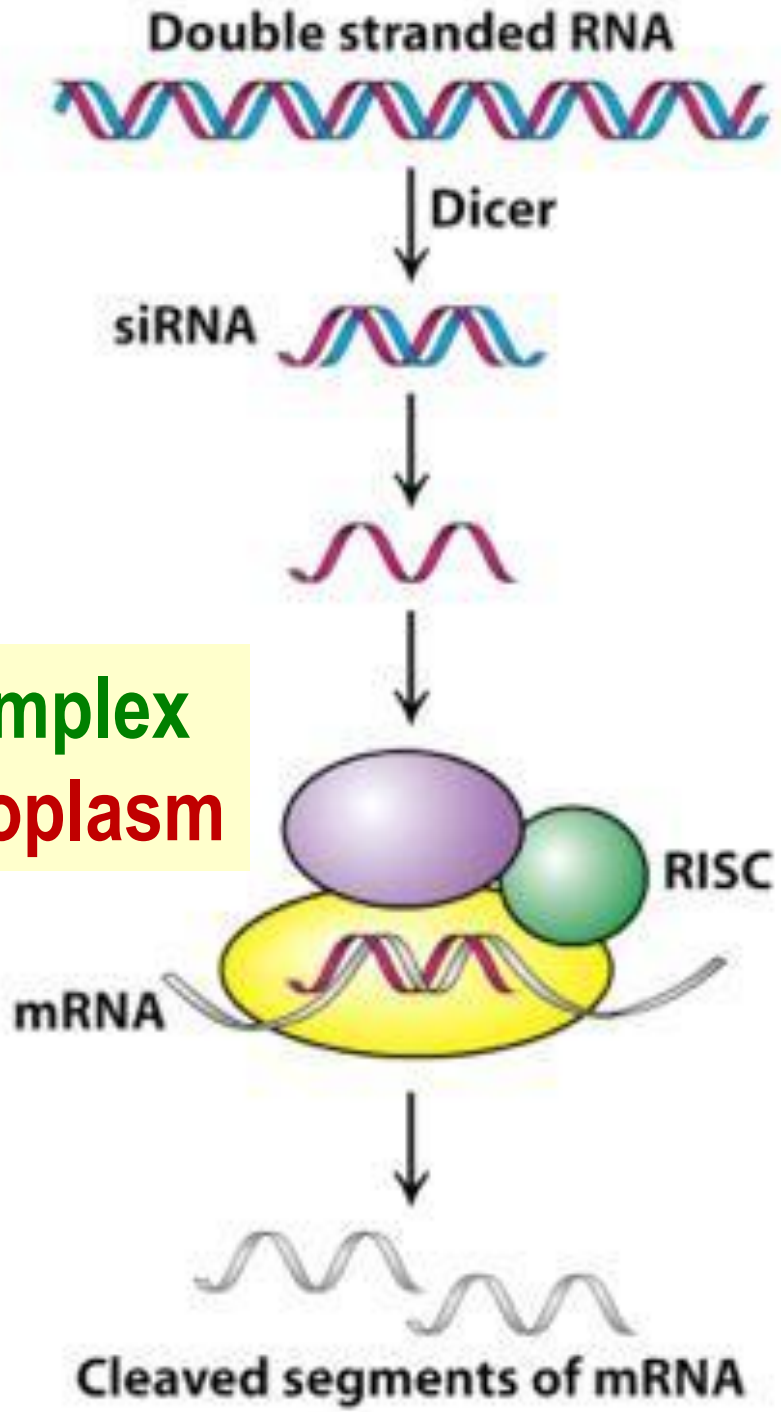
Prize share: 1/2



RNA interference

microRNA, siRNA, etc.

RISC complex
is in cytoplasm



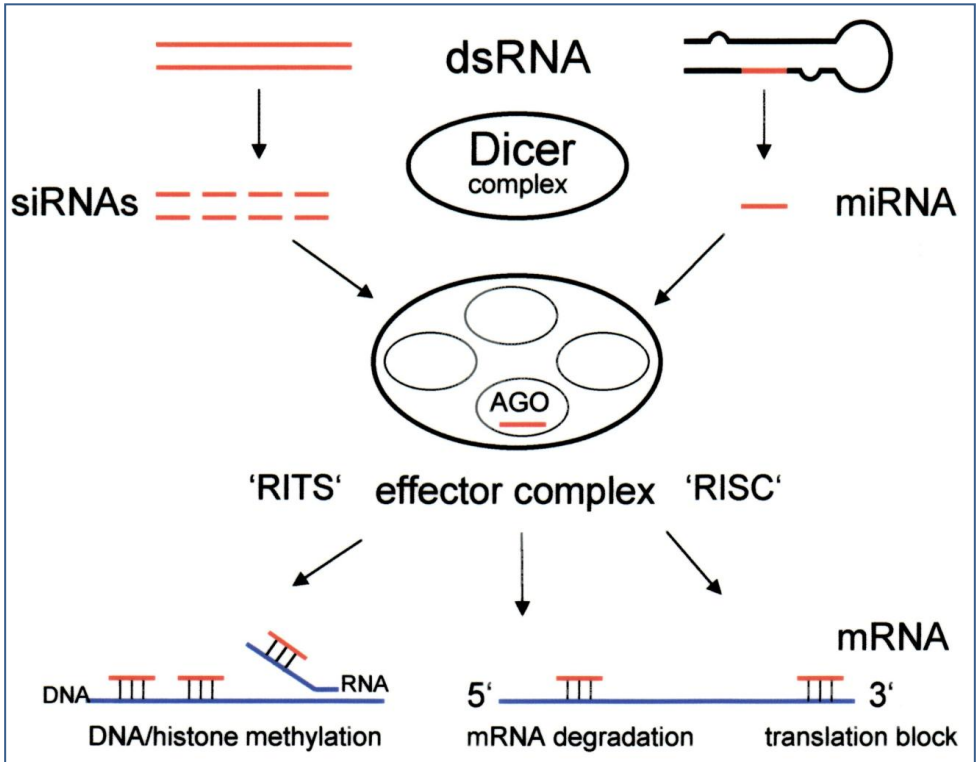
Cutting of mRNA
regions which encode
protein expression

What are we studying?

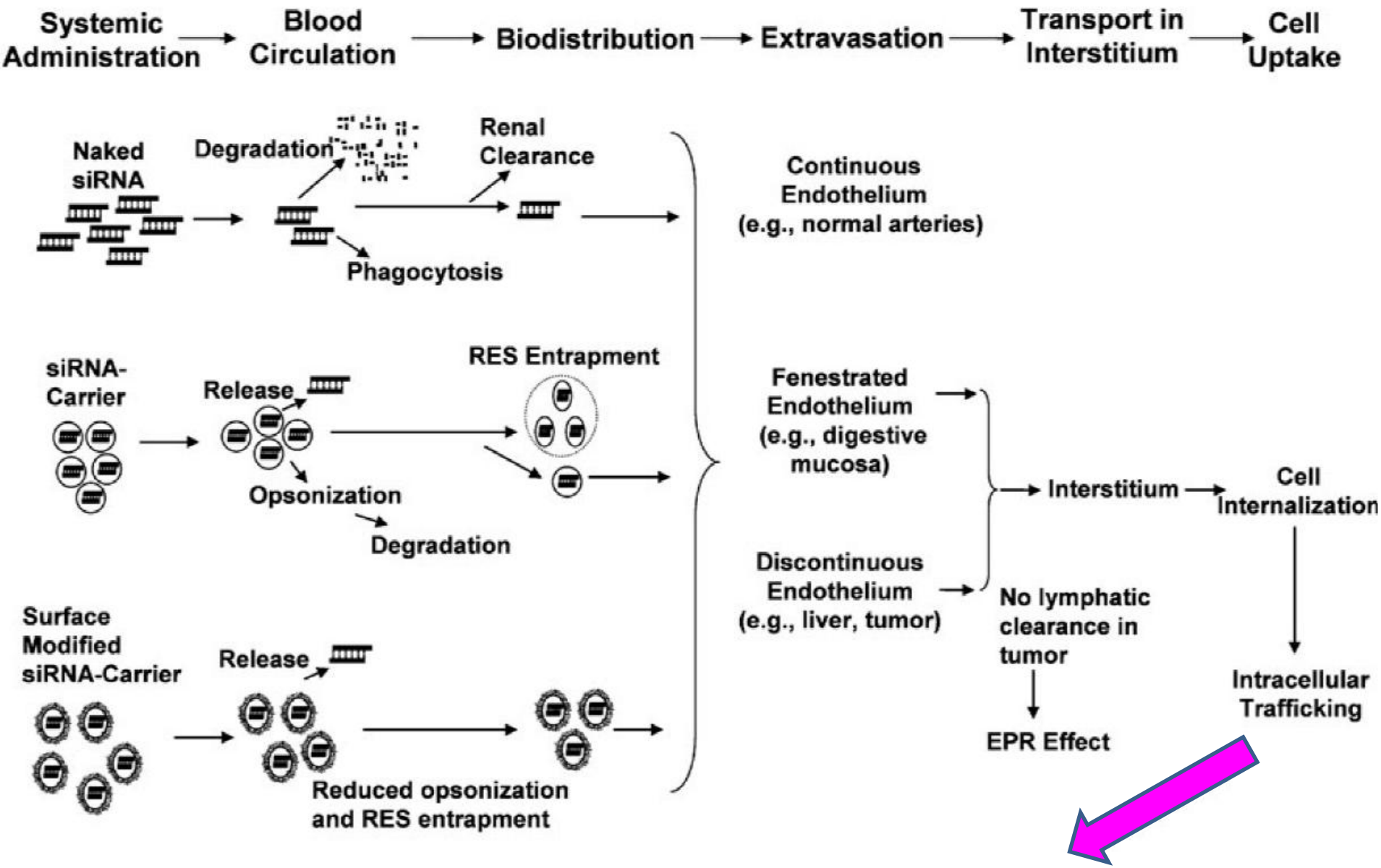
microRNA,
siRNA
+
non-viral carrier
e.g. dendrimers, dendronized Au nanoparticles, Ru dendrimers, etc.

1) To kill cancer cells

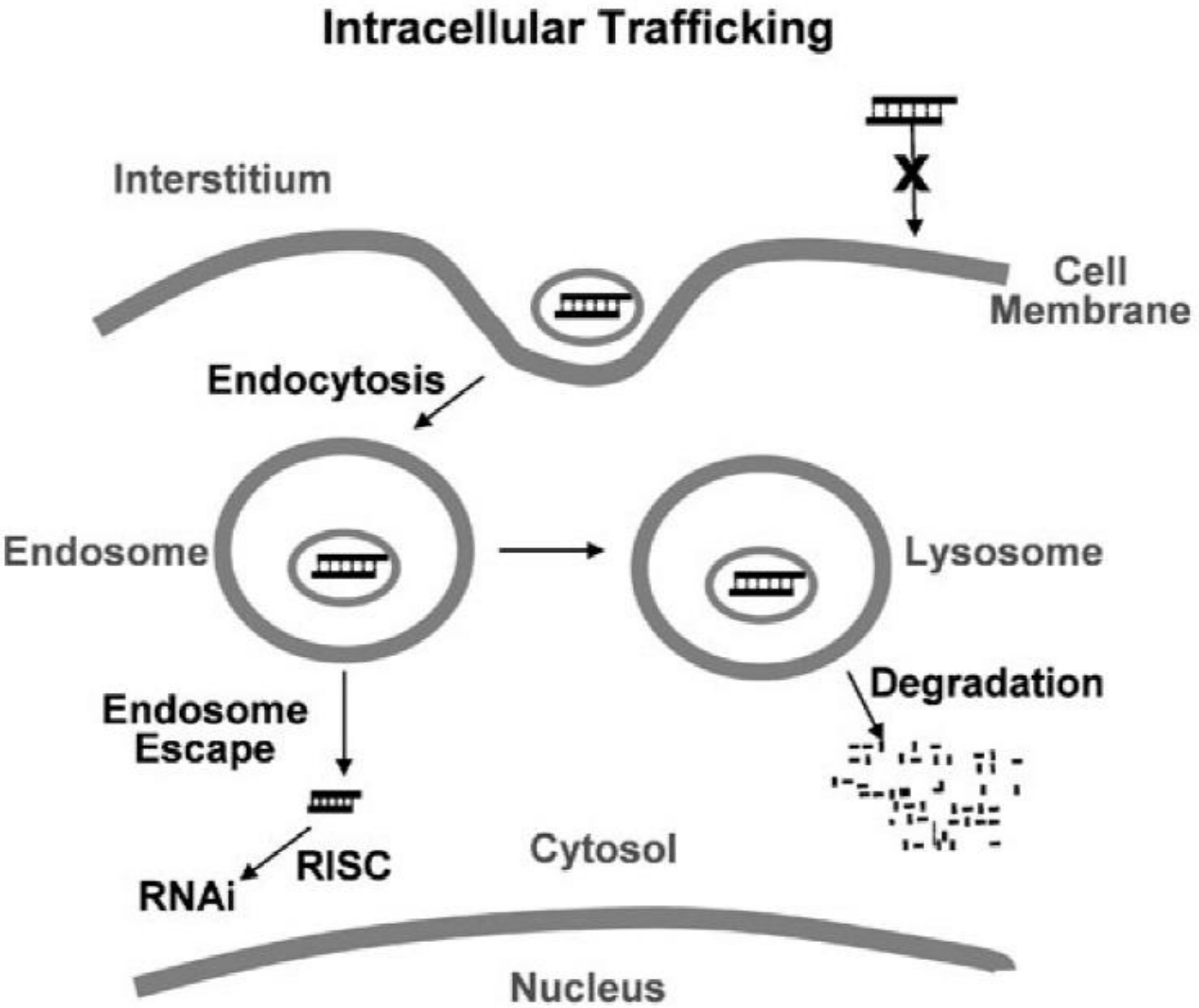
2) To regulate the protein expression in cancer cells



Problems?


Systemic Administration
Blood Circulation
Biodistribution
Extravasation
Transport in Interstitium
Cell Uptake
Naked siRNA
Degradation
Renal Clearance
Phagocytosis
siRNA-Carrier
Release
RES Entrapment
Opsonization
Degradation
Surface Modified siRNA-Carrier
Release
Reduced opsonization and RES entrapment
Continuous Endothelium (e.g., normal arteries)
Fenestrated Endothelium (e.g., digestive mucosa)
Discontinuous Endothelium (e.g., liver, tumor)
Interstitium
Cell Internalization
Intracellular Trafficking
No lymphatic clearance in tumor
EPR Effect

Problems?


Intracellular Trafficking
Interstitium
Cell Membrane
Endocytosis
Endosome
Lysosome
Degradation
Endosome Escape
RISC
RNAi
Cytosol
Nucleus

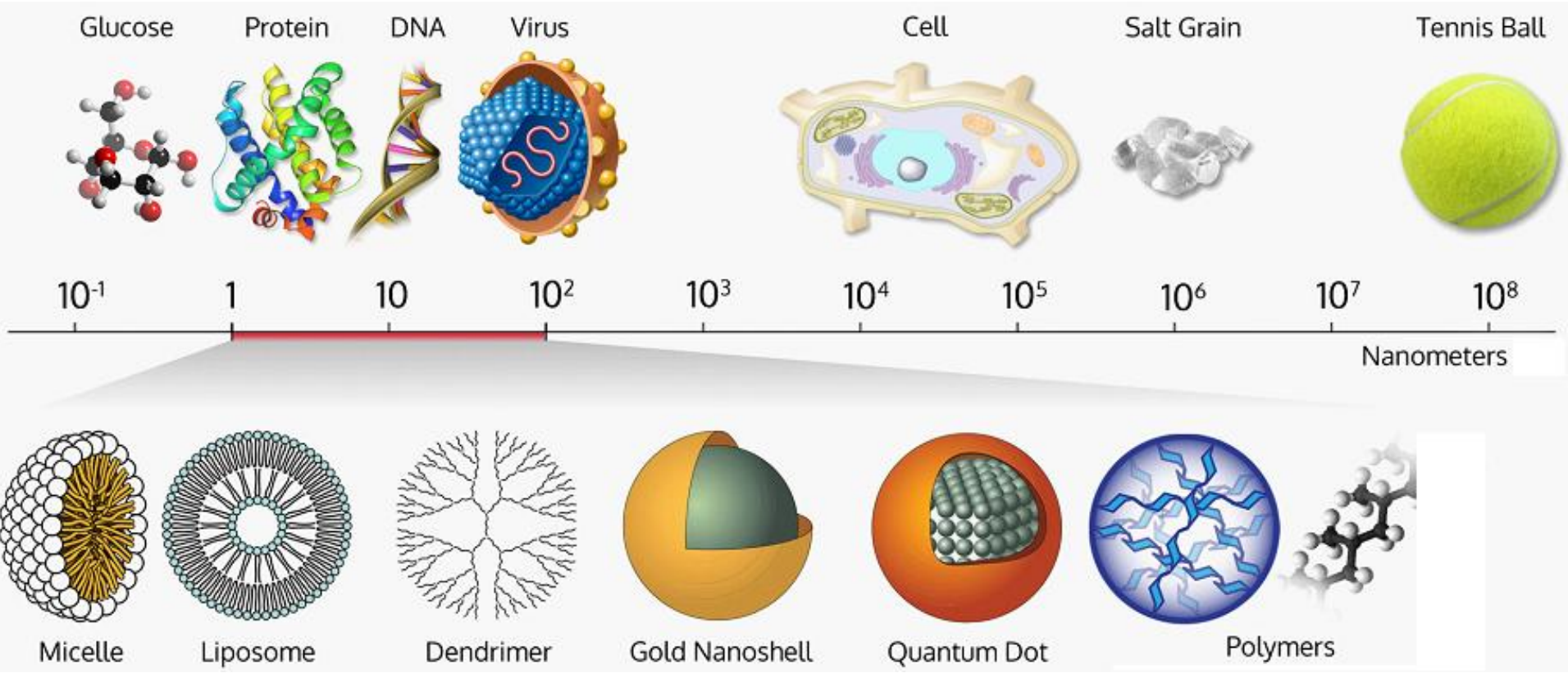
C. Gene silencing to kill cancer cells or to regulate cell proteins expression

Viral systems *versus* Non-viral systems

1) Less efficient to deliver in cytoplasm,
2) It is difficult to create viral systems for small RNAs.
3) Can be immunogenic,
4) Can penetrate in nucleous,
   e.g. risk of insertional mutagenesis.

[Simply, the best !!!]

1) More efficient to deliver in cytoplasm,
2) Selectivity in not too important,
3) Self-degrading,
4) No risk of insert. mutagenesis,
5) Non-immunogenic.



Nanomaterials

From: http://wichlab.com/research/

Toxicity

Non-modified cationic

Anionic, Neutral

Modified or complexed

Toxic (>G5)
Non-toxic (<=G5)

Non-toxic

Non-toxic
except modification by 25%-C_{12}

Biodistribution and Pharmacokinetics

Dendrimers *IN VIVO*

Non-modified cationic

Anionic, Neutral

Modified or complexed

Pancreas
liver, spleen (>G8)
kidneys (<G4)
lungs, heart

Quick removal from blood circulation.
Urinary and intestinal excretion

Liver
kidneys, lungs
blood

Longer (*vs* cationic) blood circulation times.
Urinary (mainly) and intestinal excretion

Is determined by a modification and/or a charge of complex that allows targeted delivery in any organ

Is determined by a modification and/or a charge of complex.

Enhanced, prolonged (>40 days) but non-transient therapeutic effect (dendriplexes)

Project: EU-Belarus-Russia Network in Nanomaterials-Driven Anti-Cancer Gene Therapy

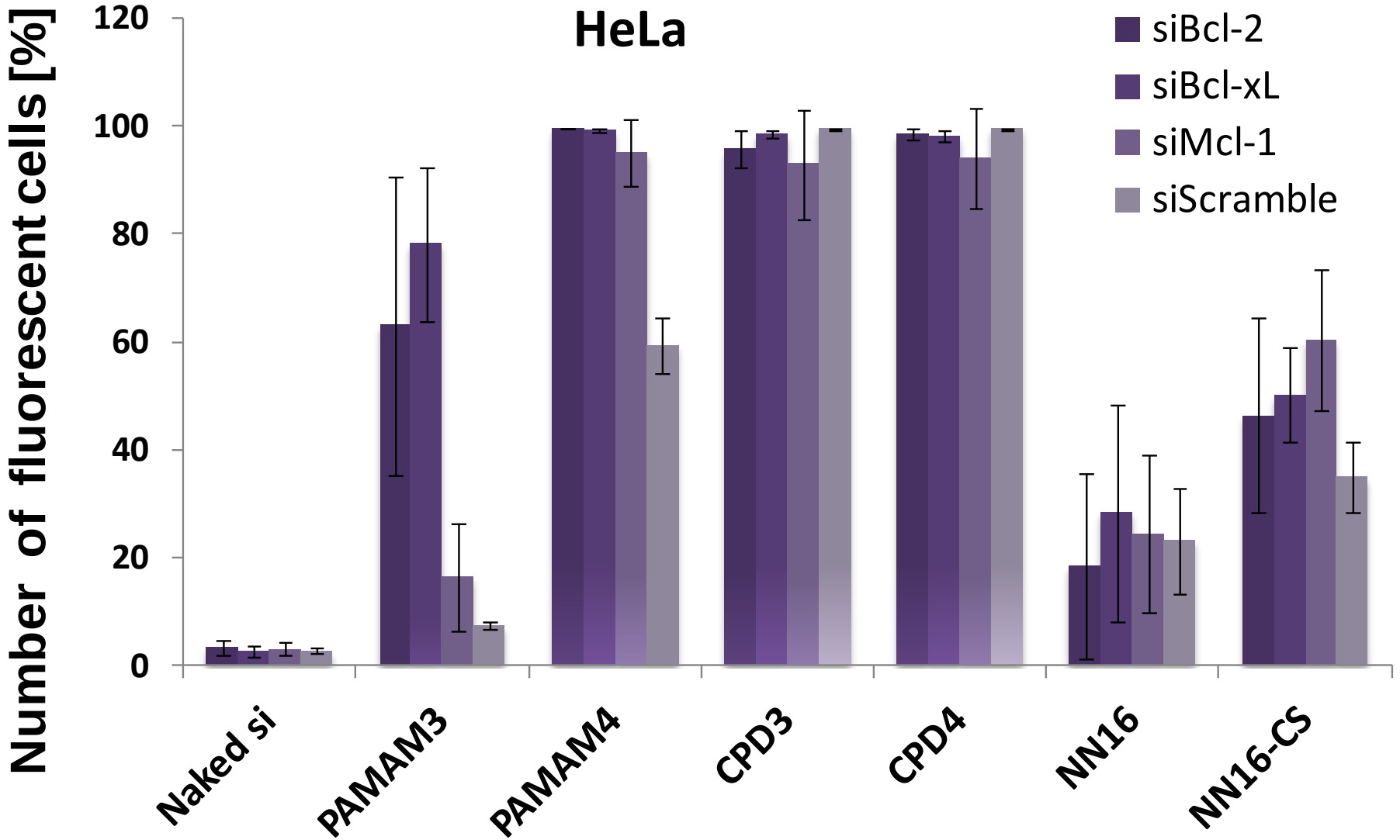
7 European Union Framework Programme (2013-2016)

- Department of General Biophysics, University of Lodz, Lodz, **Poland – coordinator**,
- Departamento de Química Inorgánica, Universidad de Alcalá, Alcalá de Henares, **Spain**,
- Laboratorio de Inmunobiología Molecular, Hospital General Universitario Gregorio Marañón, Madrid, **Spain**,
- Laboratoire de Chimie de Coordination, CNRS, Toulouse, **France**,
- Institute of Chemical Biology and Experimental Medicine of SB of Russian Academy of Sciences, Novosibirsk, **Russia**,
- Institute of Biophysics and Cell Engineering of NASB, Minsk, **Belarus**

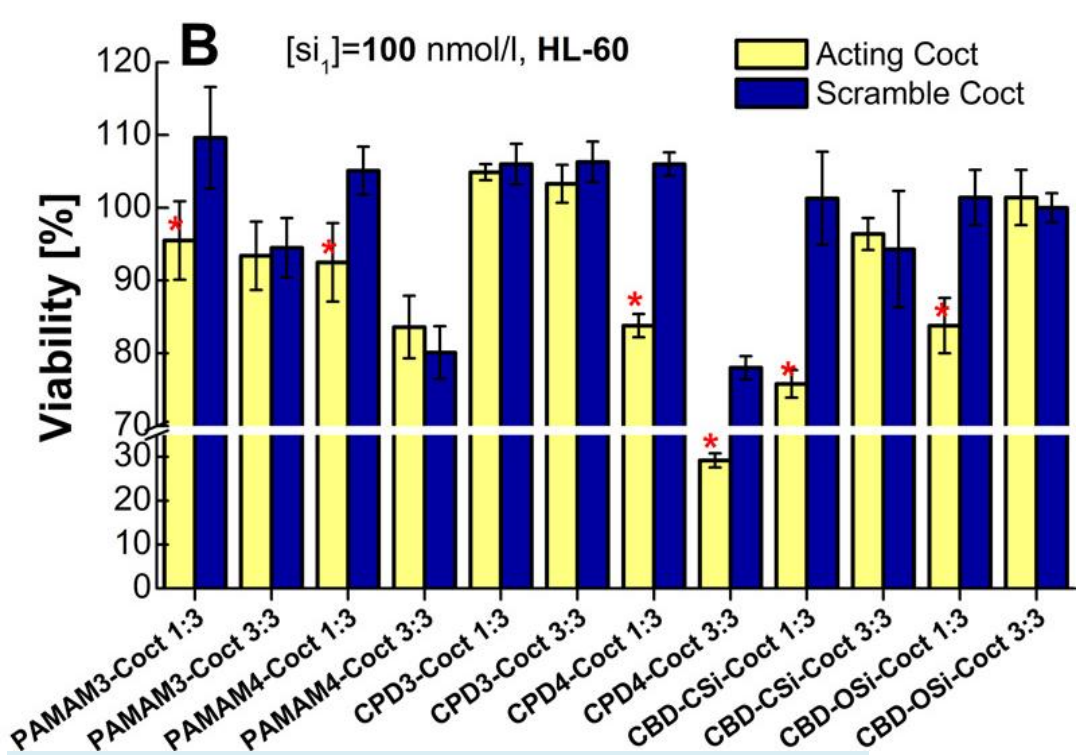
The regulation of apoptosis in cells is realized by the family Bcl-2 proteins. The family of Bcl-2 proteins is differed by pro-apoptotic and anti-apoptotic proteins. The group of apoptosis inhibitors include: Bcl-2, Bcl-xL, Mcl-xL, Bcl-w, A-1, Boo. For suppression of synthesis of anti-apoptotic proteins the newly discovered mechanism of gene expression – RNA interference - is starting to use

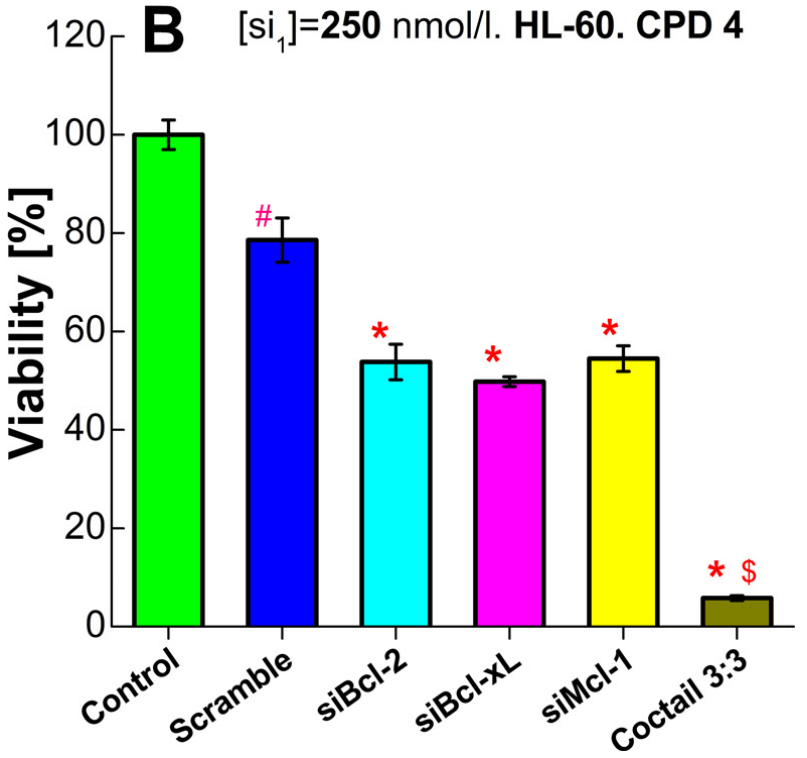
[Poeck H, Besch R, Maihoefer C, Renn M, Tormo D, Morskaya SS, Kirschnek S, Gaffal E, Landsberg J, Hellmuth J, Schmidt A, Anz D, Bscheider M, Schwerd T, Berking C, Bourquin C, Kalinke U, Kremmer E, Kato H, Akira S, Meyers R, Häcker G, Neuenhahn M, Busch D, Ruland J, Rothenfusser S, Prinz M, Hornung V, Endres S, Tüting T, Hartmann G. 5'-Triphosphate-siRNA: turning gene silencing and Rig-I activation against melanoma, Nat Med., 2008, Vol. 14(11), P. 1256-1263; Tiemann K, Höhn B, Ehsani A, Forman SJ, Rossi JJ, Saetrom P.

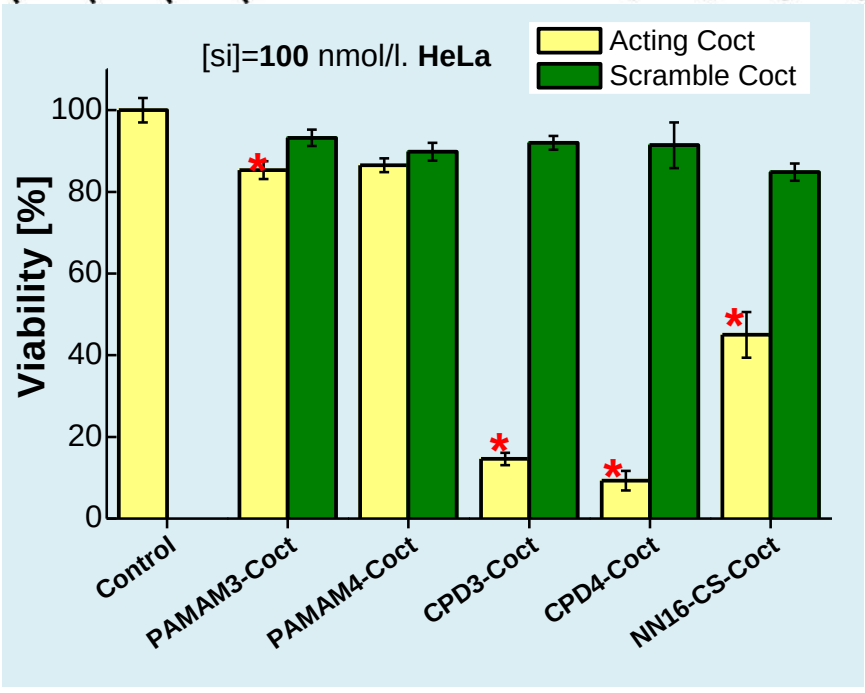
Dual-targeting siRNAs, RNA, 2010, Vol. 16(6), P. 1275-1284].



Uptake of siRNA into HeLa after 3 hours of incubation by flow cytometry







1) V. Dzmitruk, A. Szulc, D. Shcharbin, A. Janaszewska, N. Shcharbina, J. Lazniewska, D. Novopashina, M. Buyanova, M. Ionov, B. Klajnert-Maculewicz, R. Gómez-Ramirez, S. Mignani, J.-P. Majoral, M.A. Muñoz-Fernández, M. Bryszewska. Anticancer siRNA cocktails as a novel tool to treat cancer cells. Part (B). Efficiency of pharmacological action // *International Journal of Pharmaceutics. 2015 Vol. 485. P. 288-294.*

2) M. Ionov, J. Lazniewska, V. Dzmitruk, I. Halets, S. Loznikova, D. Novopashina, E. Apartsin, O. Krasheninina, A. Venyaminova, K. Milowska, O. Nowacka, R. Gomez-Ramirez, F. J. de la Mata, J.-P. Majoral, D. Shcharbin, M. Bryszewska. Anticancer siRNA cocktails as a novel tool to treat cancer cells. Part (A). Mechanisms of interaction // *International Journal of Pharmaceutics. 2015. Vol. 485. P. 261-269*

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Thank you for your attention

This work was supported by project VACTRAIN within HORIZON 2020 and by project NANOGENE within MSC IRSES of 7th EU FP, co-financed by the Polish Ministry of Science and Higher Education.