Hypersensitivity reactions (HSRs) to nano-biopharmaceuticals and COVID vaccines

Pseudo-allergic reactions to nano-biopharmaceuticals and COVID vaccines mediated by complement activation

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Nanomedicine Research and Education Center
Institute of Translational Medicine, Semmelweis University
Budapest, Hungary

Tuesday, June 16, 2021

Outline

- **Medical relevance of the Biosafety project's focus on HSRs and complement pathology**
  => i.e., complement activation-related pseudoallergy (CARPA)
- **CARPA: features**
- **Symptoms**
- **Mechanisms**
- **Prediction of reactions In vitro and in vivo**
- **Recent Progress**
  - **Experimental: Use of pigs for immunogenicity assessment and developing safe administration protocols**
  - **Hypothesis, that CARPA may be a contributing factor to the HSRs to mRNA vaccines**
  - **Conceptual: Immune stimulatory vicious cycle and CARPA as a stress reaction**

Medical relevance

(of the Biosafety project)

Biosafety - HORIZON 2020 EU project

SEMMELWEIS UNIVERSITY, INSTITUTE OF TRANSLATIONAL MEDICINE

HOME BIOSAFETY ABOUT THE PROJECT PARTICIPANTS EVENTS

PROJECT TITLE: BIOSAFETY ASSAYS FOR THE DIAGNOSIS AND PREDICTION OF DRUG-RELATED COMPLEMENT PATHOLOGY

- **HSRs represent a major safety issue, a potential biological barrier to the use of many useful drugs and contrast agents.**
- **One contributing cause is complement (C) activation (according to the CARPA theory)**
- **Expanding the arsenal of C activation tests and establishing their predictive value for HSRs requires further studies and increased dissemination of the concept.**

Why vaccines?

- **Safety has become a central issue in the ongoing large-scale anti-COVID-19 vaccination.**
- **Most (if not all) COVID vaccines consist of nanoparticles.**
- **HSR is a very rare, controllable side effect but it limits the choice of vaccines for many people with severe allergies.**
- **CARPA is hypothesized to be a contributing mechanism for vaccine-induced HSRs.**

Toxicity Clearance for Drug Registration

- Sytemic toxicity
  - Acute
  - Subacute
  - Chronic
- Carcinogenecity
- Genetic toxicology
- Reproductive toxicology
- Ecotoxicology
- ***Immune toxicity***

Species

- rat
- rabbit
- mouse
- guinea pig
- dog
- mini-pig
- pig

Role of C in HSRs, rationale of focusing on "complement pathology"

- **Most iv-administered reactogenic (i.e., HSR-causing drugs) can trigger complement (C) activation that explains some, or most symptoms. Hence, the name, CARPA.**
- **These reactions are limited to intravenous therapies, and the reactions are also known as *infusion reactions (IRs).***
- ***IRs* are common, particularly among nano-biopharmaceuticals (nanomedicines, biologicals, immuno-therapeutics**
- **Even though these can be severe and even life-threatening, assaying for IRs before a drug gets to clinical phases is challenging because relevant biomarkers or animal models are scarce.**
- **Semmelweis University Nanomedicine Department is pioneering in CARPA research.**

Nanomedicine-induced infusion reactions: Features

- Often hyperacute phenomenon
- Individual variation of symptoms
- More or less severe, can be fatal
- Intravenous therapies
- Nano-drugs and biologicals
- Therapeutic dose
- First injection
- Rare (2-10%)
- Self-tolerance (tachyphylaxis)
- Cascadic immune reaction
- Multiple, redundant pathways
- All arms of immune responses: innate/specific, humoral/cellular
- Cannot be predicted by standard allergy tests
- Hurdle for the translation of nanotechnology-based drug products
- May lead to drug withdrawal
- Regulatory authorities increasingly demand preclnical testing

nature nanotechnology

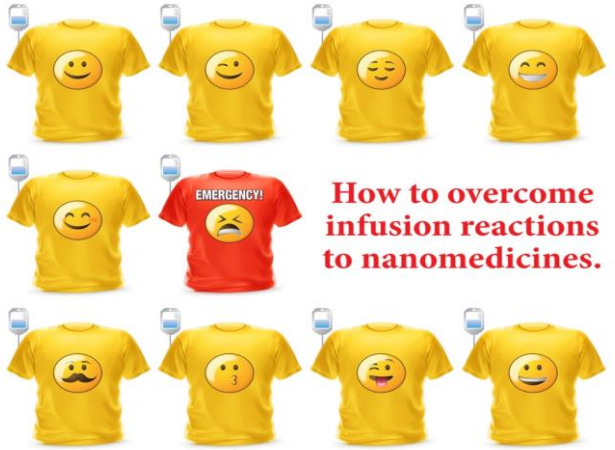
PERSPECTIVE

https://doi.org/10.1038/s41565-018-0273-1

Roadmap and strategy for overcoming infusion reactions to nanomedicines

Janos Szebeni[1,2,3], Dmitri Simberg[4], África González-Fernández[5], Yechezkel Barenholz[6] and Marina A. Dobrovolskaia[7]*

NATURE NANOTECHNOLOGY
13;12:1100-1108, 2018



Health impact of immune toxicity and pseudoallergy

Adverse Drug Events

2,2 millions / year, 5-6th cause of death

Immune toxicity

20 ± 5% (~440,000/ year)

Pseudoallergy:

≈ 77% of adverse drug effects are non-IgE mediated, hypersensitivity reactions = pseudoallergy) (~340,000/ year)

Extra health care expenses:

≈ > hundreds of millions / year

Clinical and pharmaco-industrial significance of CARPA

- **Rare, but serious –occasionally deadly- anaphylactic reactions may surface only in phase III-IV postmarket surveillance;**
  - ***can be fatal (in cardiac patients)***
  - ***cannot be predicted by standard allergy tests***
  - ***may lead to drug withdrawal***

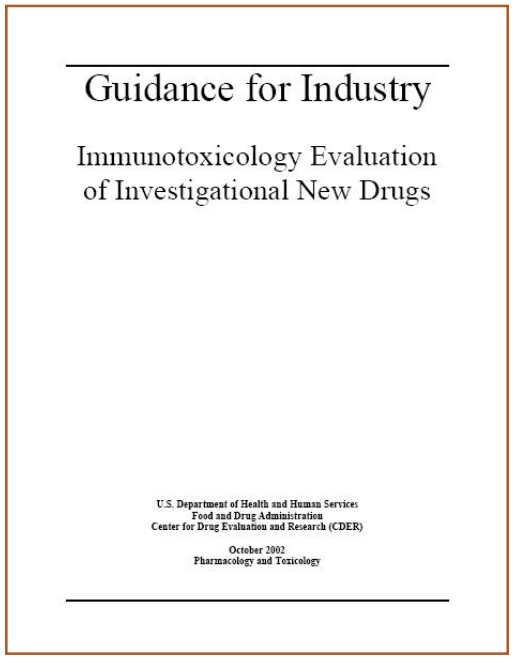
May contribute to immunogenicity

change pharmacokinetics, compromise efficacy
cause toxicity, including HSRs

Regulatory authorities increasingly demand experimental verification of short- and longterm immune tolerance

Regulatorory response to immune toxicity

2002



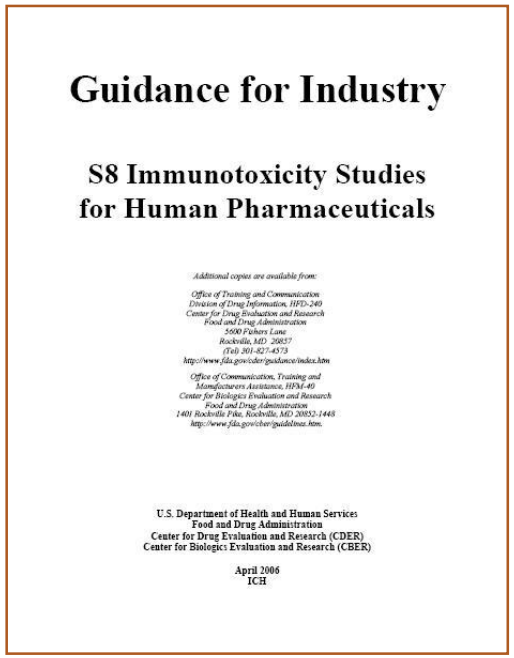
Guidance for Industry

Immunotoxicology Evaluation of Investigational New Drugs

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

October 2002
Pharmacology and Toxicology

2006



Guidance for Industry

S8 Immunotoxicity Studies for Human Pharmaceuticals

Additional copies are available from:

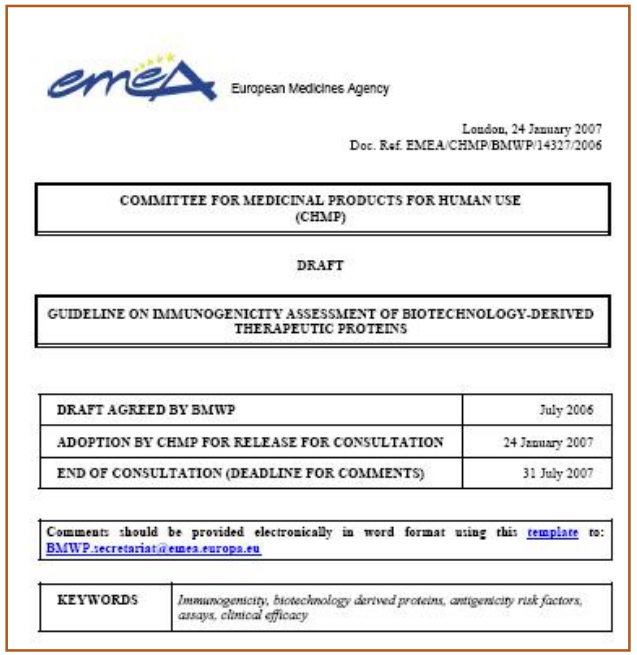
Office of Training and Communication
Division of Drug Information, HFD-240
Center for Drug Evaluation and Research
Food and Drug Administration
5600 Fishers Lane
Rockville, MD 20857
(Tel) 301-827-4573
http://www.fda.gov/cder/guidance/index.htm

Office of Communication, Training and Manufacturers Assistance, HFM-40
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike, Rockville, MD 20852-1448
http://www.fda.gov/cber/guidelines.htm.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Biologics Evaluation and Research (CBER)

April 2006
ICH

2007



European Medicines Agency

London, 24 January 2007
Doc. Ref. EMEA/CHMP/BMWP/14327/2006

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

DRAFT

GUIDELINE ON IMMUNOGENICITY ASSESSMENT OF BIOTECHNOLOGY-DERIVED THERAPEUTIC PROTEINS

DRAFT AGREED BY BMWP	July 2006
ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION	24 January 2007
END OF CONSULTATION (DEADLINE FOR COMMENTS)	31 July 2007

Comments should be provided electronically in word format using this template to: BMWP.secretariat@emea.europa.eu

KEYWORDS	*Immunogenicity, biotechnology derived proteins, antigenicity risk factors, assays, clinical efficacy*

„All *new human pharmaceuticals should be evaluated for the potential to produce immunotoxicity.”*

„Methods include standard toxicity studies and additional immunotoxicity studies conducted as appropriate…”

„It *is essential to adopt an appropriate strategy for the development of adequate screening and confirmatory assays to measure an immune response* against a therapeutic protein.”

The CARPA concept: Some drug-induced hypersensitivity reactions are due to C activation

- **The symptoms are**
  - **Explained by C activation – anaphylatoxin actions**
  - **Reproduced by C activators (in pigs and rats)**
  - **Blocked by C inhibitors (in pigs)**
- **No pre-exposure**
  - **No immune learning –** 

Complement activation-related pseudoallergy,

CARPA

Complex terminology of acute systemic immune reactions to IV drugs

Term
Hypersensitivity reaction
Infusion reaction
Idiosyncratic reaction
Anaphylaxis
Pseudoallergy
Non-allergic hypersensitivity
Non-immune hypersensitivity
Complement-activation-related pseudoallergy (CARPA)
Immunologic anaphylaxis
Non-immunologic anaphylaxis
Anaphylactoid reaction
Type B adverse drug reaction

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association. Learn and Live.

Hemodynamic Changes Induced by Liposomes and Liposome-Encapsulated Hemoglobin in Pigs

A Model for Pseudoallergic Cardiopulmonary Reactions to Liposomes: Role of Complement and Inhibition by Soluble CR1 and Anti-C5a Antibody

Janos Szebeni, MD, PhD; John L. Fontana, MD; Nabila M. Wassef, PhD; Paul D. Mongan, MD; David S. Morse, MD; David E. Dobbins, PhD; Gregory L. Stahl, PhD; Rolf Bünger, MD, PhD; Carl R. Alving, MD

1999;99:2302-2309

Complement activation plays a causal role in the cardiopulmonary distress of pigs injected i.v. with liposomes

The C system:

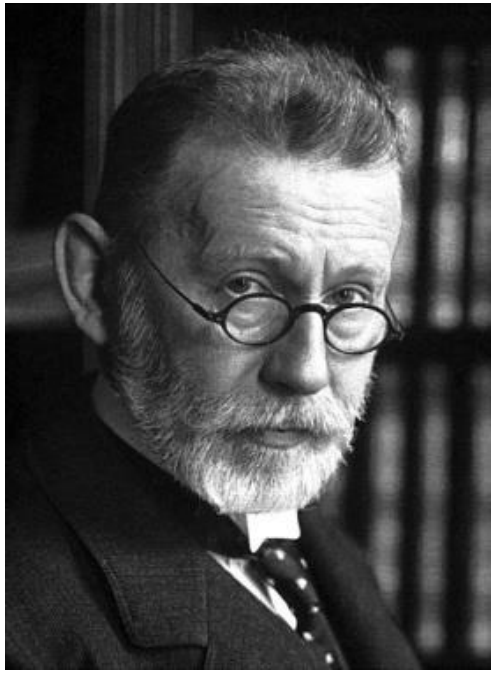
Described 131 years ago

Jules Bordet

1890 - Killing of Vibrio Cholerae by guinea pig immune serum by a heat sensitive substance:
ALEXINE (Greek *alexein* = to ward off]
Nobel Prize, 1919

Immune system

Specific (adaptive)		Nonspecific (innate)	
Humoral	**Cellular**	**Humoral**	**Cellular**
induced antibodies	**Committed T cells**	**Natural antibodies**	**PMN, NK cells, macrophages**
		Complement	



Paul Erlich

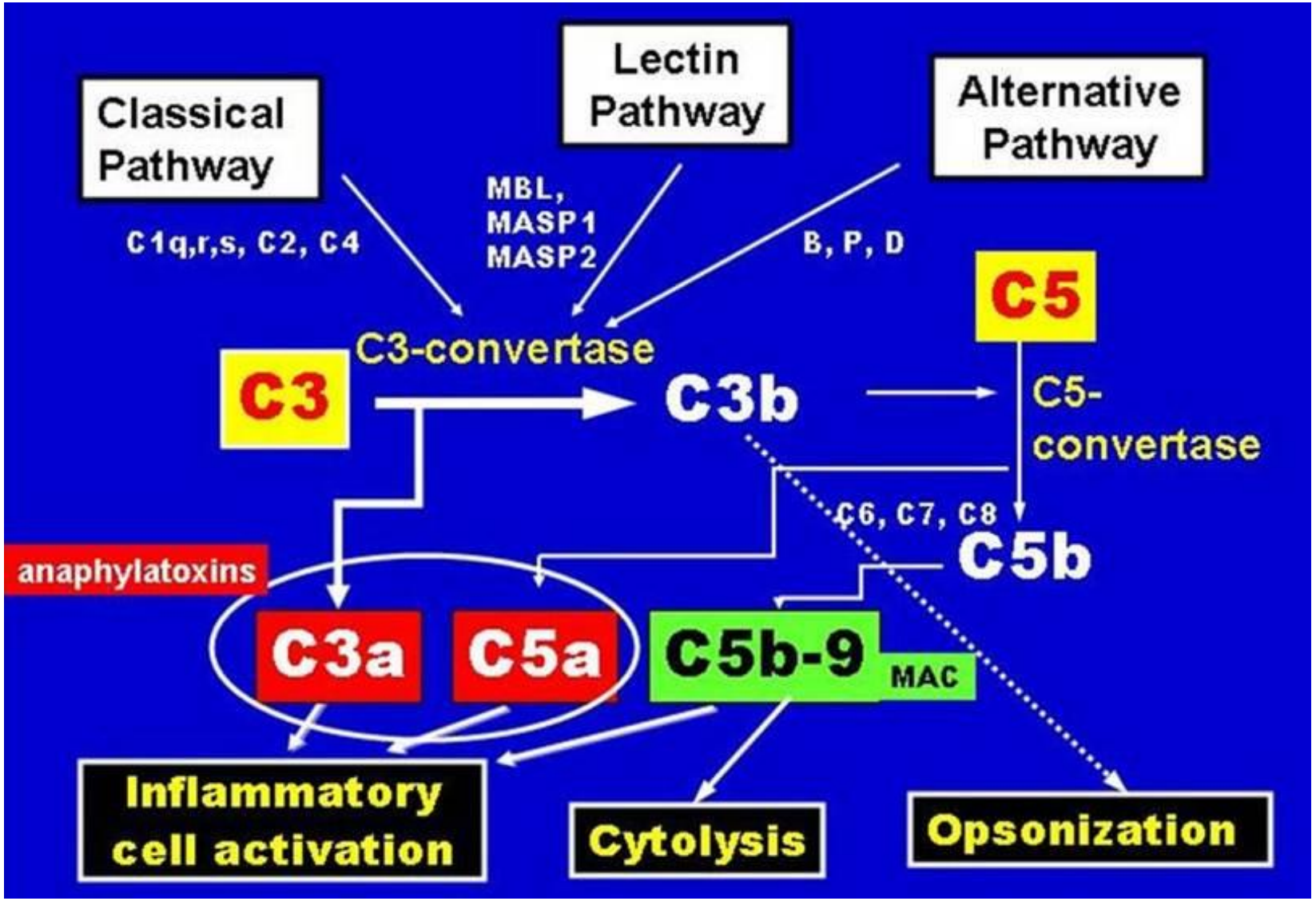
1900 - **ALEXINE**
=> **complement**

Nobel Prize 1908

- **proteolytic cascade in blood**
- **35 glycoproteins**
- **13 cell membrane-bound**
- **22 soluble**
- **14 split products**

- **Antimicrobial defense**
- **Tissue growth & repair**
- **Waste disposal**
- **Bridging innate and adaptive immunity**
- **Conception**

The C cascade



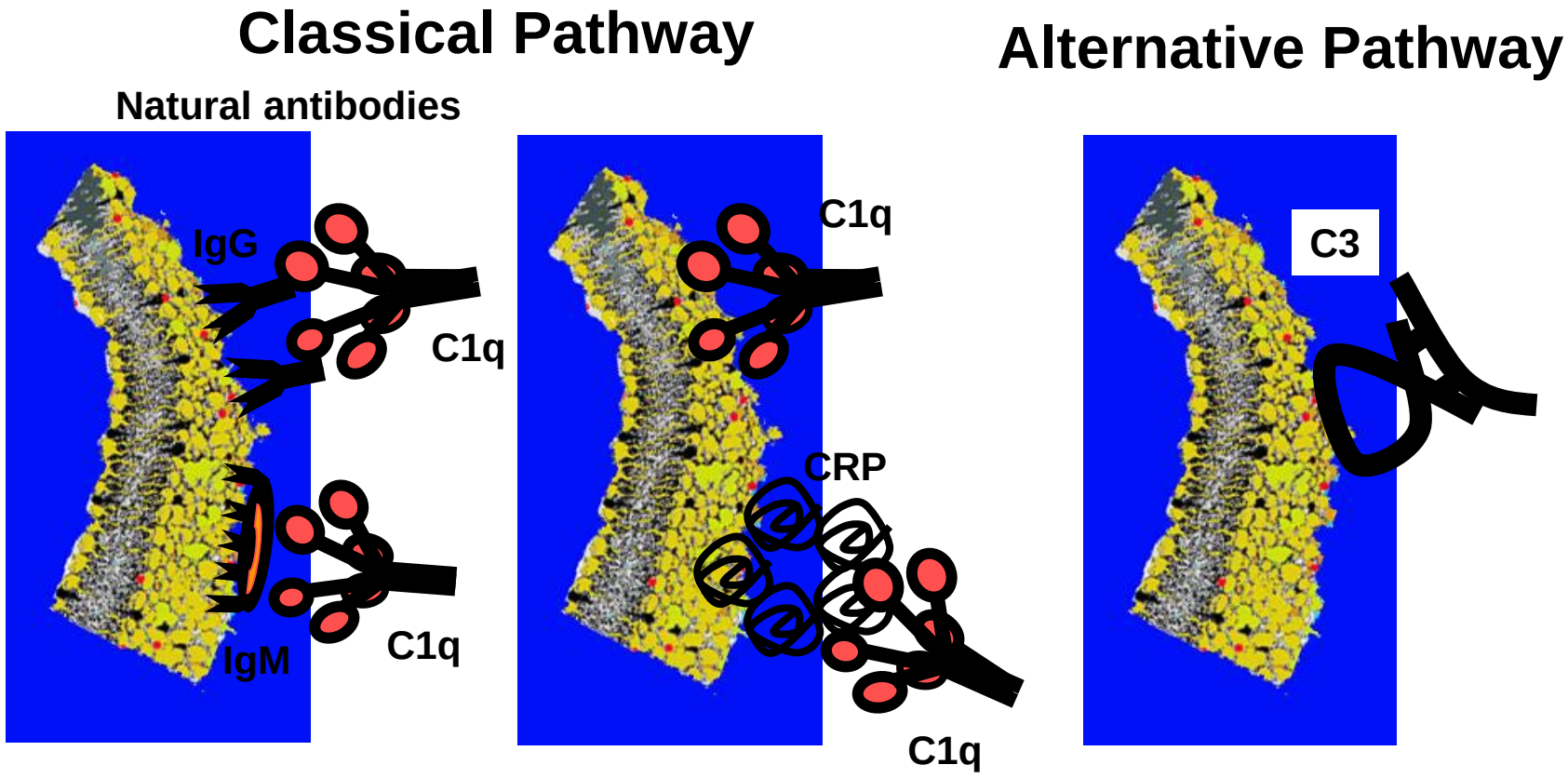
Symptoms of drug-induced acute hypersensitivity reactions (CARPA syndrome)

Cardio-vascular	Broncho-pulmonary	Hemato-logical	Muco-cutaneous	Gastro-intestinal	Neuro-psycho-somatic	Systemic
angioedema	apnea	leukopenia	cyanosis	nausea	back pain	chills
arrhythmia	bronchospasm	granulopenia	erythema	vomiting	chest pain	diaphoresis
cardiogenic shock	coughing	rebound leukocytosis	flushing	metallic taste	chest tightness	fever
hypertension	dyspnea	rebound granulocytosis	rash	diarrhea	headache	sweating
hypotension	hyper-ventilation	thrombocyto-penia	rhinitis	cramping	feeling of imminent death	wheezing
hypoxia	laryngospasm	lymphopenia	swelling	bloating	fright	rigors
myocardial infarction	stridor		urticaria		panic	feeling of warmth
tachycardia	respiratory distress		nasal congestion		rigors	loss of consciousness
ventricular fibrillation	shortness of breath		pruritus		anxiety	*death*
edema	sneezing		tearing		confusion	
syncope	hoarseness		conjunctival erythema		dizziness	

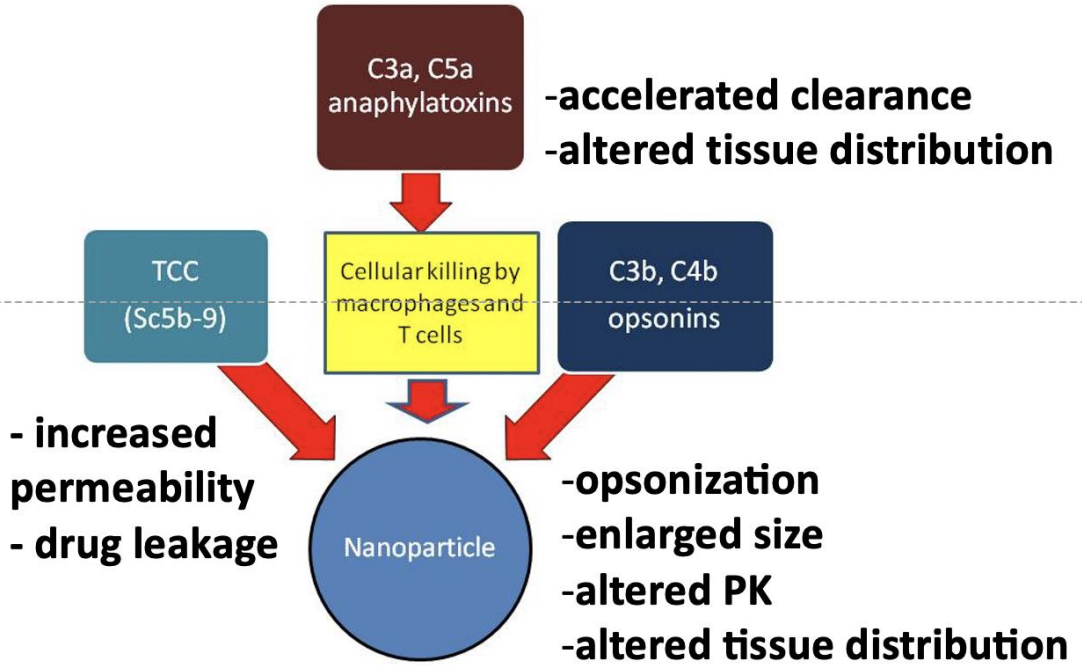
Drugs causing hypersensitivity reactions

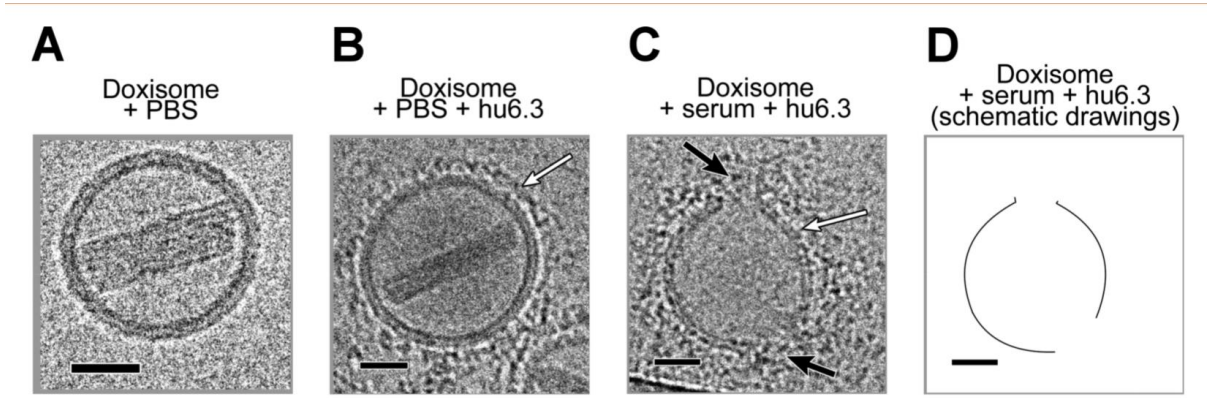
Liposomal drugs and diagnostics	Micellar drug formulations	Radio and ultrasound contrast agents	Antibody-based Therapeutics & diagnostics	Enzymes Proteins Peptides	Miscellaneous other
Doxyl (Caelix) Ambisome Amphocyl Myocet DaunoXome Tc^{99}-HINIC-PEG	Taxol Taxotere Cyclosporine Etoposide	Diatrizoate Iodixanol Iohexol Iopamidol Iopromide Iothalamate Ioversol Ioxaglate Ioxilan SonoVue Magnevist	Avastin Enbrel Herceptin Humira Raptiva Synagis Xolair Compath Erbitux Mylotarg Remicade Rituxan Vectibix Tysabri	Avonex Actimmune Abbokinase Aldurazyme Activase Zevalin Neupogen Neulasta Fasturtec Plenaxis	Cancidas Copaxone Orencia Eloxatin Salicilates

C activation by Liposomes



Impact of C activation on liposomes





ACS NANO

www.acsnano.org

Premature Drug Release from Polyethylene Glycol (PEG)-Coated Liposomal Doxorubicin *via* Formation of the Membrane Attack Complex

Even Chen, Bing-Mae Chen, Yu-Cheng Su, Yuan-Chih Chang, Tian-Lu Cheng, Yechezekel Barenholz,* and Steve R. Roffler*

ACS Nano **2020, 14, 7, 7808−7822**

Immune Toxicity Catastrophes

1999 – Death of 18 year-old Jesse Gelsinger

at Pennsylvania University in a gene therapy trial represents a major setback to the field of gene therapy in the years to come. „No one is really sure exactly why the gene therapy treatment caused his death, but it appears that ***his immune system launched a raging attack on the adenovirus carrier"****

2006 – Clinical trial of a new monoclonal antibody designated TGN 1412

leaves six volunteers badly injured. Catastrophe widely publicized as „the elephant men" trial. „British regulators … concluded that TeGenero's drug TGN 1412 appeared to cause an ***unprecedented biological reaction in humans by stimulating the immune system"**** Cytokine Storm

Drug Withdrawals

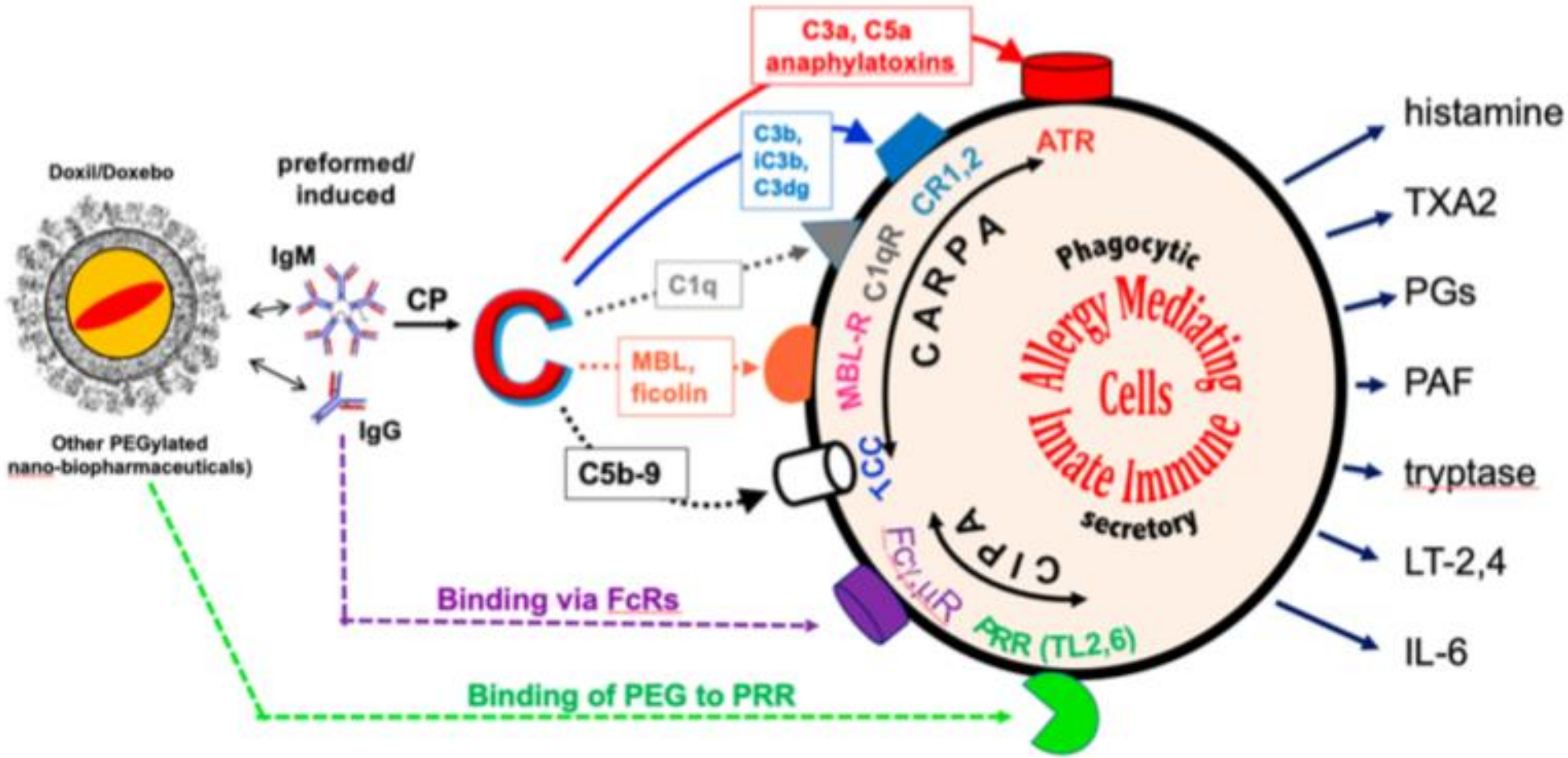
2005 - „…Palatin Technologies, the manufacturer of NeutroSpec (Technetium (99m Tc) fanolesomab) is voluntarily suspending marketing of NeutroSpec effective immediately due to serious safety concerns”

„… FDA received reports from Palatin Technologies of 2 deaths and 15 additional life-threatening adverse events in patients receiving NeutroSpec.”*

*Source: US FDA
http://www.fda.gov/CDER/drug/advisory/technetium99.htm

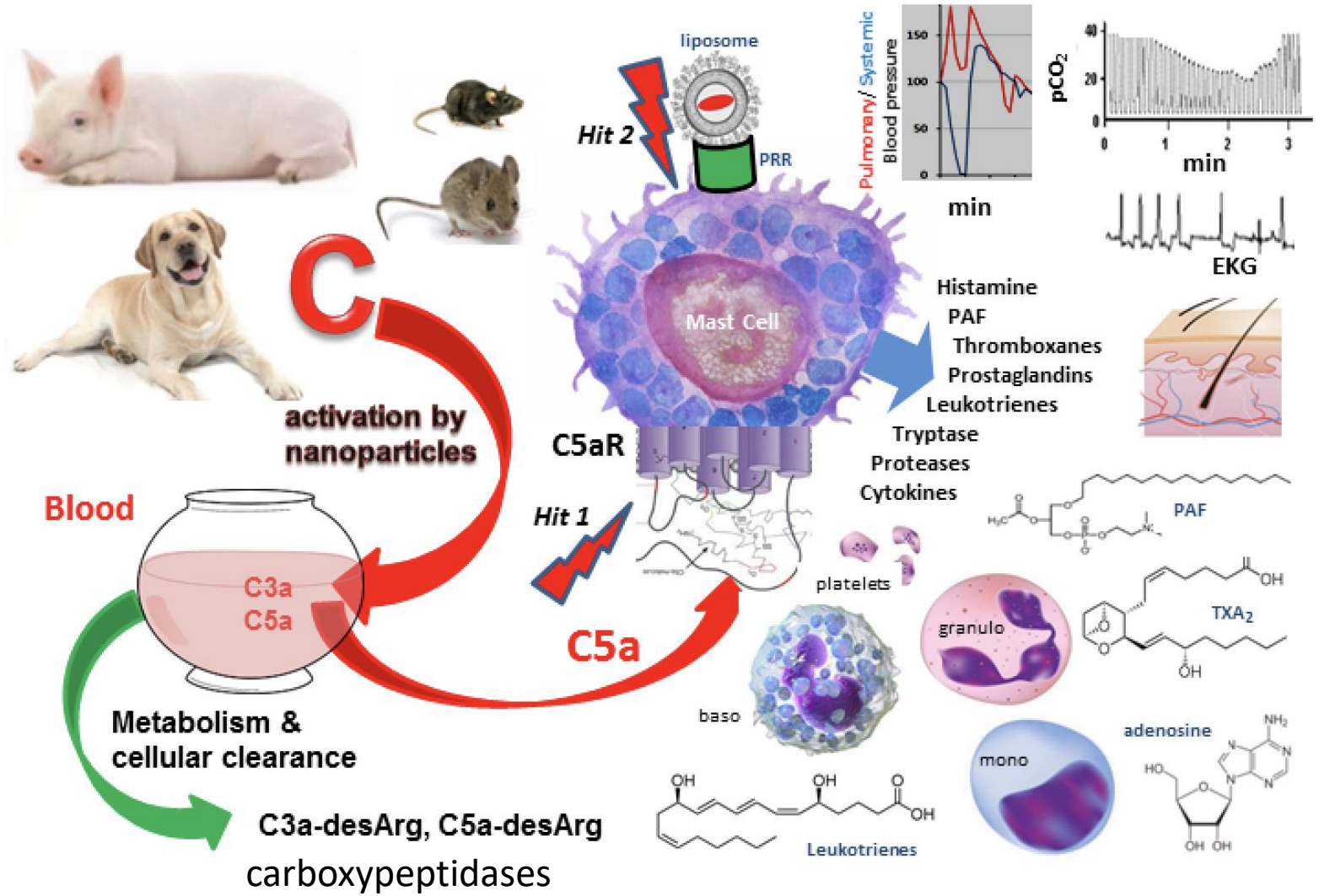
Mechanism

Double/multiple hit concept

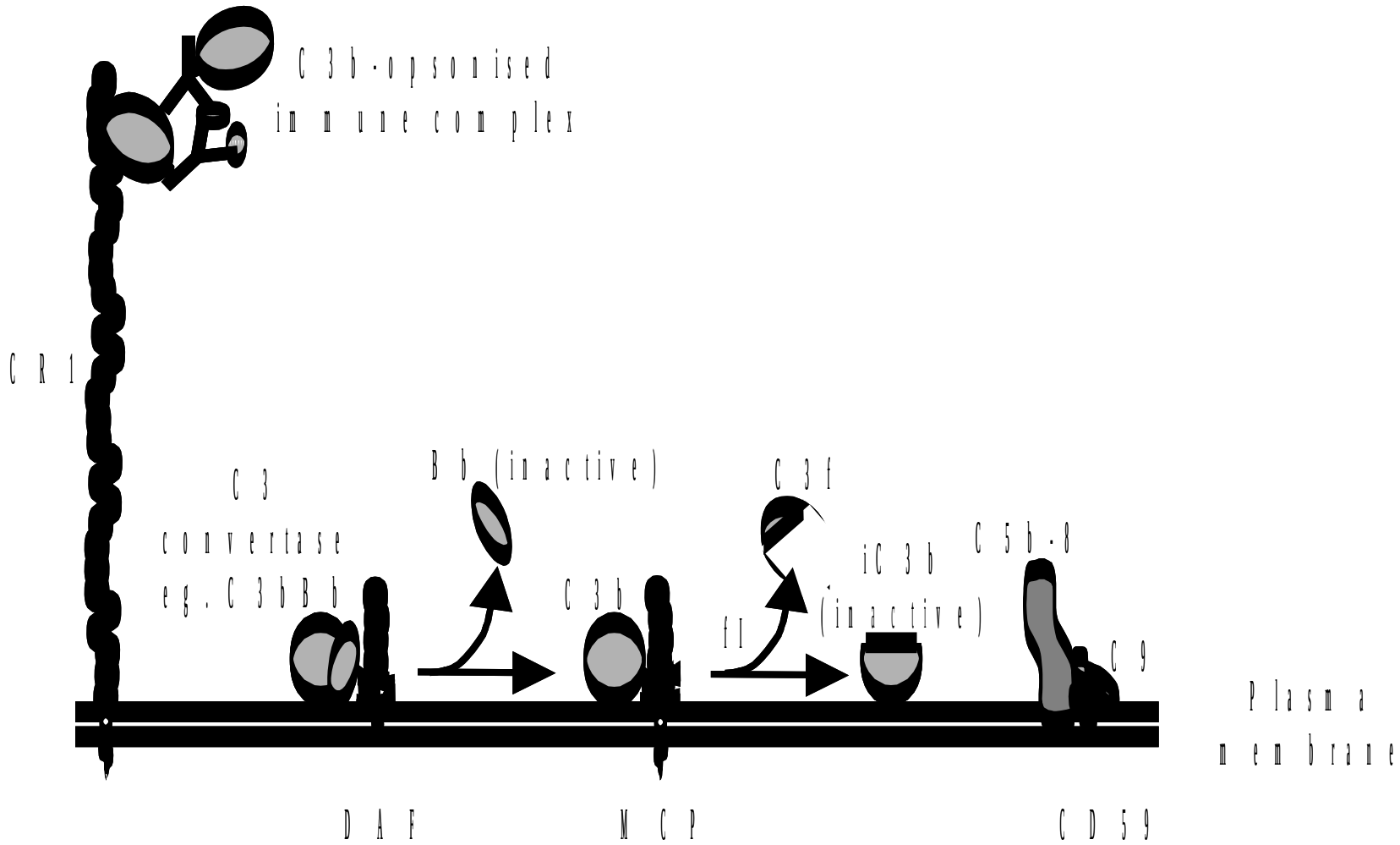


Mechanism

The anaphylatoxin “hit”



Membrane proteins protecting cells against complement attack



Prevention and Treatment of CARPA:
Current Methods

- **Emergency measures**
  - **CPR, epinephrine, oxygen, fluids**
- **Empirical**
  - **slow infusion, break or termination of infusion**
- **Parmacological**
  - **See below**

Pharmacological Prevention of CARPA

- **Commonly applied**
  - anti-inflammatory agents
    - Steroids
    - NSAID
      - Ibuprofen
      - acetaminophenol
  - Antihistamines
    - H1,H2

- **Potential**
  - IVIG
  - C1INH
  - Anti C5 mAb (Soliris)
  - Macrophage / RES / inhibitors
    - L-clodronate,
    - L-alendronate

Prevention of CARPA via desensitization

- **Theoretical basis**
  - **tachyphylactic nature of HSRs**
  - **Weak, subclinical reactions also can lead to tachyphylaxis**

- **Realization**
  - **Slow infusion of low dose of placebo (empty) liposomes**

In vitro C tests for CARPA prediction

Proof of concept

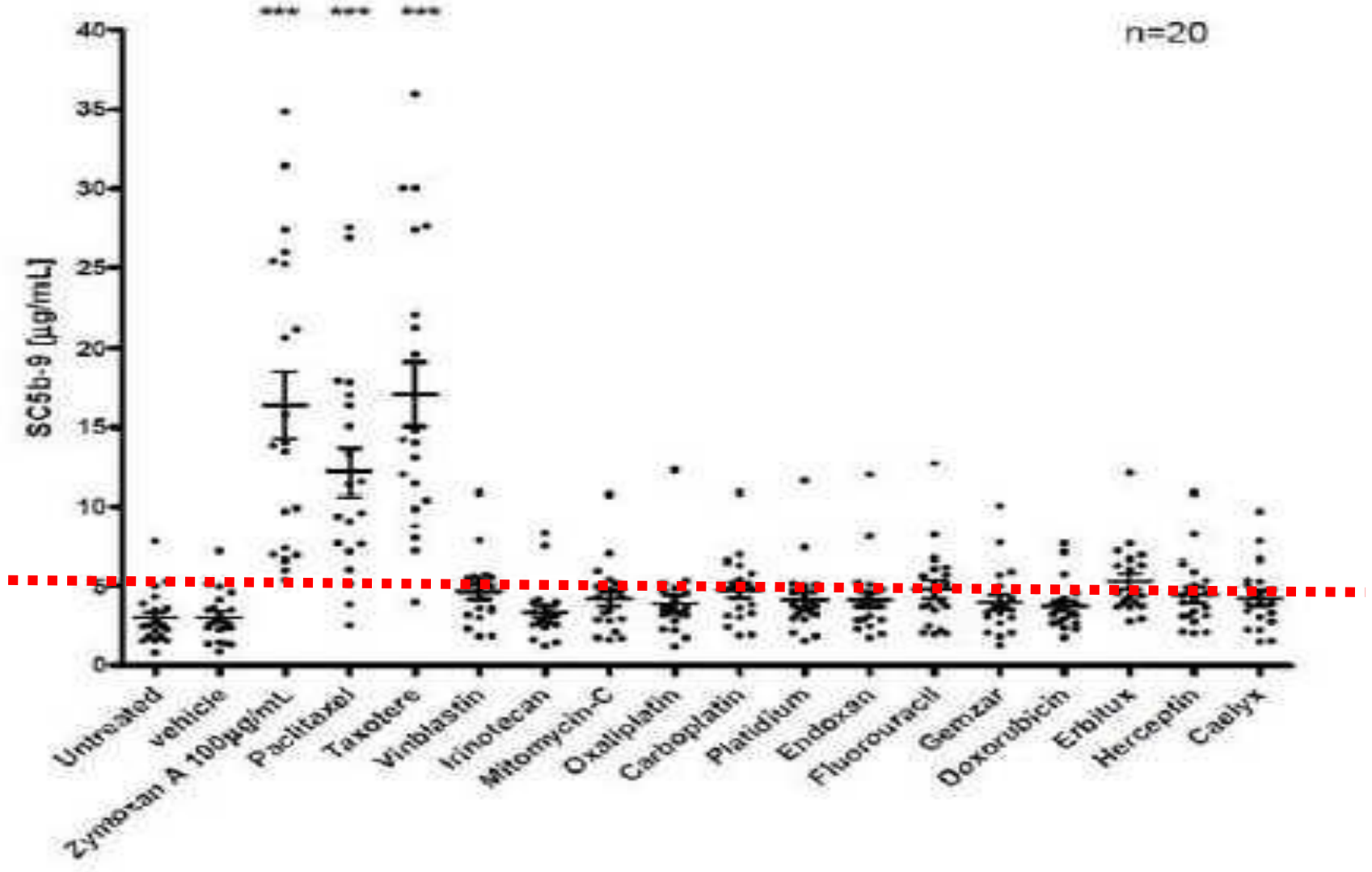
- **Animal studies**
  - Correlation between C activation by reactogenic drugs in vitro and hemodynamic and cardiopulmonary disturbance in pigs including systemic hypotension and pulmonary hypertension
  - Administration of human C5a causes cardiopulmonary and hemodynamic changes in pigs mimicking some of the hemodynamic abnormalities of human HSRs
  - Complement inhibitors sCR1 and IVIG inhibited the cardiopulmonary reaction of pigs to liposomes
- **Human studies**
  - Anaphylatoxins explain the symptoms
  - Correlation between C activation and HSRs to
    - liposomal doxorubicin (Doxil)
    - Rituximab
    - Althesin
    - Dialysis reactions
    - intravenous iron
    - radiocontrast agents

Available assays

(ELISAs, multiplex)

- **Human**
  - C3a
  - C4a
  - C5a
  - SC5b-9
  - C4a
  - C4d
  - Bb
  - CH50
- **Pig**
  - sC5b-9
  - C3a, C5a
  - C3 PAN/CH50
- **Rat**
  - C3a
  - C5a
  - C3 PAN/CH50
- **Mouse**
  - C3a
  - C3 PAN
  - CH50

Complement activation in vitro by reactogenic drugs



Latest regulatory endorsement

Received: 9 October 2019 | Revised: 16 March 2020 | Accepted: 17 March 2020

DOI: 10.1002/wnan.1633

Check for updates

ADVANCED REVIEW

WIREs NANOMEDICINE AND NANOBIOTECHNOLOGY

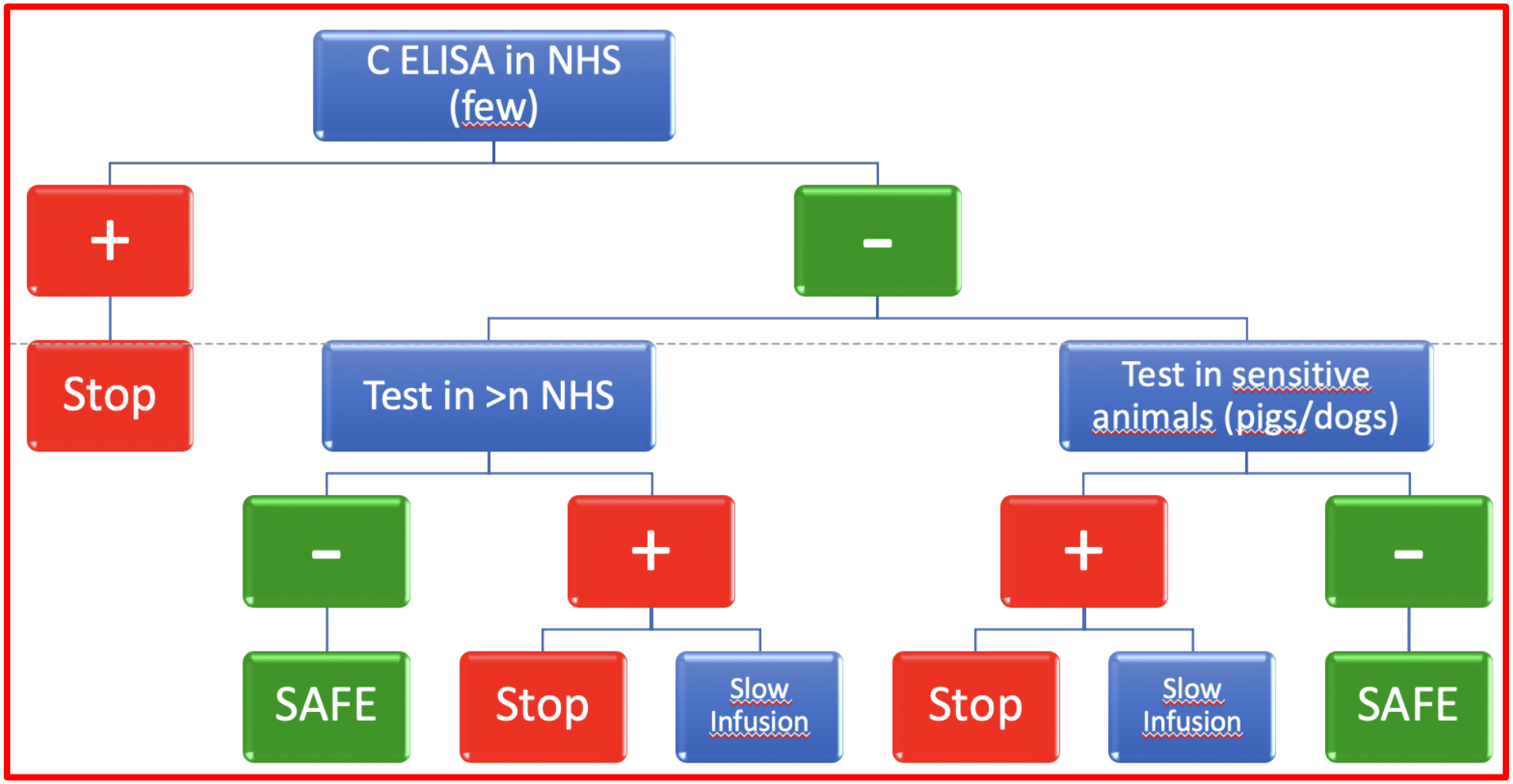
WILEY

Nonclinical regulatory immunotoxicity testing of nanomedicinal products: Proposed strategy and possible pitfalls

Christina Giannakou[1] | Margriet V. D. Z. Park[2] | Irene E. M. Bosselaers[3] | Wim H. de Jong[2] | Jan Willem van der Laan[3] | Henk van Loveren[4] | Rob J. Vandebriel[2] | Robert E. Geertsma[2]

- … immunotoxic effects relevant for NMPs such as complement activation-related pseudoallergy (CARPA) …may very well go undetected in the preclinical phase when following the ICH-S8 guideline.” …
- Therefore, we propose a predictive battery of tests for the endpoints relevant for NMPs which are currently not included in the ICH-S8 guideline, taking into account known pitfalls related to the testing of NMPs.” (

A recommended testing strategy



Szebeni, J.; Storm, G., ***Biochem Biophys Res Commun*** 2015, *468*, 490

Manifestations of porcine CARPA

Hemodynamic alterations

- rise of PAP
- rise or decline of SAP
- declince of CO and pCO_2

Cardiac abnormalities

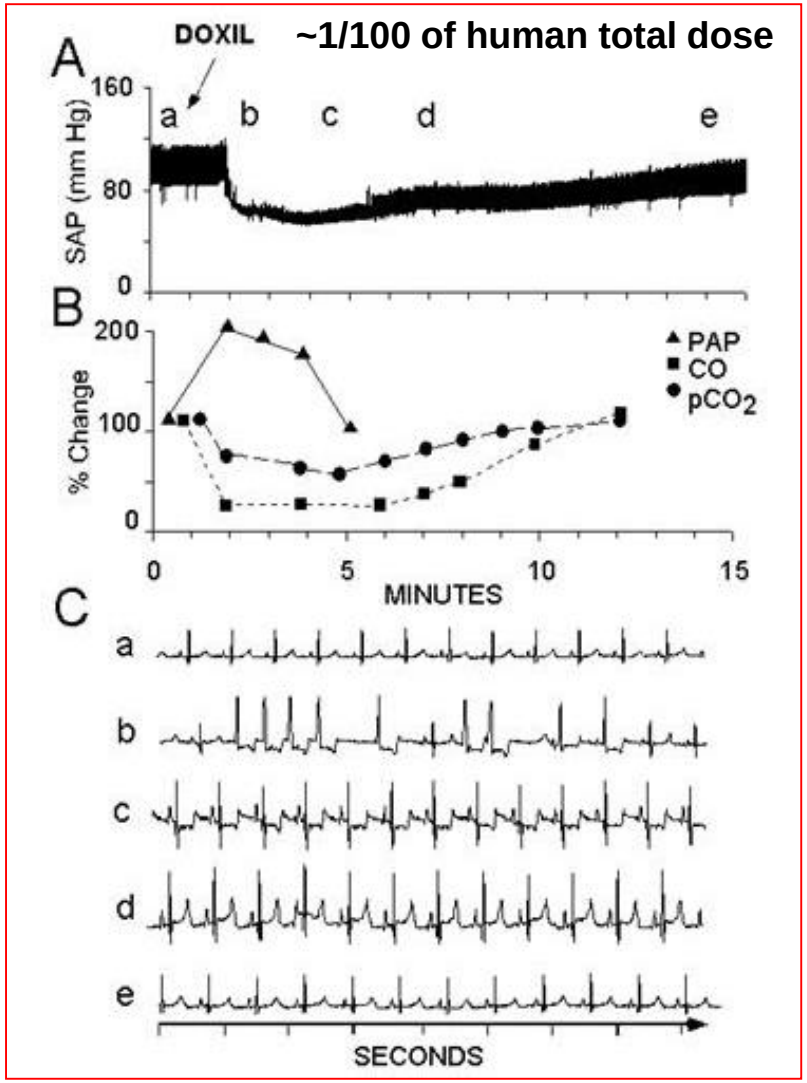
- tachycardia, bradycardia, arrhythmias
- ventricular fibrillation, arrest

Skin reaction

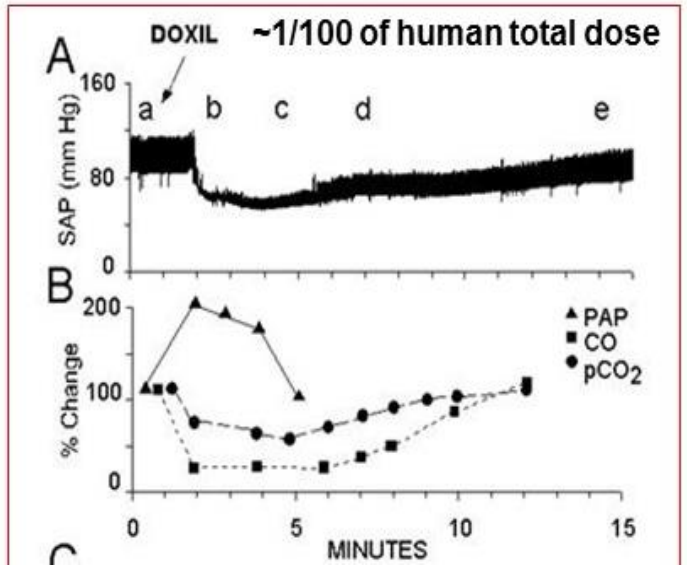
- erythema,
- rash

Blood abnormalities

- Leukocytosis
- leukopenia
- thrombocytosis
- thrombopenia



Pigs provide a sensitive and highly reproducible *in vivo* model for the acute immune (anaphylactoid) reactivity and immunogenicity of nanoparticles



Hemodynamic alterations

rise of PAP
rise or decline of SAP
declince of CO and pCO_2

Cardiac abnormalities

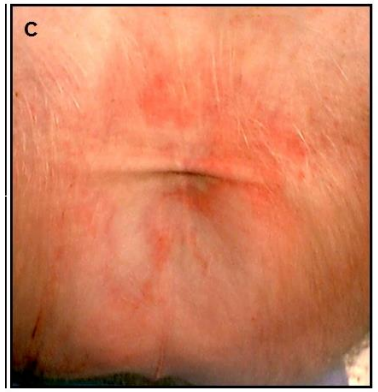
tachycardia, bradycardia, arrhythmias
ventricular fibrillation, arrest

Skin reaction

erythema,
rash

Blood abnormalities

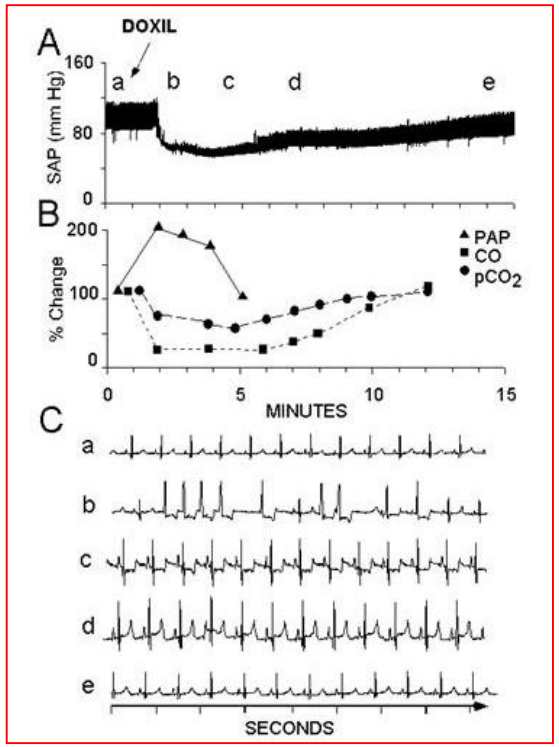
Leukocytosis
leukopenia
thrombocytosis
thrombopenia



1. *Circulation* **1999,** *99* (17), 2302-9.
2. *Am J Physiol Heart Circ Physiol* **2000,** *279* (3), H1319-28.
3. *J Liposome Res* **2002,** *12* (1-2), 165-72.
4. *Nat Med* **2003,** *9* (4), 431-8.
5. *J Liposome Res* **2005,** *15* (1-2), 3-14.
6. *Am J Physiol Heart Circ Physiol* **2006,** *290* (3), H1050-8.
7. *J Control Release* **2010,** *146* (2), 182-95.
8. *Biomaterials* **2011,** *32* (21), 4936-42.
9. *J Control Release* **2012,** *160* (2), 394-400.
10. *Adv Drug Deliv Rev* **2012,** *64* (15), 1706-16.
11. *Nanomedicine* **2012,** *8* (2), 176-84.
12. *Anesth Analg* **2014,** *119* (5), 1094-101.
13. *J Control Release* **2014,** *195*, 2-10.
14. *Nanomedicine* **2016,** *12* (4), 933-943.
15. *Nanomedicine (Lond)* **2016,** *11* (6), 597-616.
16. *J Control Release* **2017,** *264*, 14-23.
17. *Int J Nanomedicine* **2017,** *12*, 5223-5238.
18. *Nat Nanotechnol* **2017,** *12* (6), 589-594.
19. *Cardiovasc Res* **2018,** *114* (13), 1714-1727.
20. *J Control Release* **2018,** *270*, 268-274.
21. *Int J Nanomedicine* **2018,** *13*, 6345-6357.
22. *Int J Nanomedicine* **2018,** *13*, 1899-1915.
23. *Mar Drugs* **2019,** *17* (12).
24. *Control Release* **2019,** *309*, 333-338.
25. *ACS Nano* **2019,** *13* (8), 9315-9324.

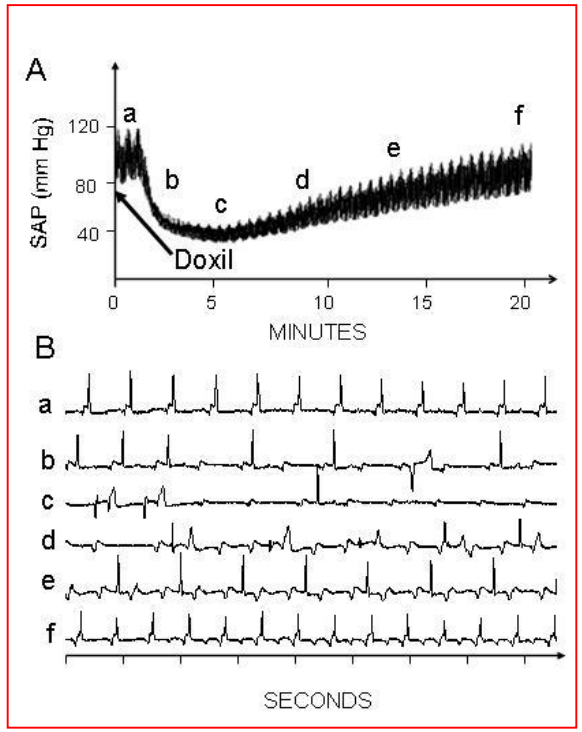
THE BACKGROUND AND EXPERTISE, AN EXAMPLE

Hemodynamic and cardiac manifestations of Doxil-induced CARPA in pigs



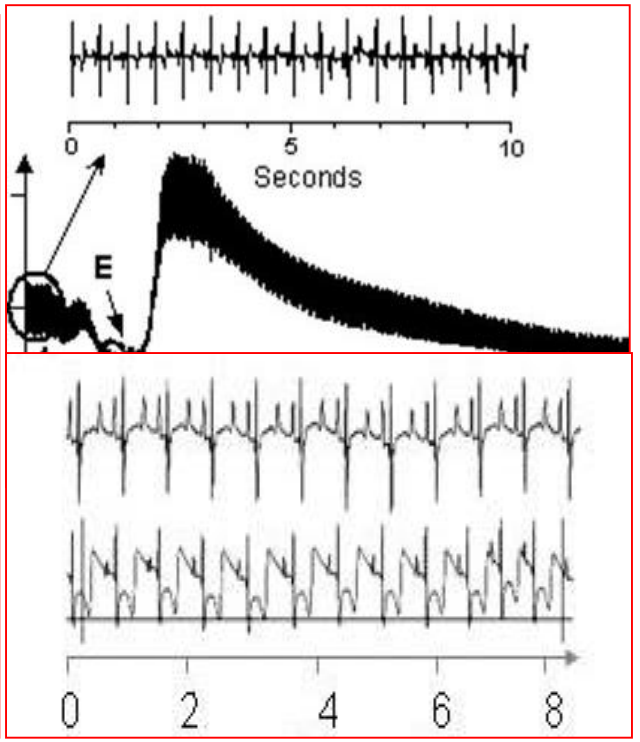
Tachyarrhythmia

T wave elevation



Paradoxical bradycardia

AV block, asystolia

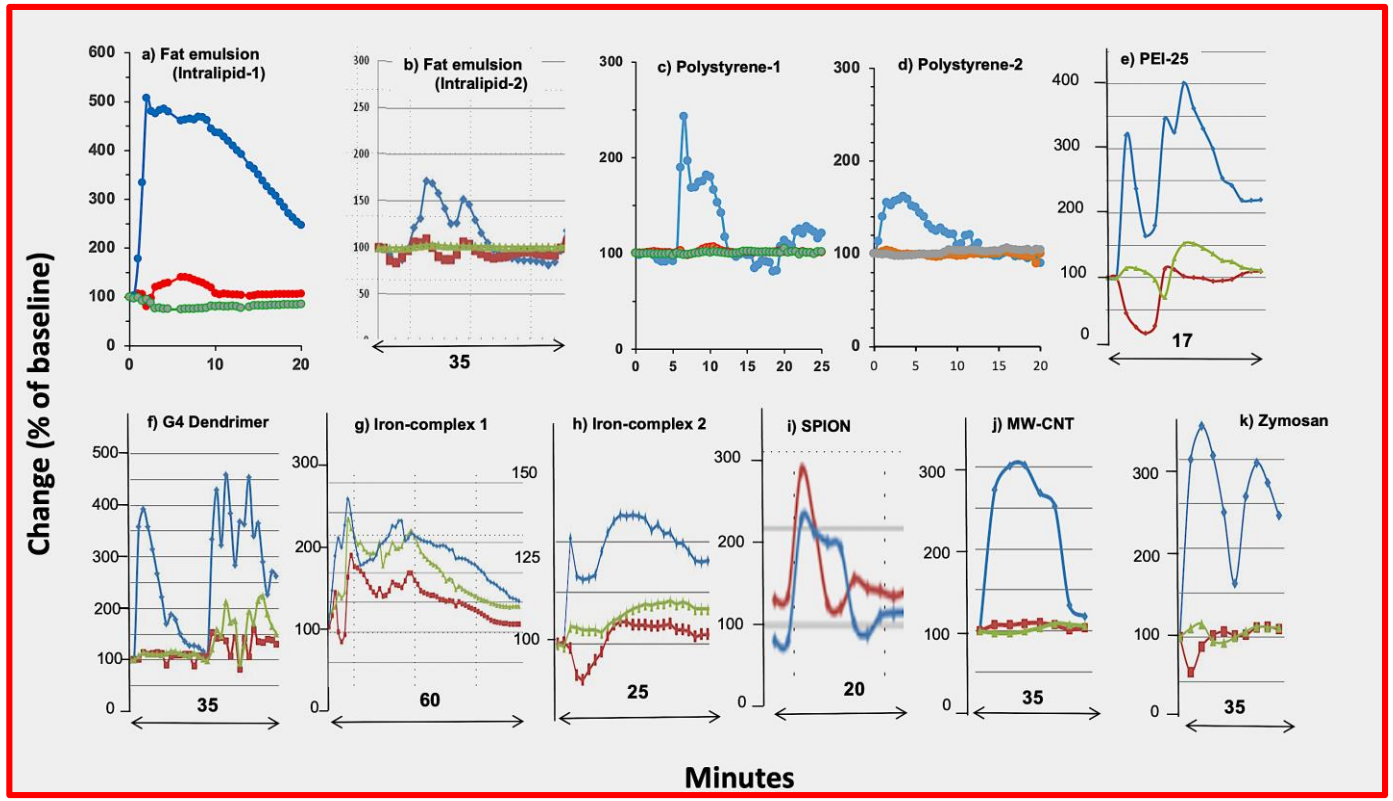


Ventricular fibrillation,

ST-depression

Am. J. Physiol. 290:H1050-8, 2006

The hemodynamic response of pigs is quantitative and specific for NPs Enables „Immuno-imaging”



Rudolf Urbanics, Péter Bedőcs and János Szebeni*

Lessons learned from the porcine CARPA model: constant and variable responses to different nanomedicines and administration protocols

Eur. J. Nanomed. 2015; 7(3): 219–231

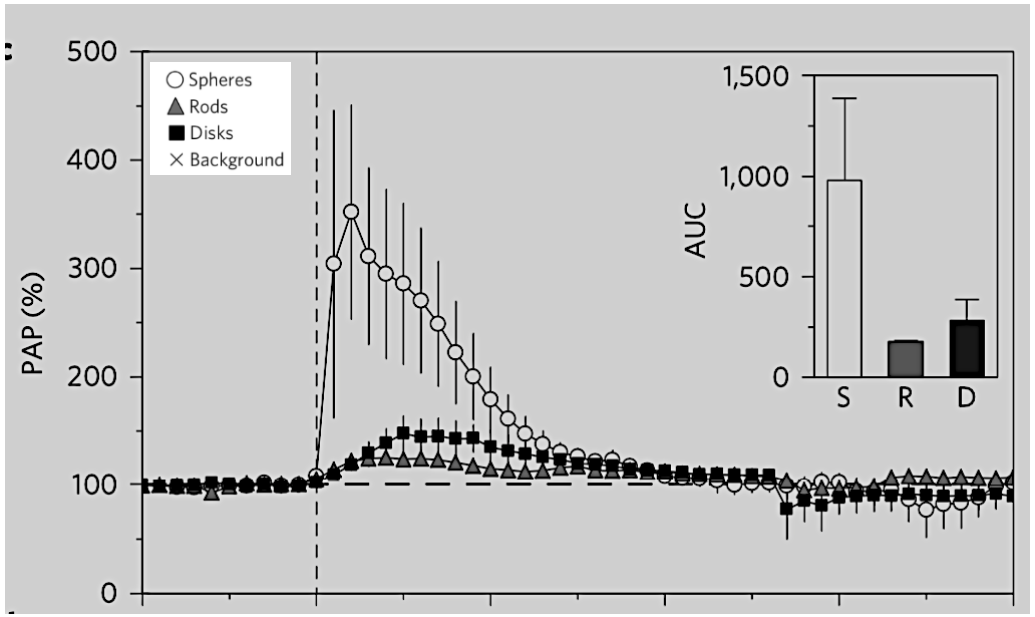
nature nanotechnology

ARTICLES

PUBLISHED ONLINE: 10 APRIL 2017 | DOI: 10.1038/NNANO.2017.47

Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes

Peter Pope Wibroe[1], Aaron C. Anselmo[2], Per H. Nilsson[3,4,5], Apoorva Sarode[2], Vivek Gupta[6], Rudolf Urbanics[7], Janos Szebeni[7], Alan Christy Hunter[8], Samir Mitragotri[2], Tom Eirik Mollnes[3,4,9,10,11] and Seyed Moein Moghimi[1,12,13]*



Nat Nanotechnol **2017**, *12* (6), 589-594.

Use of pigs for immunogenicity assessment

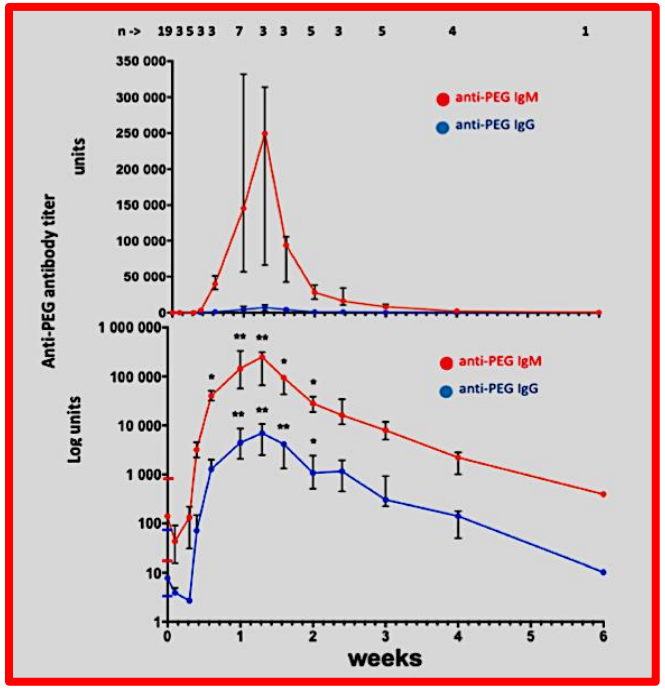
ACS NANO
Cite This: ACS Nano XXXX, XXX, XXX−XXX
www.acsnano.org

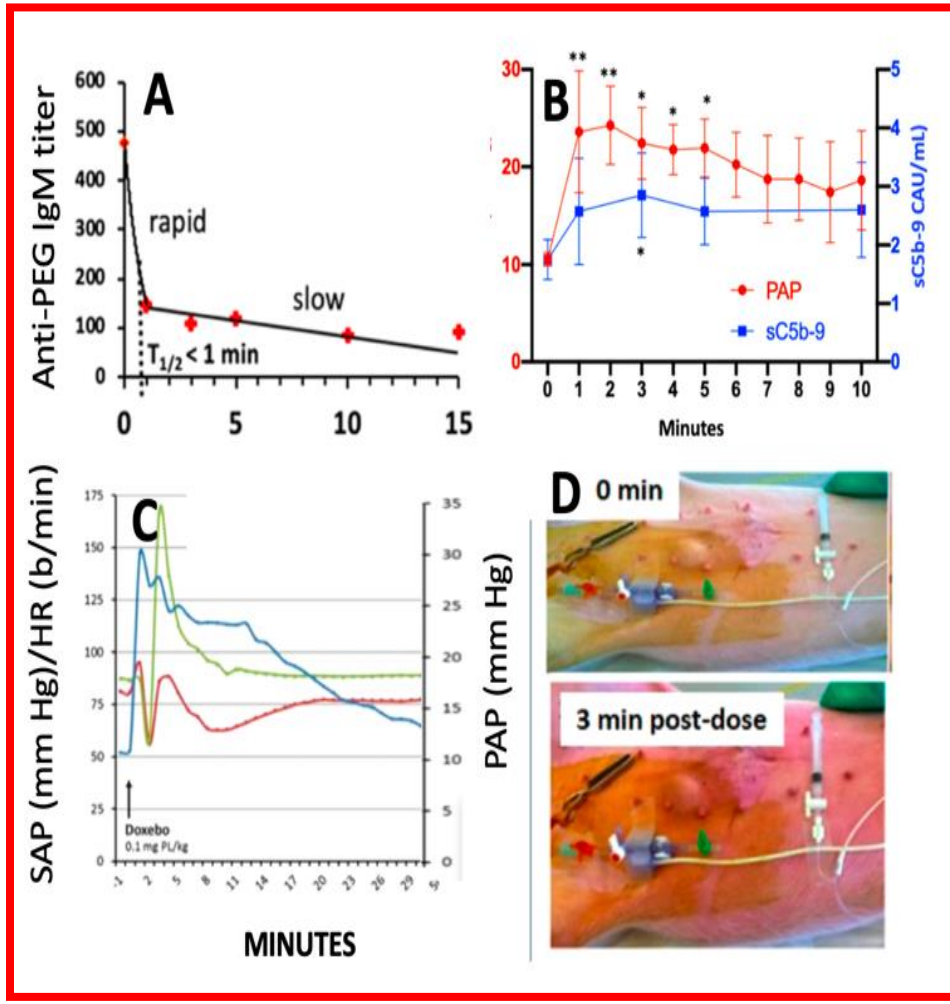
Pseudo-anaphylaxis to Polyethylene Glycol (PEG)-Coated Liposomes: Roles of Anti-PEG IgM and Complement Activation in a Porcine Model of Human Infusion Reactions

Gergely Tibor Kozma,[†,‡] Tamás Mészáros,[†] Ildikó Vashegyi,[‡] Tamás Fülöp,[†] Erik Örfi,[†] László Dézsi,[†] László Rosivall,[†,‡,§] Yaelle Bavli,[∥] Rudolf Urbanics,[†,‡] Tom Eirik Mollnes,[⊥,#,¶] Yechezkel Barenholz,[∥,□] and János Szebeni*[,†,‡,○,□]

Kozma, et al., *ACS Nano* **2019,** *13*, 9315

- **Induction of anti-PEG IgG/IgM by i.v. Doxebo**
- **After seroconversion**
  - **demonstration of drastically enhanced HSR to repeated injection of Doxebo/doxil**
  - **Its correlation with complement activation**

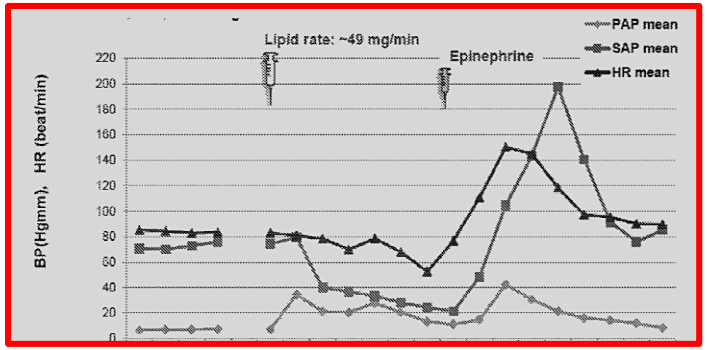


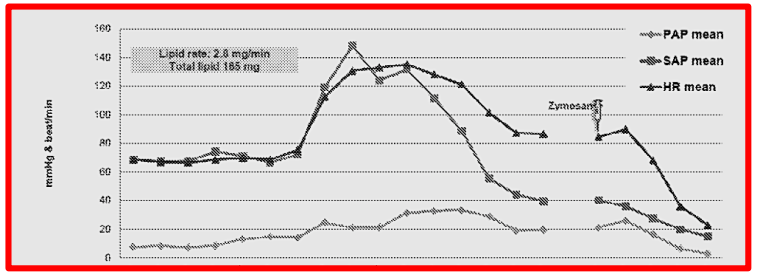


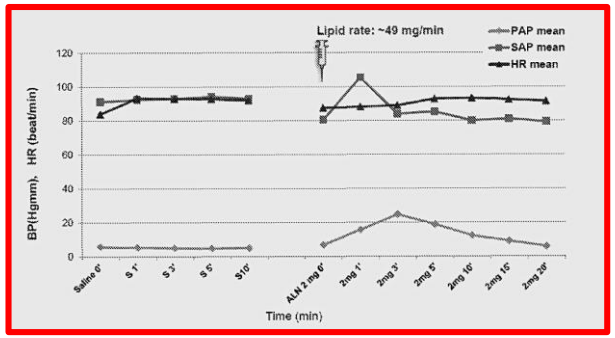
Use of pigs for developing safe infusion protocols:

the example of Onpattro

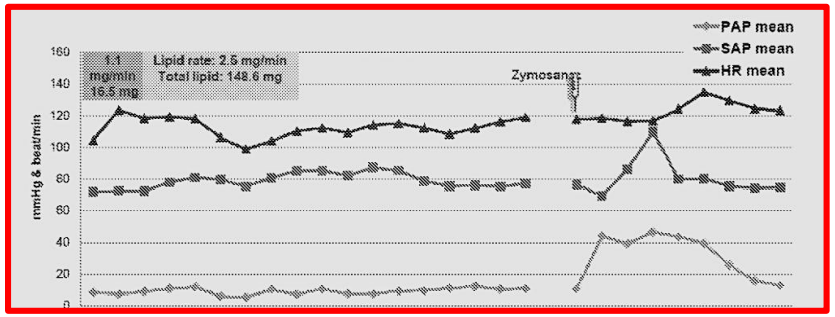
DOSAGES AND METHODS FOR DELIVERING LIPID FORMULATED NUCLEIC ACID MOLECULE
Alnylam Patent application
PCT/US2014/036915
WO/2014/182661
Helped FDA approval of Onpattro







Claim 1: Method of reducing an infusion-related response (IRR), or a hypersensitivity reaction, or both, in a subject, to a composition comprising a lipid formulation and a nucleic acid molecule, said method comprising administering to a subject:



Use of pigs for developing safe infusion protocols: *NanoCort*

Journal of Controlled Release 309 (2019) 333–338

Contents lists available at ScienceDirect

J Control Release **2019,** *309*, 333-338.

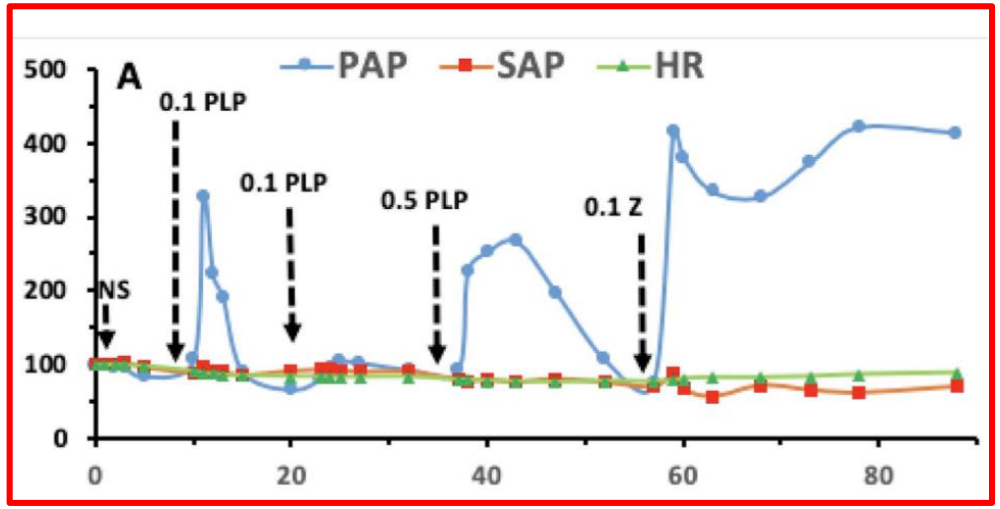
journal homepage: www.elsevier.com/locate/jconrel

Liposome-induced hypersensitivity reactions: Risk reduction by design of safe infusion protocols in pigs

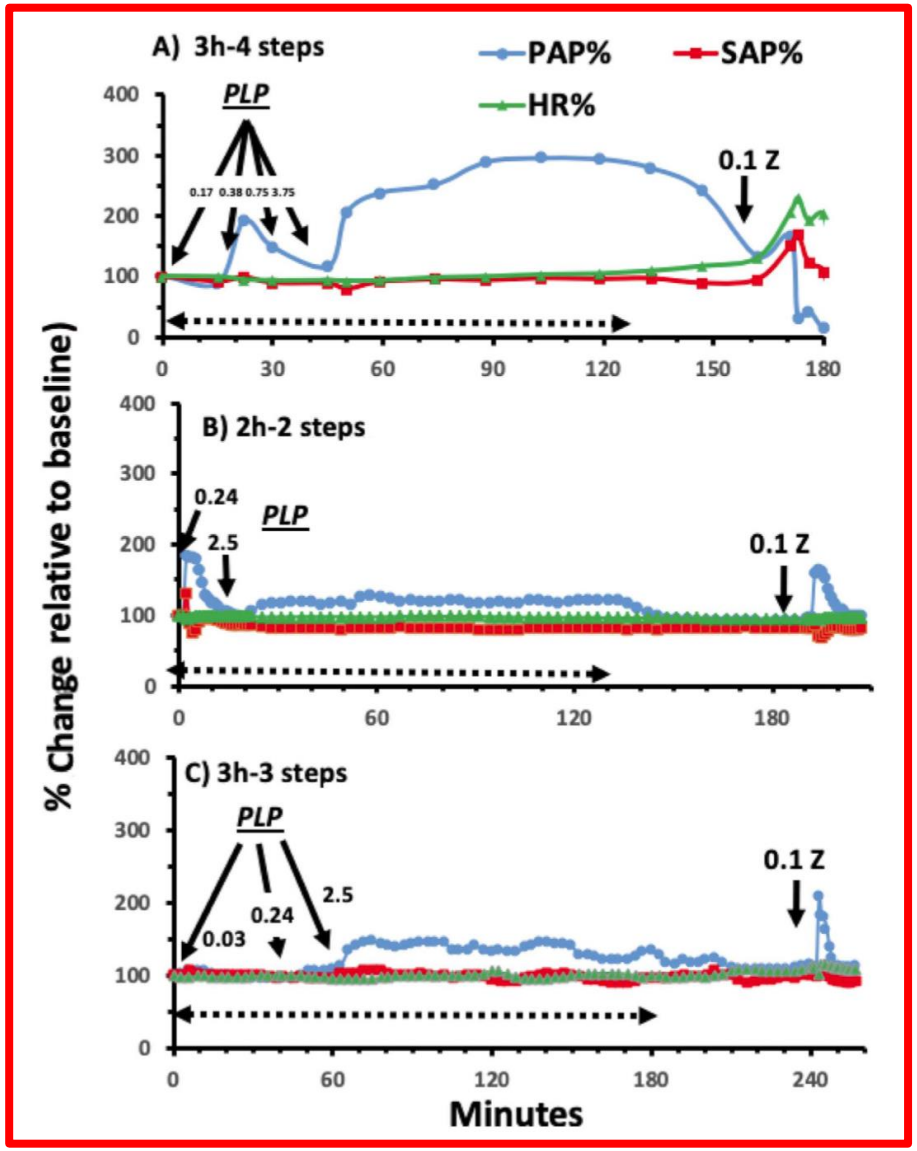
Tamás Fülöp[a,1], Gergely T. Kozma[a,b,1], Ildikó Vashegyi[b,1], Tamás Mészáros[a,b], László Rosivall[c], Rudolf Urbanics[a,b], Gert Storm[d,e], Josbert M. Metselaar[d,f,**,1], János Szebeni[a,b,g,*,1]

PLP = Pegylated liposomal prednisolone phosphate

PLP Bolus



PLP Infusion



Hypothesis on the mechanism of porcine CARPA

PIM cells

- **Removal of blood-borne particulates in calves, sheep, goats, cats, and pigs occurs predominantly in pulmonary intravascular macrophages (PIMs).**
- **Pulmonary resident macrophages of monocyte origin, adherent to the capillary endothelium, morphologically similar to hepatic Kupffer cells.**
- **Pulmonary constituent of the MPS (RES) with respect to secretory, endocytic, and functional properties.**
- **Secrete bioactive lipids, stimulated PIMs may contribute to regulation of pulmonary hemodynamics.**
- **Estimates of relative PIM numbers in porcine lung parenchyma suggest cell densities similar to that of rat hepatic Kupffer cells.**

BJA

British Journal of Anaesthesia, xxx (xxx): xxx (xxxx)

CORRESPONDENCE

Anaphylaxis to the first COVID-19 vaccine: is polyethylene glycol (PEG) the culprit?

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[1]Allergy Clinic, Department of Dermatology and Allergy, Copenhagen University Hospital, Gentofte, Denmark, [2]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark and [3]Department of Allergy, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK

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Keywords: allergy; anaphylaxis; COVID-19; drug allergy; hypersensitivity; polyethylene glycol; vaccine

Official data: 2-5 anaphylaxis to the mRNA vaccines /100,000
VAERS: 4000 death in the USA

USA statistics: >25 million people vaccinated between 2009 and 2011, 33 (i.e., 1.31 cases per 1,000,000 vaccinations) experienced anaphylaxis

20-50-fold increase

Needs to be emphasized

- **The benefits of anti-COVID vaccines far outweight the health risk of adverse effects.**

- **Investigating the adverse effects of anti-COVID vaccines aims to increase the safety, and, hence, utility of these vaccines and should not be taken as supporting vaccine-hesitancy or anti-vaccine movements**

Adverse effects of anti-COVID vaccines vs. flu vaccines

(Based on VAERS data until May 31, 2021)

Adverse Events	mRNA vaccines		All other Covid vaccines		Flu vaccines (12 years)	
	n	%	n	%	n	%
Hypersensitivity reactions/allergic reactions	942	0.000317%	19	0.000165%	213	0.000012%
anaphylaxis/anaphylactoid reaction/shock	2774	0.000932%	270	0.002344%	737	0.000043%
hyper/hypotension	3566	0.001198%	419	0.003638%	791	0.000046%
tachy/bradycardia	3183	0.001070%	279	0.002422%	526	0.000031%
dyspnoea/dyspnea	1312	0.000441%	107	0.000929%	380	0.000022%
difficulty of breathing	3670	0.001233%	426	0.003699%	1756	0.000102%
myocardial infarction	239	0.000080%	20	0.000174%	31	0.000002%
flushing/rash/skin eruptions	32701	0.010989%	2160	0.018753%	12686	0.000737%
thrombosis/thrombocytopenia/thromboembolia	1059	0.000356%	399	0.003464%	128	0.000007%
myocarditis/cardiac inflammation	508	0.000171%	9	0.000078%	32	0.000002%
death	2506	0.000842%	300	0.002605%	495	0.000029%

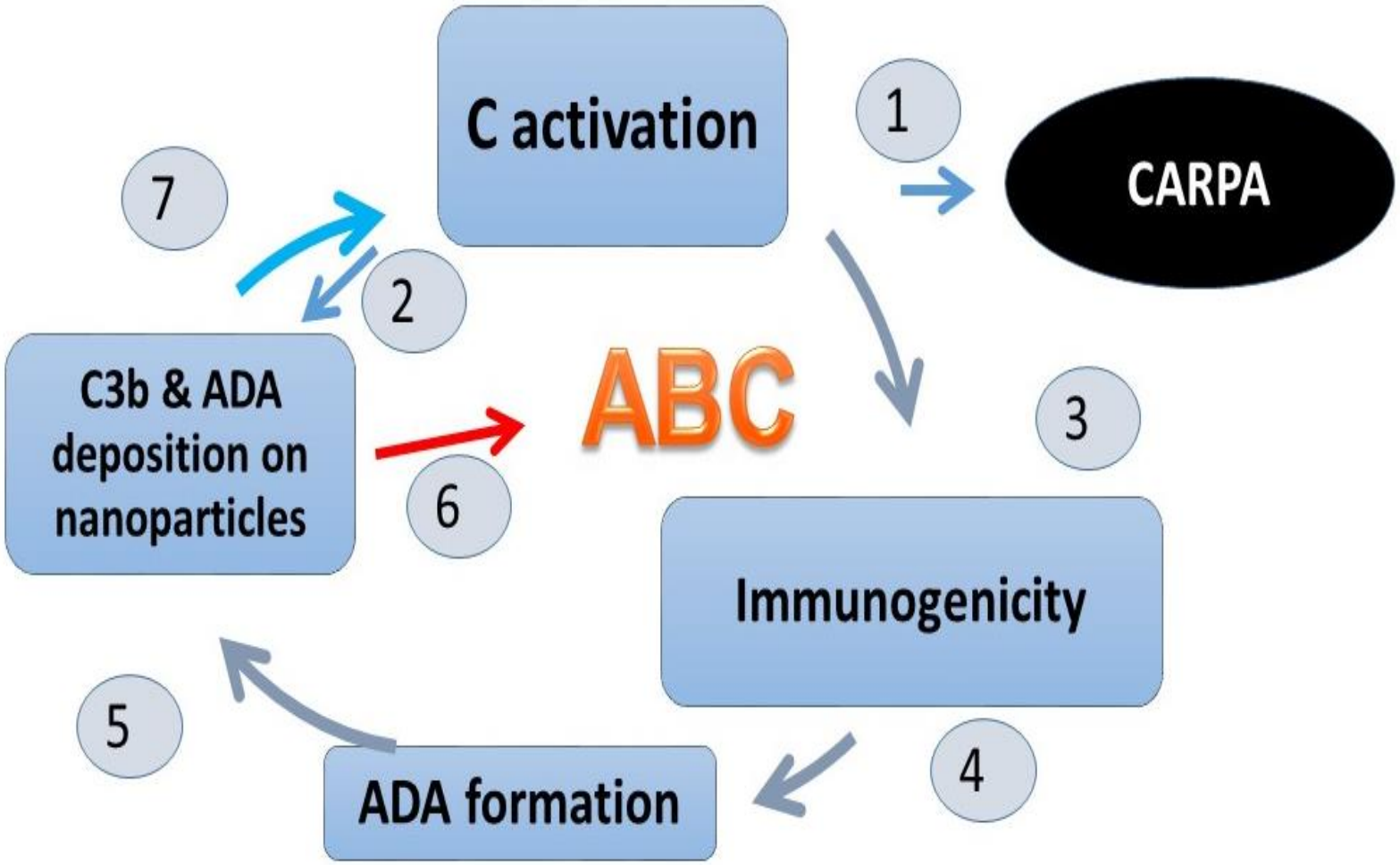
Covid mRNA vaccines given in the US todate: 297,570,484
Covid non-mRNA vaccines given in the US todate: 11,517,956
Flue Vaccines given in the US from 2009: 1,720,400,000

https://ourworldindata.org/grapher/covid-vaccine-doses-by-manufacturer?country=~USA
https://www.cdc.gov/flu/prevent/vaccine-supply-historical.htm
https://ourworldindata.org/grapher/covid-vaccine-doses-by-manufacturer?country=~USA

Preliminary information suggesting a role of CARPA in the HSRs to COVID vaccines

- The high frequency of reactions, despite exclusion of allergic people, cannot be explained with an IgE mechanism, e.g. anti-PEG allergy
- Symptoms
  - Resemble those of CARPA
  - More frequent than observed for flu vaccines (comparing half versus 12 years)
- Complement activation is a key element of immune reactivity (immunogenicity)
  - Virus-like nanoparticles, in general, can activate C
  - In the mRNA vaccines, mRNA, PEG, positively charged lipids and the applied phosphoholipids all can activate C
- Evidence that vaccine components can reach the blood

The immune stimulatory vicious cycle caused by PEGylated nanoparticles



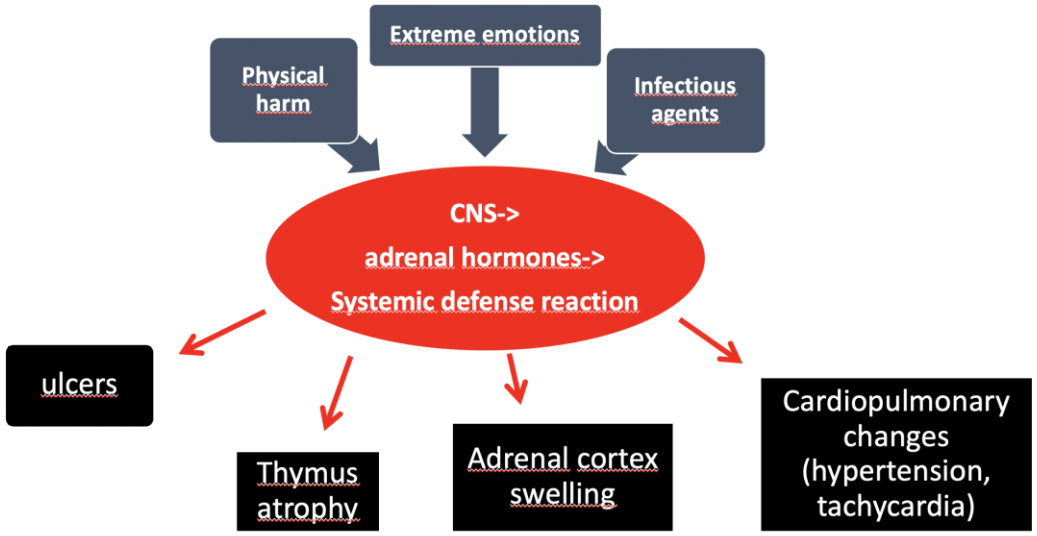
1st step, C activation leading to CARPA; Step 2, C activation results in C3b deposition on nanoparticles which initiates the alternative pathway amplification loop of the C cascade, and, thus, leads to more C activation. Furthermore, as a potent opsonin, C3b enhances the uptake of nanoparticles by the reticuloendothelial system (RES), i.e., it initiates the ABC phenomenons; Step 3, C activation promotes immunogenicity via large number of different effects on antigen-presenting cells (APCs); Step 4, Production of anti-drug antibodies (ADAs) by B cells; Step 5, deposition of these antibodies on nanoparticles causing classical pathway C activation; Step 6, opsonized particles undergo ABC; Step 7, Amplification of C activation via the alternative pathway and C1q binding (classical pathway activation).

Is CARPA a stress reaction in blood?

Similarity between conventional stress and CARPA

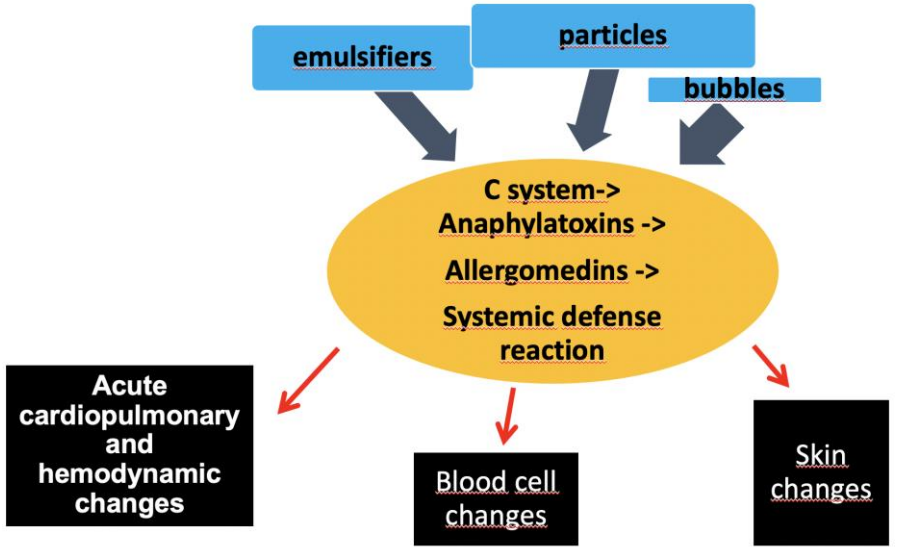
Conventional stress: a variety of noxious stimuli trigger a systemic defense reaction manifested in a specific pattern of physiological changes

hypothalamo-pituitary-adrenal axis



CARPA: a variety of noxious stimuli exposed to blood trigger a systemic defense reaction manifested in a specific pattern of physiological changes

C system-allergic cell-cardiovascular system axis



Szebeni, J., Complement activation-related pseudoallergy: a stress reaction in blood triggered by nanomedicines and biologicals. ***Mol Immunol* 2014,** *61* (2), 163-73.

Conceptual progress in HSR cause and modeling

Question	Challenge	Clarification
Role of complement activation in HSRs	**Questionable because** ➢ **there is no clear relationship between complement activation and HSRs** ➢ **The reactions are linked to rapid phagocytic response by macrophages**	➢ **Complement activation may be a significant contributor but not sole cause of HSRs. In mice HSRs is "CIPA"*** ➢ **Reactions have double or multiple simultaneous activation triggers on many cells, including macrophages**
Relevance of the pig model	**Inappropriate because** ➢ **"global reaction" of PIM cells** ➢ **nonquantitative, nonspecific** 	➢ **Pigs provide a disease (HSR) hazard identification model, not general toxicity** ➢ **Response is highly specific, dose dependent and quantitative**
References	Moghimi et al., **Nature Nanotechnol**, 2017*; 12,* 589*;* **Drug Discov Today** 2018;23:1034, **Nanomedicine** 2018;13:973; **J Pharmacol Exp Ther**. 2019;370:581. **Adv. Drug Deliv. Rev.** 2020**,** In press	Szebeni et al., **Drug Discov Today** 2018;23:487; **Int J Nanomedicine** 2018;13:6345**; Nature Nanotechnol** 2018**;** 13;1100**; Biomedicines 2**020, 8 (4); **Frontiers in Immunology** 2020, Vol. 11

*Orfi et al., *Int J Nanomedicine* **2019,** *14*, 1563-1573.

Conclusions

- **Infusion reactions remain an unsolved problem for many therapeutic or diagnostic nanomedicines.**
- **Current experimental evidence supports the causal or contributing role of C activation.**
- **Animal models, complemented with in vitro C assays, enables the prediction of CARPA and elaboration of safe administration protocols**
- **CARPA may be a contributing factor to the HSRs to certain COVID vaccines**

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- **Milosevits Gergely**
- **Őrfi Erik**
- **Rosivall Laszlo**
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- **Szénási Gabor**
- **Urbanics Rudolf**
- **Velkei Maria**