Outline

- The immune system of pigs/minipigs
  - Focus on pseudoallergy and complement
    - the history of complement (C) activation-related pseudoallergy (CARPA)
    - features of human infusion reactions clained to be CARPA
      - symptoms, drugs, incidence, regulatory endorsements
- Studies in the porcine model
  - symptoms corresponding to human infusion reactions
  - the role of C activation
- Studies in minipigs, justifying their use
- Mechanism: double hit
- The utilty of the porcine CARPA model for developing safe infusion protocols
- Rare anaphylaxis caused by mRNA vaccines: can it be CARPA?

The immune system of pigs/minipigs: human relevance

Cell and Tissue Research (2020) 380:287–304
https://doi.org/10.1007/s00441-020-03206-9

REVIEW

The pig as a model for immunology research

Reinhard Pabst[1]

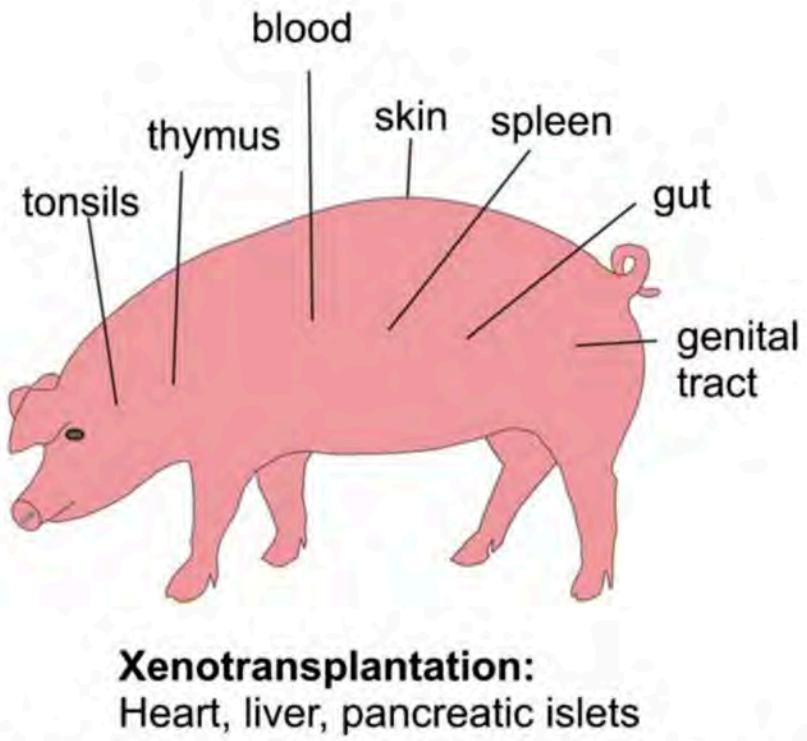


Fig. 1 Schematic drawing of important organs of the pig as models for immunology

The pigs' immune system resembles man for more than 80% of analyzed parameters (mouse 10%).

- **More or less differences amply studied**
  - Immunoglobulins, antibody repertoire
  - T and B Cell subclasses, development
  - Host defense peptides (defensins)
  - Toll-like receptors
  - Tonsils, lymph nodes, spleen structure
- **Best animal model for xenotransplantation (organ transplantation**
- **Widely used for ICH S8-mandated immunotoxicity studies, mainly immune suppression studies (e.g., TDAR) assay**

- ***Can be used for immuno-stimulation studies***
  - ***unique model of pseudo-allergic infusion reactions in man & pseudo-anaphylaxis***

Organ manifestations of pseudoallergic infusion reactions

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	

Infusion reactions classified by strength

Mild (GRADE 1)	Moderate (GRADE 2-3	Severe GRADE (4-5)
Nausea	Chest discomfort	Hypo/hypertension (>40 mm Hg)
Dizziness	Shortness of breath	Chest pain
Headache	Hypo/hypertension (>20 mm Hg)	Back pain
Flushing	Increased temperature	Increased temperature with rigors
Diaphoresis	Urticaria	Stridor
Palpitations		Shock, death
No intervention needed	Intervention needed	Infusion needs to be abandoned

CHEIFETZ et al., **Monoclonal Antibodies, Immunogenicity, and Associated Infusion Reactions**
THE MOUNT SINAI JOURNAL OF MEDICINE Vol. 72 No. 4 July 2005, 250-56

Nanomedicine-induced infusion reactions (IRs): Features

- Acute allergy syndrome
- 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 involved: 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 preclinical testing

nature nanotechnology

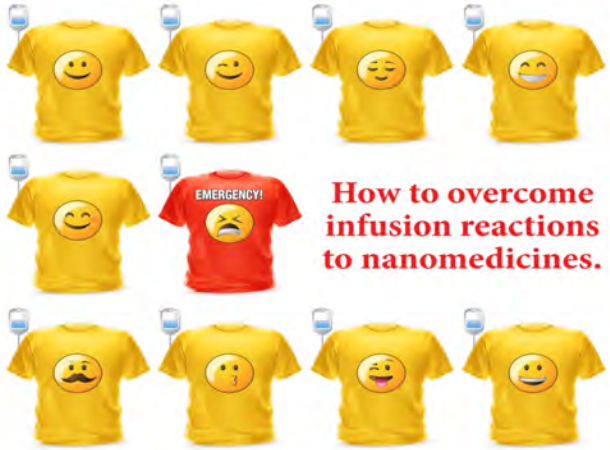
PERSPECTIVE

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



frontiers in Immunology

Why pigs?

The Critical Choice of Animal Models in Nanomedicine Safety Assessment: A Lesson Learned From Hemoglobin-Based Oxygen Carriers

Peter Bedőcs and János Szebeni

REVIEW
published: 26 October 2020
doi: 10.3389/fimmu.2020.584966

Front. Immunol., 26 October 2020
| https://doi.org/10.3389/fimmu.2020.584966

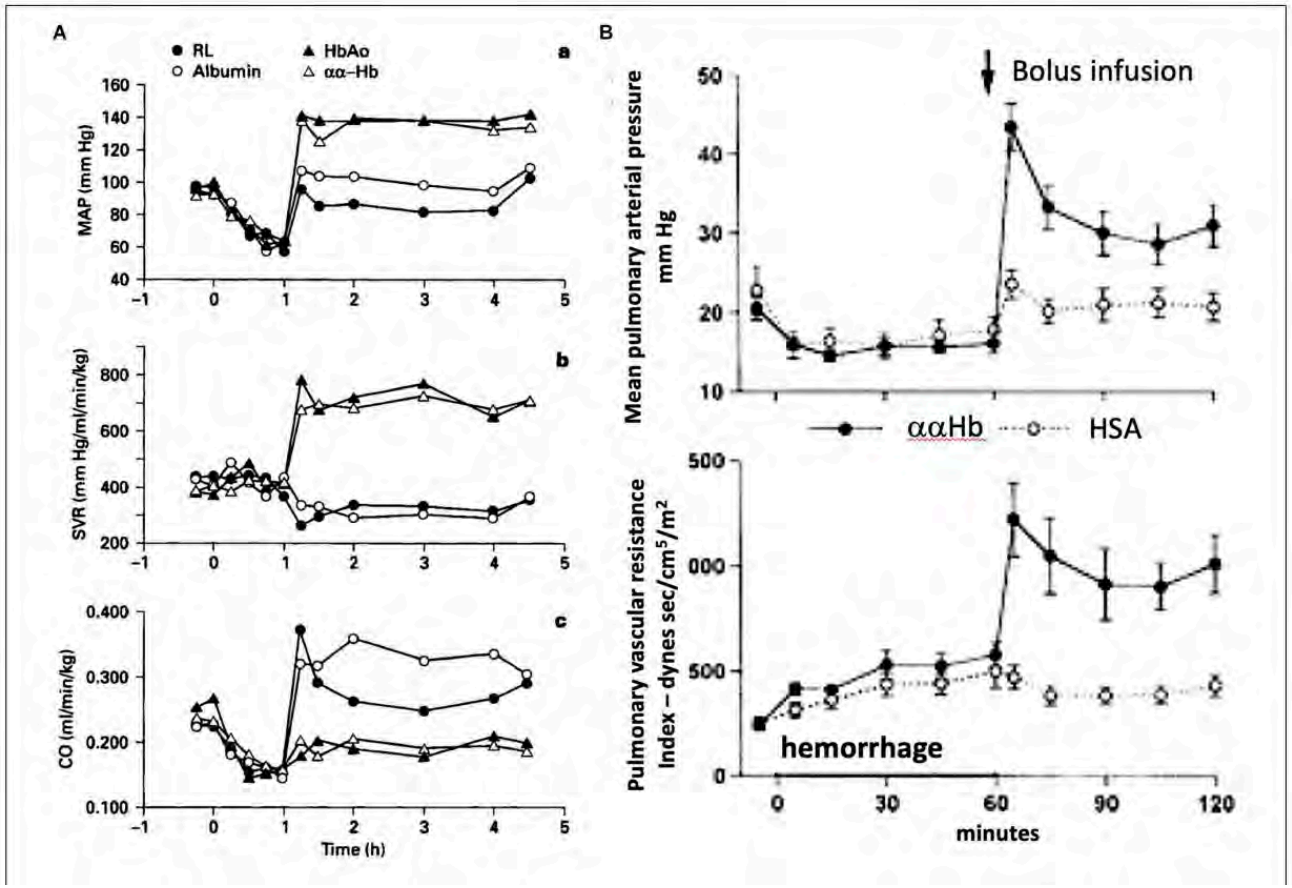


Figure 1. Hemodynamic changes in pigs infused with ααHb or control albumin (HSA), lactated Ringer or purified HbA solutions.

Hemoglobin-based oxygen carriers **(HBOCS)** caused major hemodynamic changes **ONLY in pigs**, predicting the failure of Baxter's Phase III clinical trial of HemeAssist (1996), showing increased death in trauma patients.

How did C and pigs get involved in 118 years' anaphylaxis research?

- Anaphylaxis-anaphylactic reactions (ana=against, phylaxis= protection)
  - the most severe manifestations of hypersensitivity, better known, allergy
- 1902 Portier and Richet dog experiments
- Tom Hugli (1981) associated anaphylaxis with the liberation of complement (C) activation byproducts (C3a, C4a and C5a) called "anaphylatoxins".
- US Army study (1999) described a role of C activation in the hypersensitivity reactions, also known as infusion reactions, to intravenously administered liposomes in pigs
  - a model for infusion reactions caused by liposomes and other nano-bio pharmaceuticals in humans
  - Complement mediation + No IgE =>

C activation-related pseudo-allergy (CARPA)

The birth of the CARPA concept

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

(*Circulation*. 1999;99:2302-2309.)

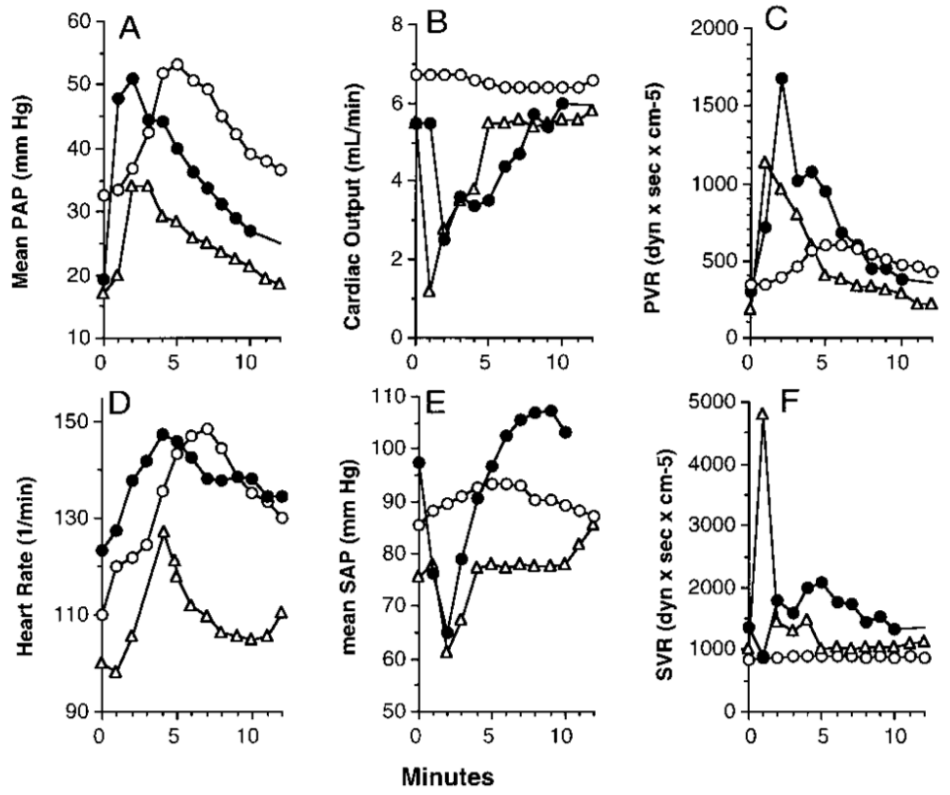


Figure 1. Hemodynamic changes induced by intravenous injection of 5-mg liposome boluses in pigs. Representative curves obtained in 3 pigs. Different symbols designate different pigs.

I.V. injection of tiny amounts of liposomes induced transient, significant hemodynamic changes

- **first treatment (no prior exposure)**
- **milder or absent upon re-exposure**
- **spontaneous resolution**
- **pulmonary infiltration**
- **manifested in 100% of pigs**

The CARPA concept

- A large fraction of acute hypersensitivity (allergic) reactions to I.V. drugs is caused by complement (C) activation, or at least C activation is a key contributor to these reactions.

- Many state-of-art nanomedicines and therapeutic antibodies have heightened risk to cause CARPA

Yearly incidence of CARPA in the USA

≈ 2 million adverse drug reactions (ADRs)

≈ 25 % of all ADRs is immune mediated (0.5 M)

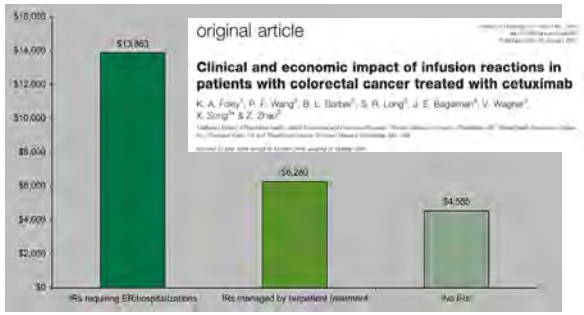
≈ 77% of all immune reactions to drugs are not IgE-mediated (0.4 M)

≈ 25-50% of pseudoallergies are due to C activation (10-200 death)

≈ 0.01-0.1% of ADRs are lethal: 40-400 CARPA death

=> Lethal CARPA is an orphan disease

Drugs causing infusion reactions



economic impact

- Liposomal drugs
- Micellar drugs
- Monoclonal antibodies
- PEGylated nanoparticles
- Contrast media

Doxil, Caelyx (Johnson & Johnson)
Myocet (Elan)
Abelcet (Elan, Enzon)
AmBisome (Gilead, Fujisawa)
Amphotec, Amphocyl (Elan)
DaunoXome (Gilead)
Visudyne (Novartis)
Onivyde (Merrimack Pharmaceuticals)
Vyxeos (Jazz Pharmaceuticals)

Fasturec, Elitec (Sanofi Aventis)
Taxol (Bristol-Myers Squibb)
Cyclosporine injection, USP (Draxis Pharma)
Vumon injection (Bristol- Myers Squibb)
Etoposide (Gensia Sicor Pharmaceuticals)
Taxotere (Sanofi-Aventis)

Pegaspargase	Oncaspar	Enzon
Pegfilgrastim	Neulasta	Amgen
Pegaptanib	Macugen	Eye Tech/Pfizer
Mono-mPEG-epoetin-b	Mircera	Hoffmann-LaRoche
Certolizumab pegol	Cimzia	UCB, Inc., Smyrna
Pegvisomant	Somavert	Pfizer
Pegloticase	Krystexxa	Horizon Pharma
Peginesatide	Omontys//Hematide	Affymax/Takeda
Pegnivacogin + Anivamersen	Revolixys kit	Regado/Tobira

Avastin (Genentech/Roche)
Campath (Genzyme)
Erbitux (Bristol-Myers Squibb, Eli Lilly)
Herceptin (Genentech)
Mylotarg (Pfizer/Wyeth) Pharmaceuticals)
Vectibix (Amgen)
Rituxan (Genentech)

Iatrizoate
Iodixanol
Iohexol
Iopamidol
Iopromide
Iothalamate
Ioversol
Ioxaglate
Ioxilan
SonoVue

Magnevist
Feraheme
Sinerem
Resovist

CARPAgenic factors

C reactogenicity

- Enabling surface
  - size
  - charge
  - assymetry
  - inhomogeneity
  - presence of pathogen-associated molecular patterns (PAMPs)
  - presence high density molecular arrays
- Lack of local control of C activation

Individual hypersensitivity

- Natural proneness for amplified C response
  - Natural antibodies
  - C1q
  - ficolin
  - factor H
  - CRP
  - PIM cells?
- Increased response at the mast cell level
  - costimulation

Regulatorory response to immune toxicity

EMA

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*

2013

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

21 February 2013
EMA/CHMP/806058/2009/Rev. 02
Committee for Human Medicinal Products (CHMP)

Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudoallergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event.

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

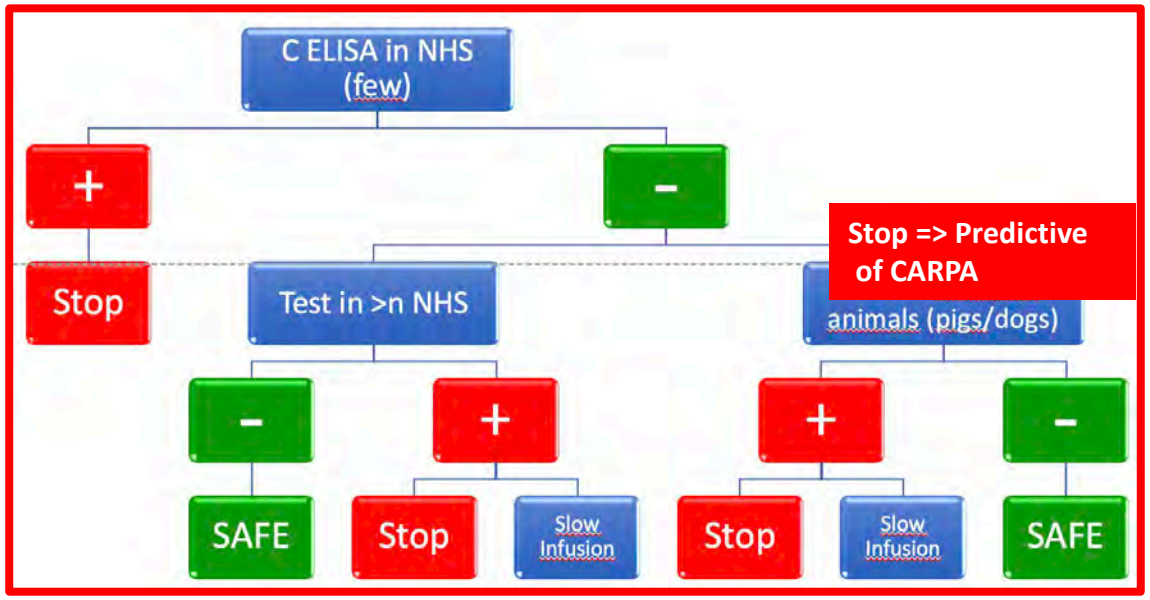
WIREs WILEY

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.”

Testing scheme



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

A glimpse into tabulated information on pseudoallergic drug reactions

Hypersensitivity reactions to marketed liposomal drugs

Szebeni J. Haemocompatibility testing for nanomedicines and biologicals: Predictive assays for complement mediated infusion reactions. Eur. J. Nanomedicine, 4 (1), 33-53

Brand name (manufacturer)	Active ingredient	Indication	Type of particle (Size)	Symptoms
Doxil, Caelyx (Johnson & Johnson)	doxorubicin	ovarian cancer, Kaposi sarcoma, myeloma multiplex	liposomes (80-100 nm)	flushing, shortness of breath, facial swelling, headache, chills, back pain, tightness in the chest or throat, hypotension
Myocet (Elan)				flushing, dyspnea, fever, facial swelling, headache, back pain, chills, tightness in the chest and throat, hypotension
Abelcet (Elan, Enzon)	amphotericin B	fungal infections	solid microparticles (1.6-11 mm)	shortness of breath, change in blood pressure
Ambisome (Gilead, Fujisawa)			liposomes (45-80 nm)	chills, rigors, fever, nausea, vomiting, cardiorespiratory events
Amphotec, Amphocyl (Elan)			disk shape solid nanoparticles (115 nm)	hypotension, tachycardia, bronchospasm, dyspnea, hypoxia, hyperventilation
DaunoXome (Gilead)	daunorubicin	Kaposi sarcoma	liposomes (45 nm)	back pain, flushing, chest tightness
Visudyne (Novartis)	verteporfin	age-related macular degeneration	multilamellar liposomes (multimicron)	chest pain, syncope, sweating, dizziness, rash, dyspnea, flushing, changes in blood pressure and heart rate, back pain

Hypersensitivity reactions to marketed micellar drugs

Brand name (manufacturer)	Active ingredient	Indication	Micelle-forming excipient (size)	Symptoms
Fasturec, Elitec (Sanofi Aventis)	rasburicase	hyperuricemia	Poloxamer-188 (~15 nm)	anaphylaxis, bronchospasm, chest pain, diarrhea, dyspnea, fever, headache, hypotension, nausea, rash, rhinitis, urticaria, vomiting
Taxol (Bristol-Myers Squibb)	paclitaxel	cancer	Cremophor EL® (8-20 nm)	acute respiratory distress, anaphylaxis, angioedema, arrhythmias bronchospasm, chills, dyspnea, facial flushing, fever, flushing of the face and flushing of the upper thorax, hypertension, hypotension, rash, sudden death, tachycardia, urticaria, wheezing
Cyclosporine Injection, USP (Draxis Pharma, Inc.)	cyclosporine	Immuno-suppression		
Vumon Injection (Brystol-Myers Squibb)	teniposide	leukemias		
Etoposide (Gensia Sicor Pharmaceuticals, Inc.)	podophyllotoxin	different cancers	Polysorbate 80 (8-16 nm)	apnea, back pain, bronchospasm, chills, coughing, cyanosis, diaphoresis, dyspnea, fever, flushing, facial swelling, hyper and/or hypotension, laryngospasm, loss of consciousness, rash, tachycardia, tightness in throat, tongue swelling, urticaria
Taxotere (Sanofi-Aventis)	docetaxel			back pain, bronchospasm, chest tightness, chills, dyspnea, erythema, fatal anaphylaxis, fever, flushing, generalized rash, hypotension

Hypersensitivity reactions to some marketed monoclonal antibodies

Szebeni J, Eur. J. Nanomedicine, 4 (1), 33-53, 2012

Brand name (manufacturer)	mAb, type (target antigen)	Indication	Incidence	Symptoms
Anti-cancer use				
Avastin (Genentech/Roche)	bevacizumab, recombinant humanized IgG_1 (VEGF-A)	Combination chemotherapy of metastatic colon, lung, kidney cancer and glioblastoma	< 3%, severe: 0.2%	chest pain, diaphoresis, headache, hypertension, neurologic signs and symptoms, oxygen desaturation, rigors, wheezing
Campath (Genzyme)	alemtuzumab)–IH, recombinant, humanized $IgG_1\kappa$ (CD52 on T and B cells)	B cell chronic lymphocytic leukemia (B-CLL)	4-7%	bronchospasm, chills, dyspnea, emesis, fever, hypotension, nausea, pyrexia, rash, rigors, tachycardia, urticaria
Erbitux (Bristol-Myers Squibb, Eli Lilly)	cetuximab, chimeric $IgG_1\kappa$ (human EGFR)	metastatic colorectal cancer, head and neck cancer, squamous cell carcinomas	< 3%, fatal <0.1%	anaphylaxis, angioedema, bronchospasm, cardiac arrest, chills, dizziness, dyspnea, fever, hoarseness, hypotension, pruritus, rash, rigor, stridor, urticaria, wheezing
Herceptin (Genentech)	trastuzumab , humanized $IgG_1\kappa$ (human EGFR receptor 2, HER2/neu / erbB2)	metastatic breast and gastric cancer	<1%	asthenia, bronchospasm, chills, death within hours, dizziness, dyspnea, further pulmonary complications, headache, hypotension, hypoxia, nausea, pain, rash, severe hypotension, vomiting
Mylotarg (Pfizer Inc./Wyeth Pharmaceuticals)	gemtuzumab ozogamicin. recombinant humanized $IgG_4\kappa$ (CD33 on hematopoetic cells)	CD33 positive acute myeloid leukemia in first relapse	<8%	acute respiratory distress syndrome, anaphylaxis, dyspnea, fatal anaphylaxis, hypotension, pulmonary edema
Vectibix (Amgen)	panitumumab, recombinant humanized $IgG_2\kappa$ (human EGFR)	KRAS+ metastatic colorectal carcinoma	1-4%	anaphylactic reaction, bronchospasm, chills, fever, hypotension
Rituxan (Genentech)	Rituximab, chimeric IgG_1k (CD20 on B cells)	B cell leukemias, rheumatoid arthritis and non-Hodgkin's B-cell lymphoma	>80% severe: <10%	ARDS, bronchospasm, cardiogenic shock, flushing, hypotension, hypoxia, itching, myocardial infarction, pain (at the site of the tumor), pulmonary infiltrates, runny nose, swelling of the tongue or throat, ventricular fibrillation, vomiting
Anti-inflammatory use				
Remicade (Janssen Biotech. Inc.)	Infliximab, chimeric $IgG_1\kappa$ (TNFα)	Crohn's disease, rheumatoid arthritis, spondylitis ankylopoetica, arthritis psoriatica, ulcerative colitis	18%	Bronchospasm, laryngeal edema, pharyngeal edema, dyspnea, hypotension, urticaria, serum sickness-like reactions
Xolair (Genentech)	Omalizumab, recombinant, humanized IgG_4 (IgE)	atopia, asthma	39% Severe: 0.2%	Anaphylaxis, bronchospasm, hypotension, syncope, urticaria, and/or angioedema of the throat or tongue, delayed anaphylaxis (with onset two to 24 hours or even longer) beyond 1 year after beginning regularly administered treatment

FDA-approved PEGylated drugs

Table 1
FDA-approved PEGylated drugs.

Trade names	API	Indication	Producer	Approval	Ref to immunogenicity
Jivi®	60K-PEG recombinant Factor VIII antihemophilic factor	Hemophilia A	Bayer	2018	
Palynziq®	2K-PEG-rhu-Phenylalanine ammonia-lyase, Pegvaliase-pqpz	Phenyl-ketonuria	Biomarin	2018	[14]
Revolixys® kit[a]	40K-PEG-RNA aptamer + reverse agent, a Factor-IXa blocker, Pegnivacogin /Anivamersen	Anti-coagulation	Regado/Tobira	2016	[15]
Adynovate®	20K-PEG-Factor VIII Antihemophilic Factor VIII	Hemophilia A	Baxalta	2015	
Onivyde®	2K-PEG-Liposomal irinotecan hydrochloride trihydrate	Metastatic pancreatic cancer	Ipsen	2015	
Plegridy®	20K-PEG-Interferon beta-1a	Relapsing forms of multiple sclerosis.	Biogen	2014	
Movantic®	<1K-PEG-Naloxegol	Opioid-induced constipation	AstraZeneca	2014	
Omontys®[a]	*40K-PEG-Erythropoietin-mimetic peptide, Peginesatide*	*Anemia associated with chronic kidney disease*	*Affymax/Takeda*	*2012*	[16]
Sylatron™	12K-PEG-Interferon alpha 2b	Melanoma	Merck	2011	
Krystexxa®[a]	*10K-PEG-Uricase, Pegloticase*	*Gout*	*Savient*	*2010*	[17]
Cimzia®	40K-PEG-Certolizumab	Rheumatoid arthritis, Crohn's disease, Axial spondyloarthritis and psoriatic arthritis	Nektar/UCB Pharma	2008	
Mircera®	30K-PEG- erythropoietin (epoetin) beta	Anemia associated with chronic kidney disease	Hoffman-La Roche	2007	[18]
Macugen®	40K-PEG-anti-VEGF aptamer, Pegaptanib	Age-related macular degeneration	Pfizer	2004	[19]
Somavert®	5K-PEG-rhuGH (human growth hormone), Pegvisomant	Acromegaly	Pfizer	2003	
Neulasta®	20K-PEG-Granulocyte colony stimulating factor, Pegfilgrastim	Neutropenia	Amgen	2002	[20]
Pegasys®	40K-PEG-interferon alpha-2	Hepatitis C and B	Hoffmann-La Roche	2001	
PegIntron®	12K-PEG-interferon alfa-2b	Hepatitis C and B	Schering-Plough/Enzon,	2000	
Doxil®/Caelyx®	2K-PEG-Liposomal doxorubicin HCl	Cancer	Alza	1995	[21]
Oncaspar	5K-PEGylated L-asparaginase, Pegaspargase	Acute lymphoblastic leukemia	Enzon	1994	[22]
Adagen®	5K-PEG-adenosine deaminase (bovine), Pegademase	Severe combined immunodeficiency disease (SCID)	Enzon	1990	

[a] Withdrawn from the market.

Anti-PEG antibodies: Properties, formation, testing and role in adverse immune reactions to PEGylated nano-biopharmaceuticals

Gergely Tibor Kozma [a,b], Taro Shimizu [c], Tatsuhiro Ishida [c], Janos Szebeni [a,b,d,*]

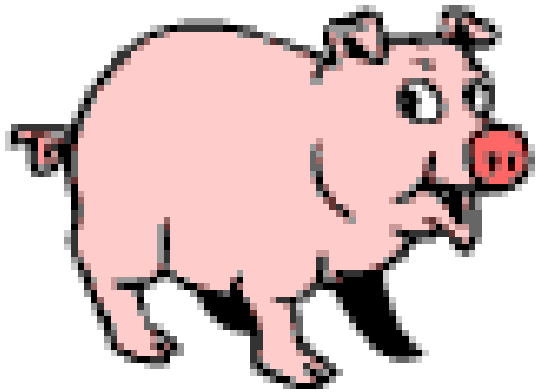
Advanced Drug Delivery Reviews 154–155 (2020) 163–175

Animal models of CARPA

Animal species	Sensitivity to HSR	Advantages	Disadvantages
Mouse	Low	Simple and relatively cheap	Insensitive; not generally accepted for preclinical safety studies
Rat	Low	Simple and relatively cheap; generally accepted for preclinical safety studies	Insensitive
Rabbit	Medium-to-high	Simple and relatively cheap; generally accepted for pyrogen screening	Unknown relevance to IRs in human patients except for cytokine release in response to pyrogens
Pig	High	Reproduces clinical symptoms of human patients; consistent response between individual animals	Skills- and labour-intensive; not generally accepted for preclinical safety studies
Minipig	High	Reproduces clinical symptoms of human patients; consistent response between individual animals	Skills- and labour-intensive; not generally accepted for preclinical safety studies
Dog	High	Reproduces clinical symptoms of human patients; generally accepted for preclinical safety studies	High interanimal variability; expensive; ethical and logistic hurdles
Non-human primate	Medium-to-high	Reproduces clinical symptoms of human patients; generally accepted for preclinical safety studies	Expensive, ethical and logistic hurdles

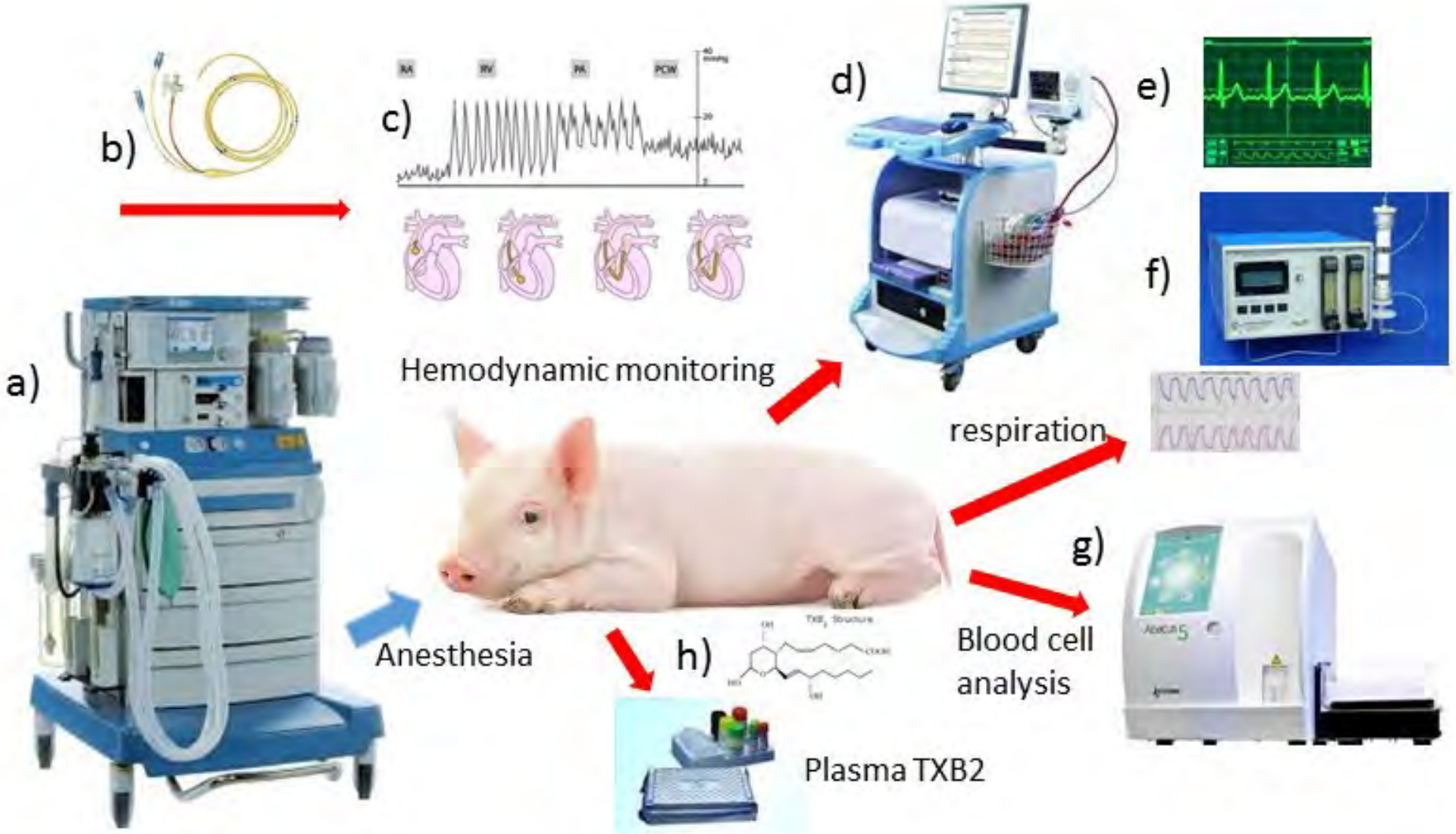
Comparison of haemodynamic and other manifestations of HSRs in animal models. The summary is based on ref. [61].

Szebeni et al, ***Nat Nanotechnol*** 2018, *13*, 1100



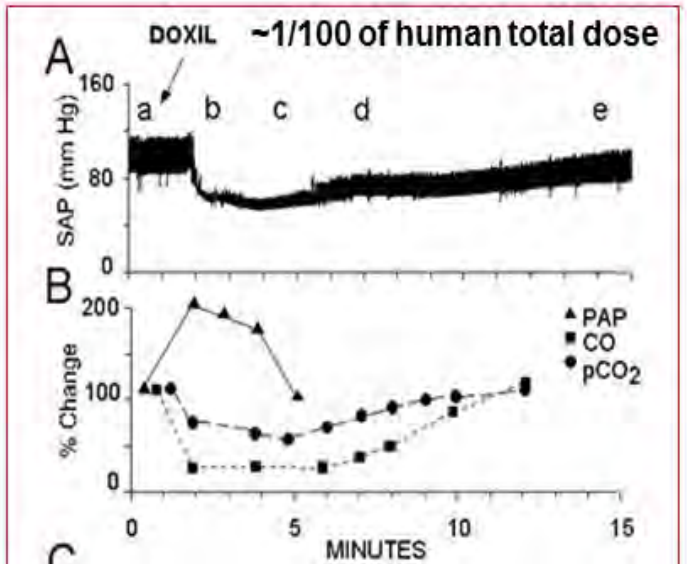
The porcine CARPA model

Setup: Instrumentation for hemodynamic, hematological and biochemical monitoring



Szebeni J et al., A porcine model of complement-mediated infusion reaction to drug carrier nanosystems and other medicines ***Adv Drug Deliv Rev. 2012;64:1406-1416***

Symptoms of porcine CARPA



Hemodynamic alterations

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

Cardiac abnormalities

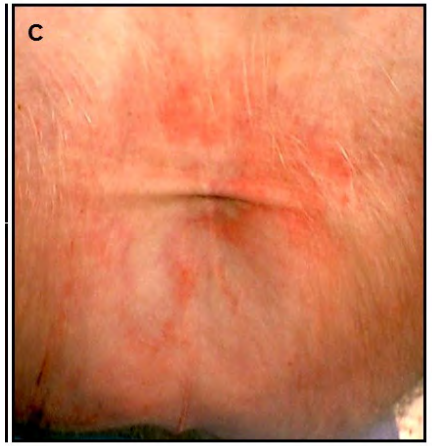
- tachycardia, bradycardia, arrhythmias
- ventricular fibrillation, arrest

Skin reaction

- erythema,
- rash

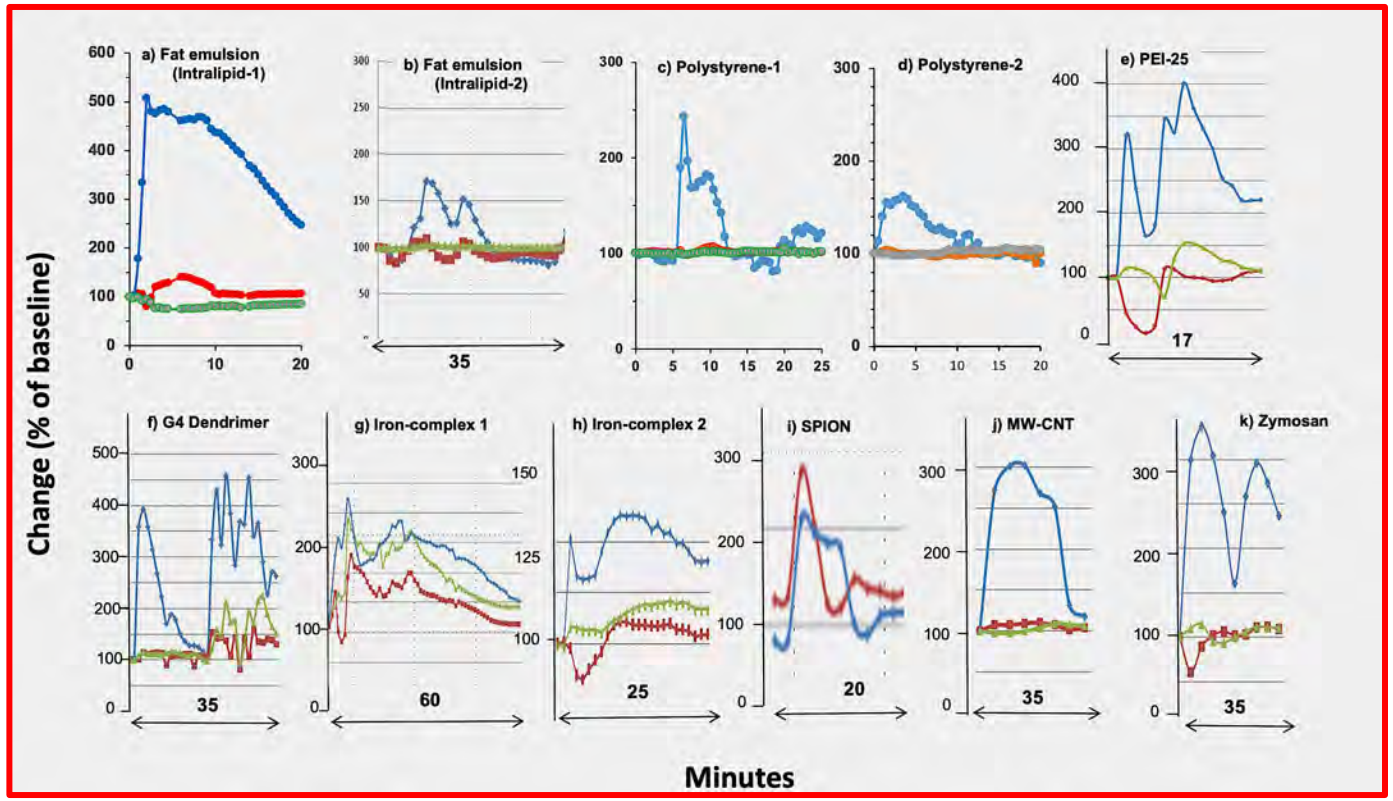
Blood abnormalities

- Leukocytosis
- leukopenia
- thrombocytosis
- thrombopenia



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



Rudolf Urbanics, Péter Bedőcs and János Szebeni*

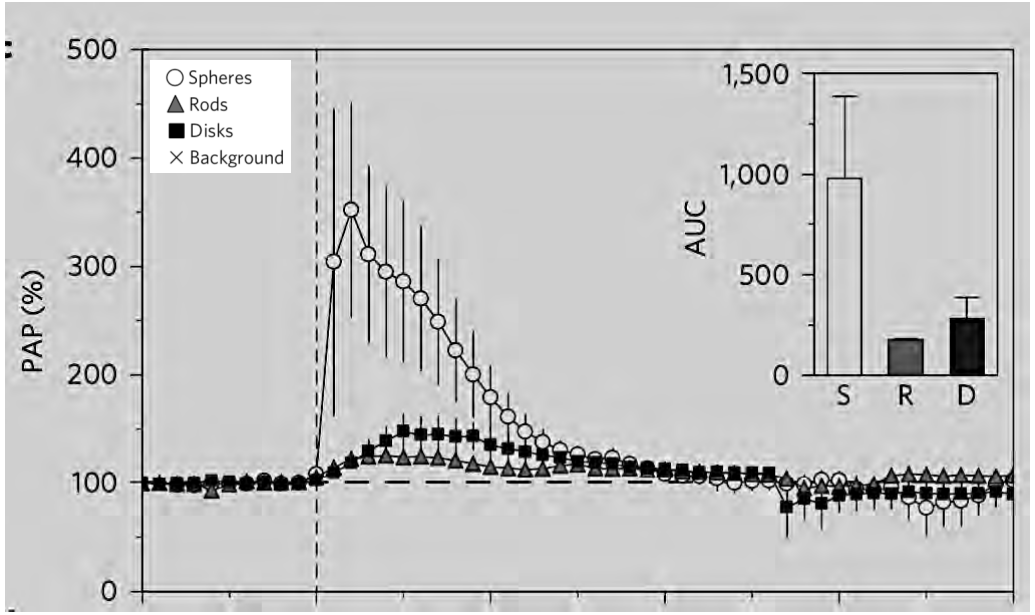
Lessons learned from the porcine CARPA model: constant and variable responses to different nanomedicines and administration protocols

Eur. J. Nanomed. 2015; 7(3): 219–231

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

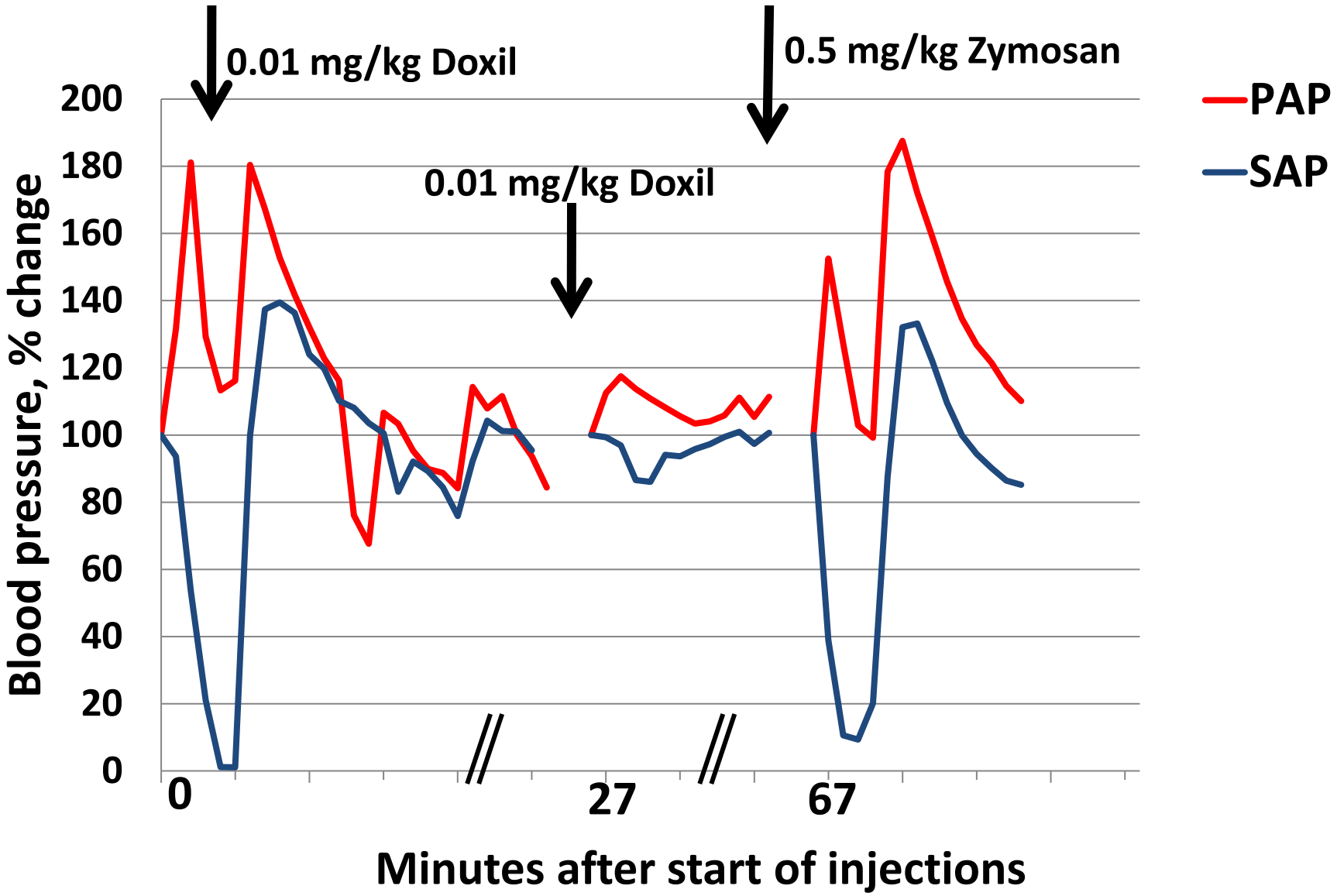
Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes

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]*

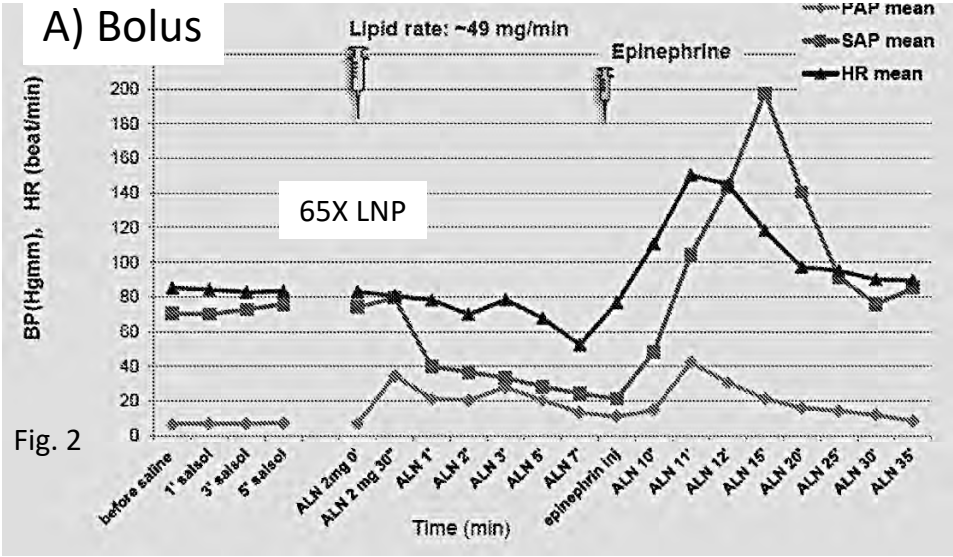


Nat Nanotechnol **2017**, *12* (6), 589-594.

Doxil causes self-tolerance via tachyphylaxis

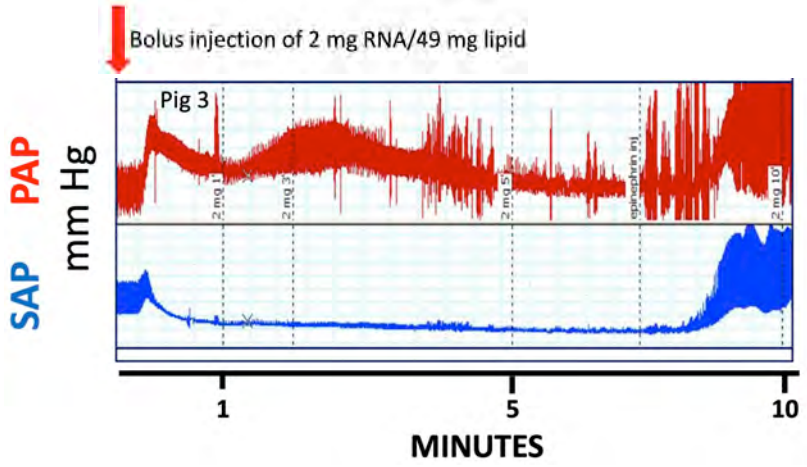


Anaphylactoid reactions to LNPs in pigs

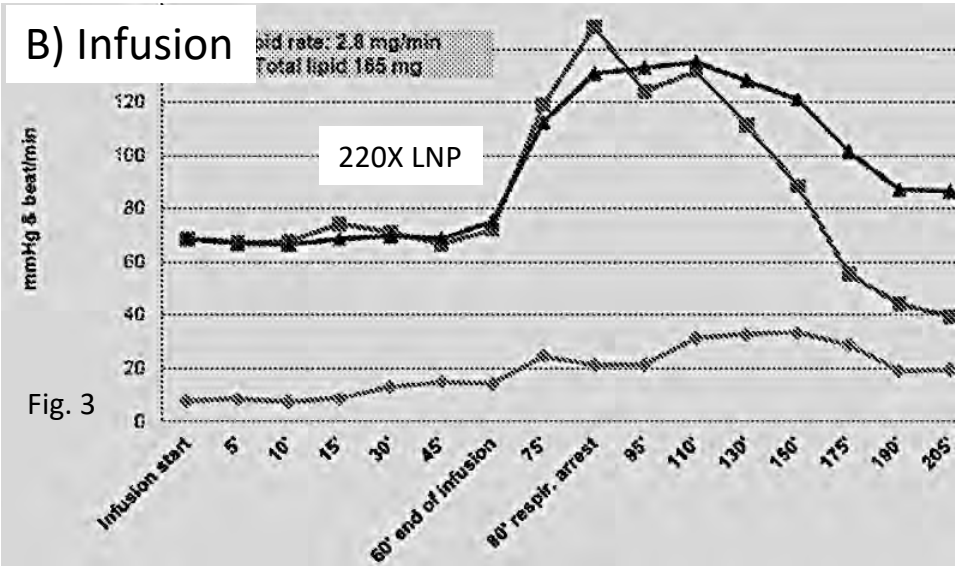


Hypertension + tachycardia

7 min after bolus lasting ~ 25 min (1 of 3)



Pulmonary hypertension + systemic hypotension + shock (2 of 3)



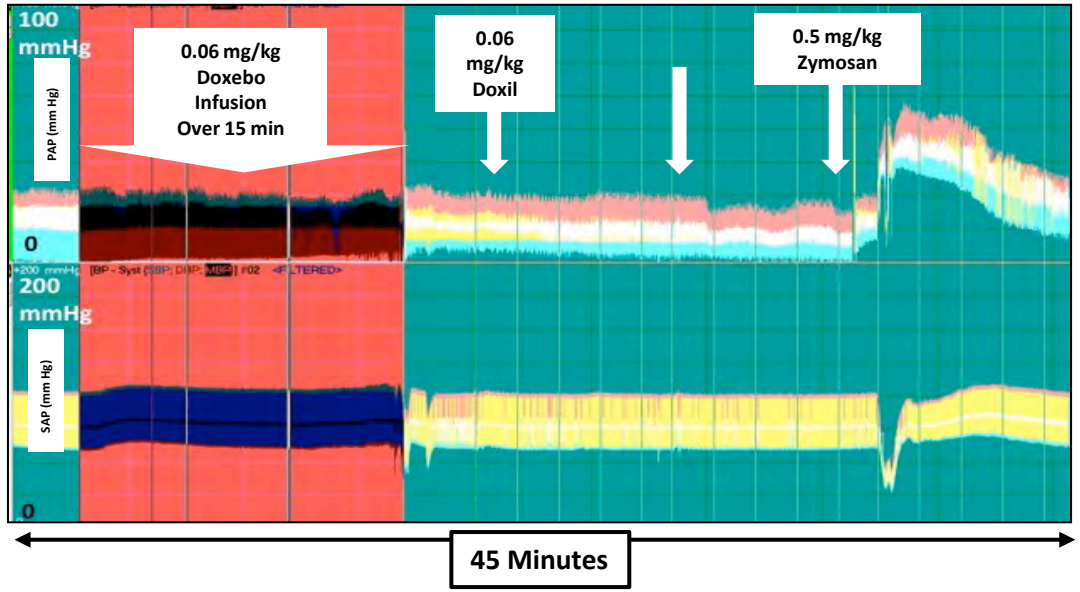
Immediately after 1 h infusion lasting for 2 h

Lipid content of LNP = ~0.75 mg

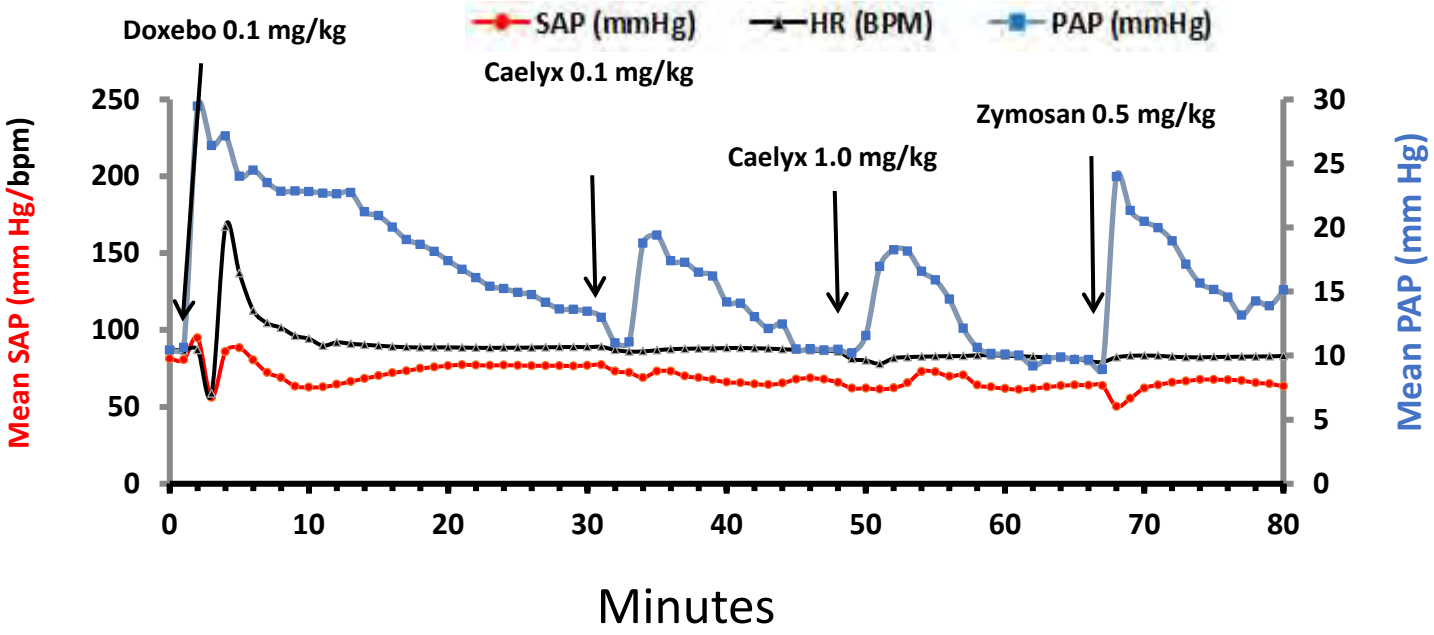
1) Iv-administered LNPs can caused major hemodynamic changes in pigs typical in anaphylaxis
2) Doses are >> higher than vaccine, reaction needs dose-titration

Reactogenicity of Doxebo and Doxil in naive and immunized pigs

Day 0, Naive pigs



Day 7-42, immunized pigs



Use of pigs for immunogenicity assessment

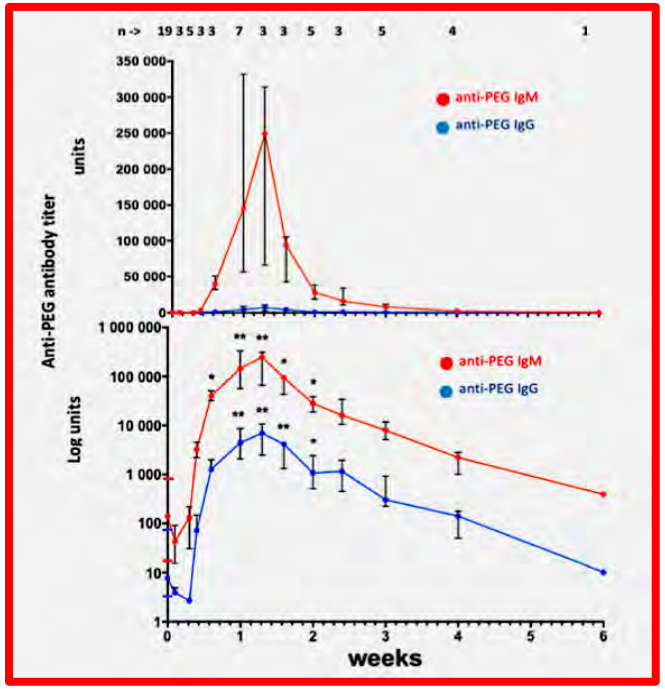
ACSNANO

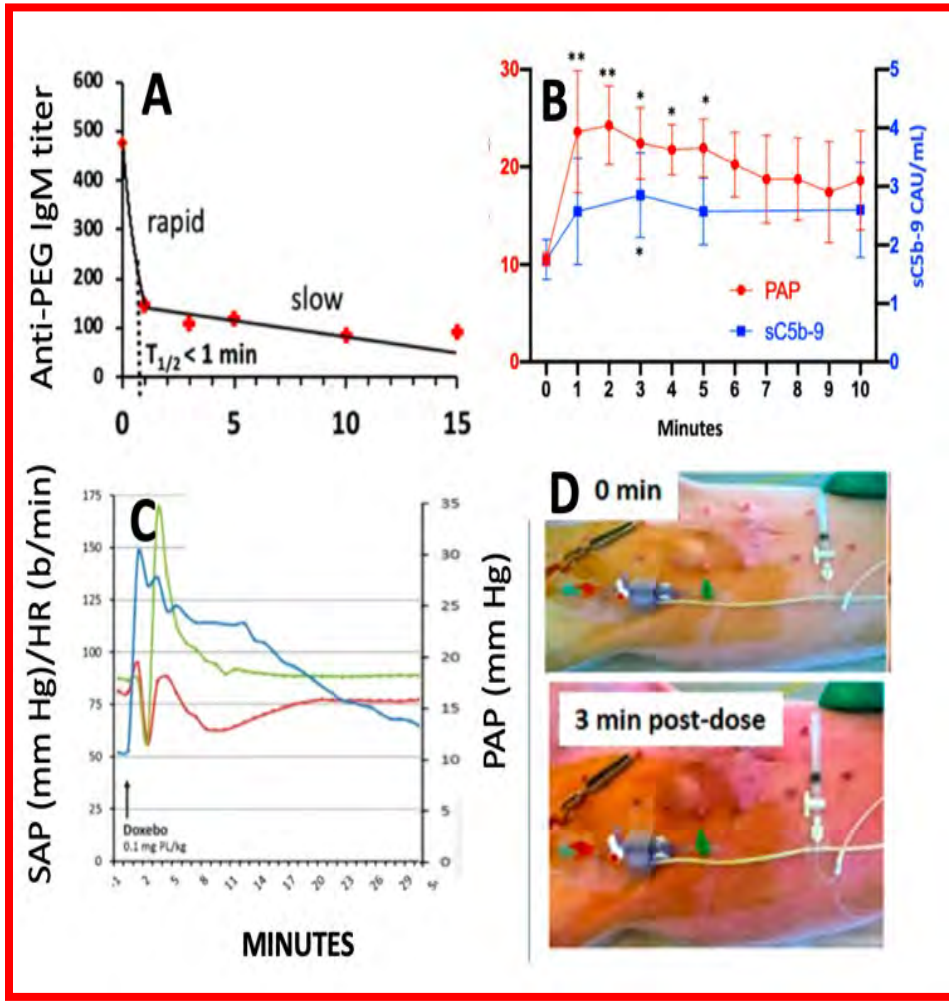
Pseudo-anaphylaxis to Polyethylene Glycol (PEG)-Coated Liposomes: Roles of Anti-PEG IgM and Complement Activation in a Porcine Model of Human Infusion Reactions

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


Journal of Controlled Release 309 (2019) 333–338

Contents lists available at ScienceDirect

ELSEVIER

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

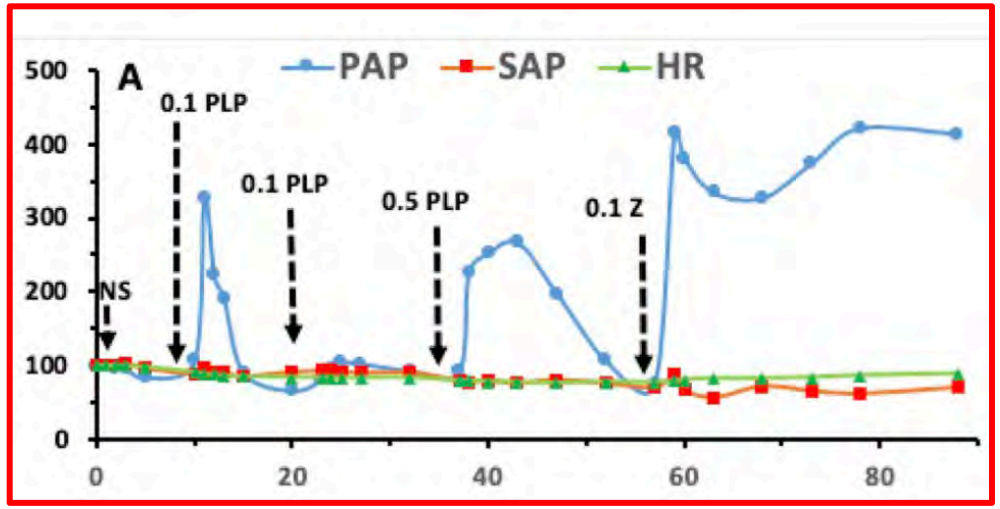
J Control Release **2019,** *309*, 333-338.

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

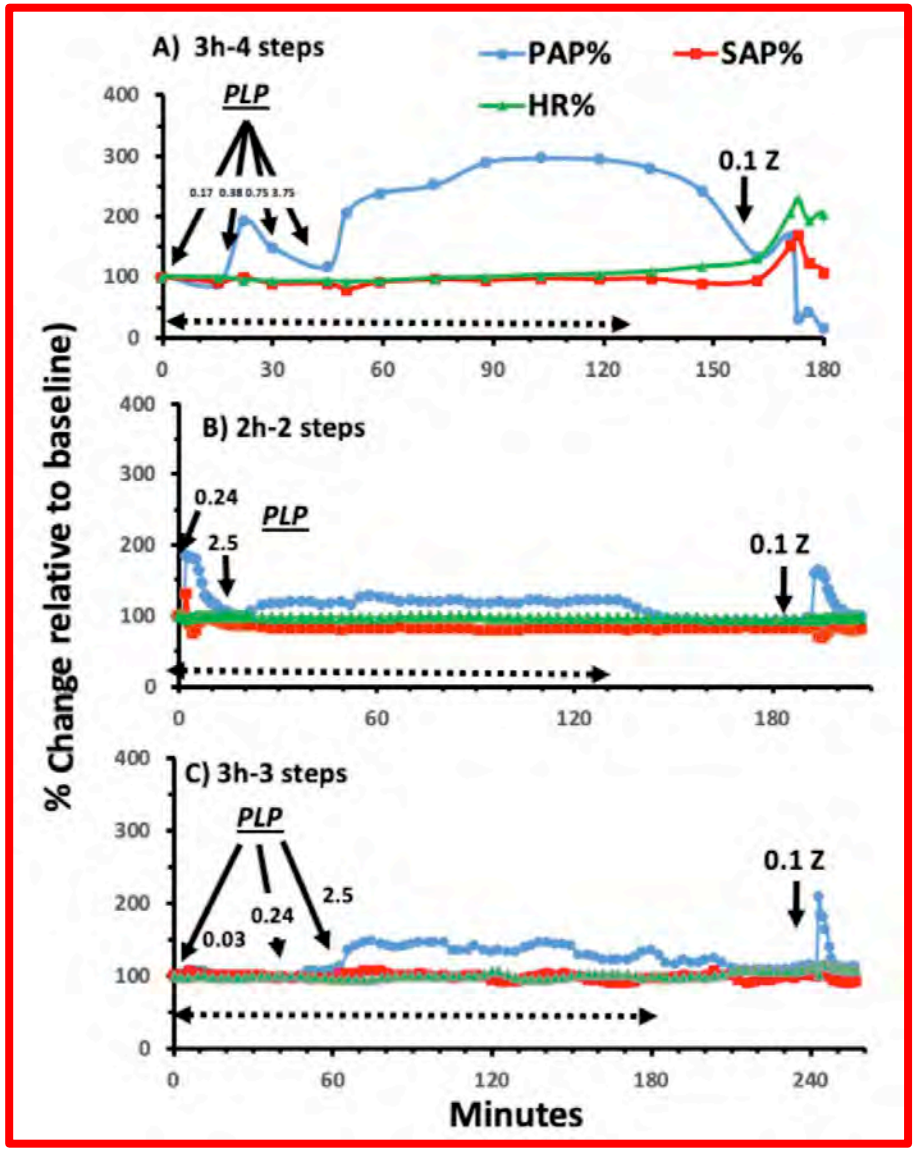
Tamás Fülöp[a,1], Gergely T. Kozma[a,b,1], Ildikó Vashegyi[b,1], Tamás Mészáros[a,b], László Rosivall[c], Rudolf Urbanics[a,b], Gert Storm[d,e], Josbert M. Metselaar[d,f,*,1], János Szebeni[a,b,g,*,1]

PLP = Pegylated liposomal prednisolone phosphate

PLP Bolus



PLP Infusion



(54) Title: DOSAGES AND METHODS FOR DELIVERING LIPID FORMULATED NUCLEIC ACID MOLECULES

PCT/US2014/036915
Alnylam Pharm Inc.
on safe administration of LNPs

Uses the porcine "CARPA" model

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, ...

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 November 2014 (13.11.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/182661 A2

(51) International Patent Classification:
A61K 48/00 (2006.01) *A61K 9/10* (2006.01)

(21) International Application Number:
PCT/US20 14/0369 15

(22) International Filing Date:
6 May 2014 (06.05.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/820,036 6 May 20 13 (06.05.2013) US

(71) Applicant: ALNYLAM PHARMACEUTICALS, INC [US/US]; 300 Third Street, Cambridge, MA 02142 (US).

(72) Inventors: KASPERKOVITZ, Pia; 300 Third Street, Cambridge, MA 02142 (US). GOLLOB, Jared; 300 Third Street, Cambridge, MA 02142 (US).

(74) Agent: LU, Shihua; Lando & Anastasi LLP, Riverfront Office Park, One Main Street, Suite 1100, Cambridge, MA 02142 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(H))*

— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(in))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

Chronological list of pig studies which included the analysis of hemodynamic changes and other endpoints of hypersensitivity reactions to i.v. drugs. (I)

Year	Tested Drugs/Agents *	Major Findings on HSR **	Ref.
1989	Cholesterol-containing liposomes used for immunizing pigs against hypercholesterolemia-induced arteriosclerosis	Liposomes caused major cardiopulmonary distress and TXA2 release via anti-cholesterol antibody-mediated C activation in pig blood.	[21]
1992	Albunex microspheres used as ultrasound contrast agents	HSR involves TXA2-mediated pulmonary hypertension in pigs.	[22]
1994		NPs are cleared mainly by pulmonary intravascular macrophages (PIMs) in the lung of pigs.	[23]
1999	Liposome-encapsulated hemoglobin used for blood substitution and control liposomes	Hemodynamic changes are due to C activation with subsequent secretion of TXA2. PAP is dose-dependent, highly reproducible endpoint of HSRs.	[15]
2000	Different liposomes applied to dissect the structural factors contributing to pulmonary hypertension in pigs	Vesicle size, lamellarity, charge and infusion speed are all critical determinants of the rise of PAP.	[24]
	PEGylated liposomal doxorubicin (Doxil/Caelyx), a reactogenic anticancer drug	Doxil activates C in vitro and its dose-dependent hemodynamic effects in pigs mimic the human HSRs to this drug.	[25]
2005	Negatively charged multilamellar vesicles applied as a model for reactogenic liposomes	The hemodynamic disturbance during HSRs is also manifested in cerebrovascular changes, explaining the psychic symptoms of HSRs.	[26,27]
2006		The symptoms of HSRs reproduce those of cardiac anaphylaxis. The reaction can be reproduced only partially with injection of C5a.	[28]
2008	Oversulfated chondroitin sulfate (OCS), a contaminant of heparin that caused a US nationwide outbreak of severe adverse reactions during 2007–2008	OCS induced contact and complement system activation and cardiopulmonary distress only in pigs but not in other species, mimicking the human symptoms of severe heparin reactions.	[29]
2010	Liposomal bisphosphonates (LBPs) developed for the prevention of myocardial infarction via macrophage inhibition	LBPs triggered no or minor HSRs in pigs, which correlated with their C activating capability in vitro.	[30]
2011	PEI-PEG block-copolymers used as models for polymeric drug carrier nanosystems	25K-PEI activated C in vitro and caused HSRs in pigs; its PEGylation decreased, but did not eliminate these effects.	[31]
2012	PEGylated liposomal doxorubicin (Doxil, Caelyx) and liposomal amphotericin-B (AmBisome), both are reactogenic in patients	Doxil and AmBisome activated C in vitro and caused proportional HSRs in pigs. The effect of Doxil, but not of AmBisome, was tachyphylactic.	[32,33]
	Hemoglobin vesicles (HbVs) used as an oxygen carrier blood substitute	By optimizing the lipid composition of HbVs both C activation and the HSR of pigs could be attenuated. The reaction was tachyphylactic.	[34]

J. Szebeni and R. Bawa: Human Clinical Relevance of the Porcine Model of Pseudoallergic Infusion Reactions ***Biomedicines*** 2020, *8*, 82

biomedicines

MDPI

Chronological list of pig studies which included the analysis of hemodynamic changes and other endpoints of hypersensitivity reactions to i.v. drugs. (II)

Year	Tested Drugs/Agents *	Major Findings on HSR **	Ref.
2014	TRO40303, a cardioprotective sterane compound, inhibitor of transitional permeability pores in mitochondria	Consistent with the safety and tolerance in a phase I trial, TRO40303 did not activate C and caused HSR in pigs.	[35]
	Intralipid, used for reversing the symptoms of local anesthetic overdose	Intralipid caused major HSR in pigs, although C activation could not be detected in pig blood in vitro.	[36]
	Inclisiran, a siRNA-containing LNP formulation inhibiting PCSK9 protein to reduce plasma LDL	A stepwise micro-dosing protocol is reaction-free in pigs, suggesting safety in patients.	[37]
2016–2018	Known reactogenic nanomedicines (Doxil, AmBisome, Cremophor EL)	The hemodynamic derangement and other changes caused by reactogenic nanomedicines were similar in pigs and Göttingen miniature pigs.	[38]
	Nano-systems intended for cardiovascular applications (liposomes, LNPs, polymeric and iron oxide NPs)	The non-reactive NPs were suggested to have the least risk for HSRs in man.	[39]
	Nitroglycerin encapsulated in 1,3-diamidophospholipid-containing, shear-responsive liposomes developed to alleviate coronary vasoconstriction	Despite irregular size, these NPs did not activate C and were not reactogenic in pigs.	[40,41]
	A new type of superparamagnetic iron oxide NPs (SPIONdex) used as MRI contrast agents	C activation and HSR in pigs can be eliminated by reducing the size of SPIONdex NPs.	[42,43]
	Polystyrene NPs (PS-NPs) used as a model for reactogenic drug delivery nano-systems	PS-NP-induced cardiopulmonary distress depends on the shape of particles, spheres being more reactogenic than rods or disks. C activation was not measurable in pig whole blood.	[44]
		Spherical PS-NP-induced cardiopulmonary distress in pigs showed significant correlation with C activation in human serum. PS-NPs were opsonized in pig serum by C3 derivatives, indicating C activation.	[45]
	Hemostatic NPs based on PEG-PLGA-PLL-PEG-cRGD copolymers, developed to control traumatic blood loss	In a porcine liver injury model, these NPs led to massive vasodilation and exsanguination due to CARPA. This adverse effect could be attenuated by tailoring the zeta potential of NPs.	[46]

J. Szebeni and R. Bawa: Human Clinical Relevance of the Porcine Model of Pseudoallergic Infusion Reactions ***Biomedicines*** 2020, *8*, 82

biomedicines

MDPI

Chronological list of pig studies which included the analysis of hemodynamic changes and other endpoints of hypersensitivity reactions to i.v. drugs. (III)

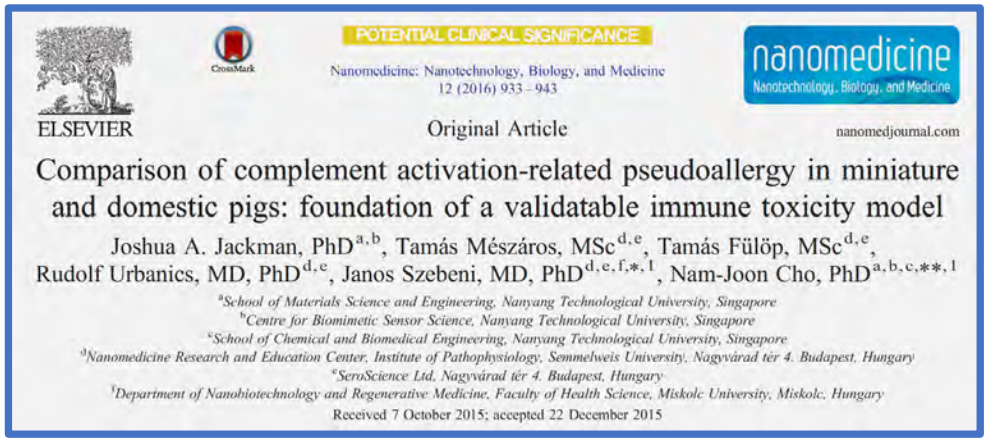
Year	Tested Drugs/Agents *	Major Findings on HSR **	Ref.
2019	Doxil and placebo Doxil (Doxebo) used to clarify the mechanism of HSRs to Doxil and other PEGylated NPs	C activation and the HSR caused by Doxil was greatly amplified in Doxebo-immunized animals in which the anti-PEG IgM levels were increased. This provides evidence for the causal role of classical pathway C activation in Doxil reactions.	[47]
	Liposomal cortisol phosphate developed against chronic inflammatory diseases	Consistent with the human practice, slow, stepwise infusion with micro-dosing minimizes the risk for HSRs.	[48]
	TC^{99m}-Fucoidan, a sulfated fucose-rich polysaccharide developed for the detection of P-selectin expression in cardiovascular diseases	The drug did not cause C activation or HSR in pigs, suggesting safety for human use for the imaging of activated endothelium.	[49]

* Trade names and abbreviations: AmBisome, liposomal amphotericin-B; C, complement; Doxil, PEGylated liposomal doxorubicin; HSR, hypersensitivity reaction; HbVs, hemoglobin vesicles; LEH, liposome-encapsulated hemoglobin; LNPs, lipid nanoparticles; LDL, low density lipoprotein; NPs, nanoparticles; OCS, oversulfated chondroitin sulfate; PEI, polyethylene-imine; PEG-PLGA-PLL-PEG-cRGD, cyclic peptide (arginine-glycine-aspartic-glutamic-valine acid, cRGD)-modified monomethoxy (polyethylene glycol)-poly (D,L-lactide-*co*-glycolide)-poly (L-lysine) nanoparticles; siRNA, small inhibitory ribonucleic acid; PS-NPs, polystyrene NPs; SPIONs, superparamagnetic iron oxide nanoparticles; TRO40303, 3,5-seco-4-nor-cholestan-5-one oxime-3-ol-containing liposomes; PCSK9, proprotein convertase subtilisin/kexin type 9 (LDL uptake blocker). ** Conclusions on hemodynamic, TXA2, and other physiological changes observed in response to i.v. administration of test agents.

J. Szebeni and R. Bawa: Human Clinical Relevance of the Porcine Model of Pseudoallergic Infusion Reactions ***Biomedicines*** 2020, *8*, 82

biomedicines MDPI

CARPA in minipigs


POTENTIAL CLINICAL SIGNIFICANCE

Nanomedicine: Nanotechnology, Biology, and Medicine
12 (2016) 933–943

ELSEVIER

nanomedicine
Nanotechnology, Biology, and Medicine

Original Article

nanomedjournal.com

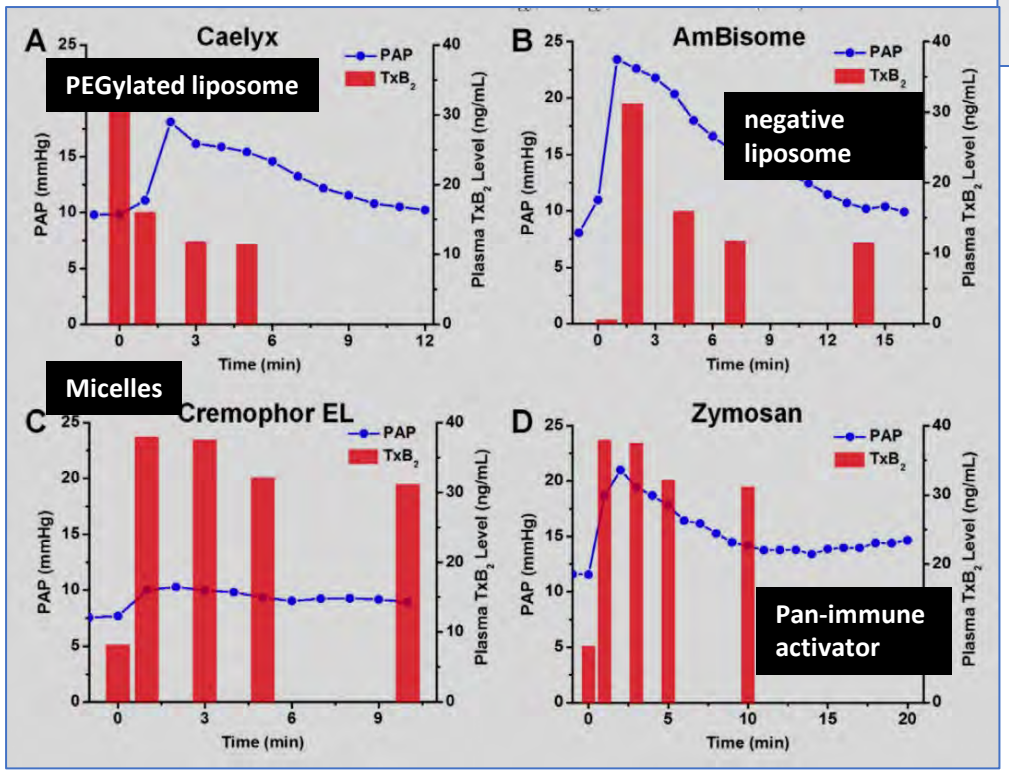
Comparison of complement activation-related pseudoallergy in miniature and domestic pigs: foundation of a validatable immune toxicity model

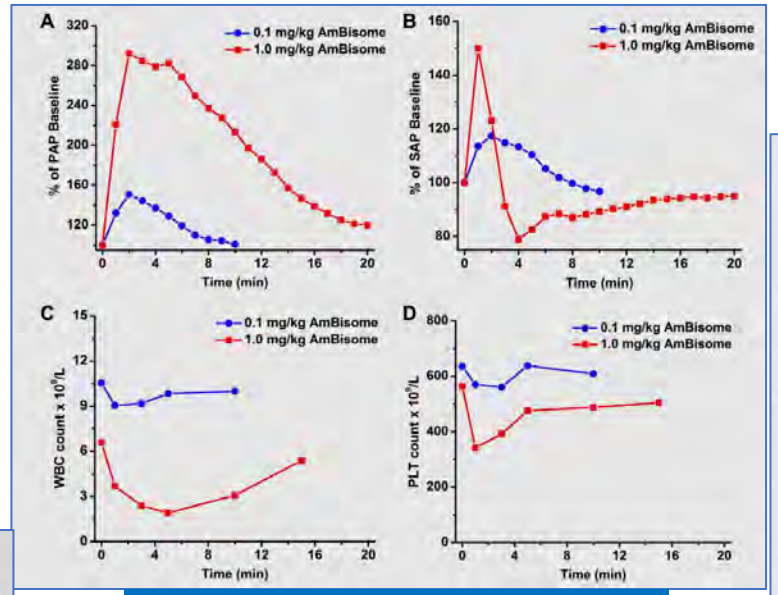
Joshua A. Jackman, PhD[a,b], Tamás Mészáros, MSc[d,e], Tamás Fülöp, MSc[d,e], Rudolf Urbanics, MD, PhD[d,e], Janos Szebeni, MD, PhD[d,e,f,*,1], Nam-Joon Cho, PhD[a,b,c,**,1]

[a]School of Materials Science and Engineering, Nanyang Technological University, Singapore
[b]Centre for Biomimetic Sensor Science, Nanyang Technological University, Singapore
[c]School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore
[d]Nanomedicine Research and Education Center, Institute of Pathophysiology, Semmelweis University, Nagyvárad tér 4. Budapest, Hungary
[e]SeroScience Ltd, Nagyvárad tér 4. Budapest, Hungary
[f]Department of Nanobiotechnology and Regenerative Medicine, Faculty of Health Science, Miskolc University, Miskolc, Hungary

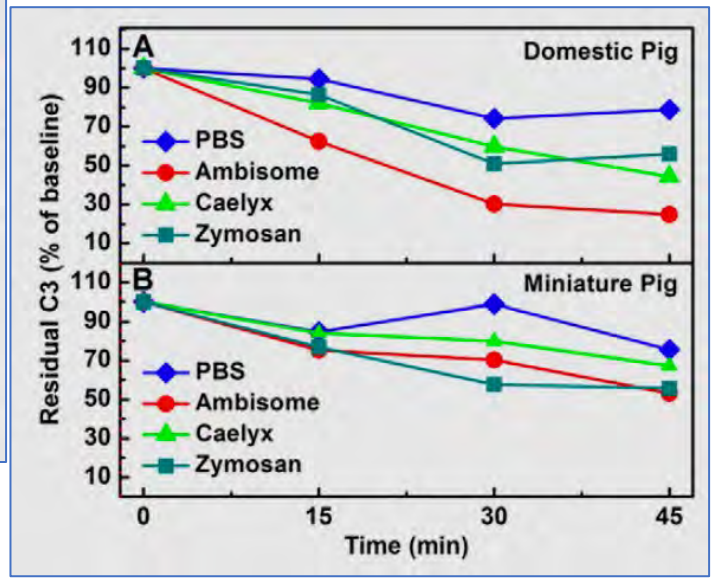
Received 7 October 2015; accepted 22 December 2015

Pulmonary hypertension
Thromboxane release

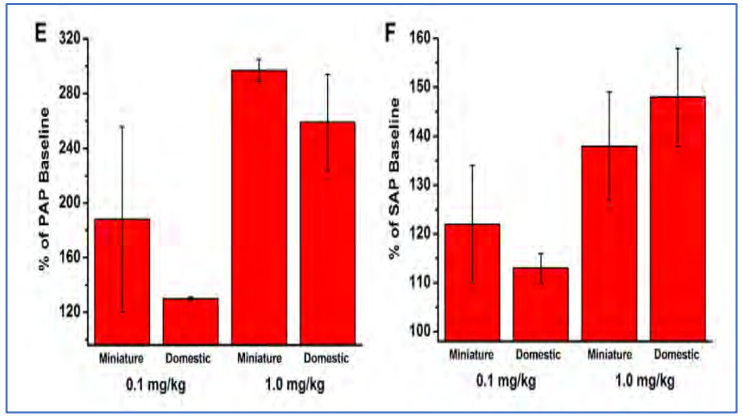




Dose dependence
- ***Pulmonary hypertension***
- ***Systemic hypotension***
- ***Leukopenia***
- ***Thrombocytopenia***



In vitro C consumption

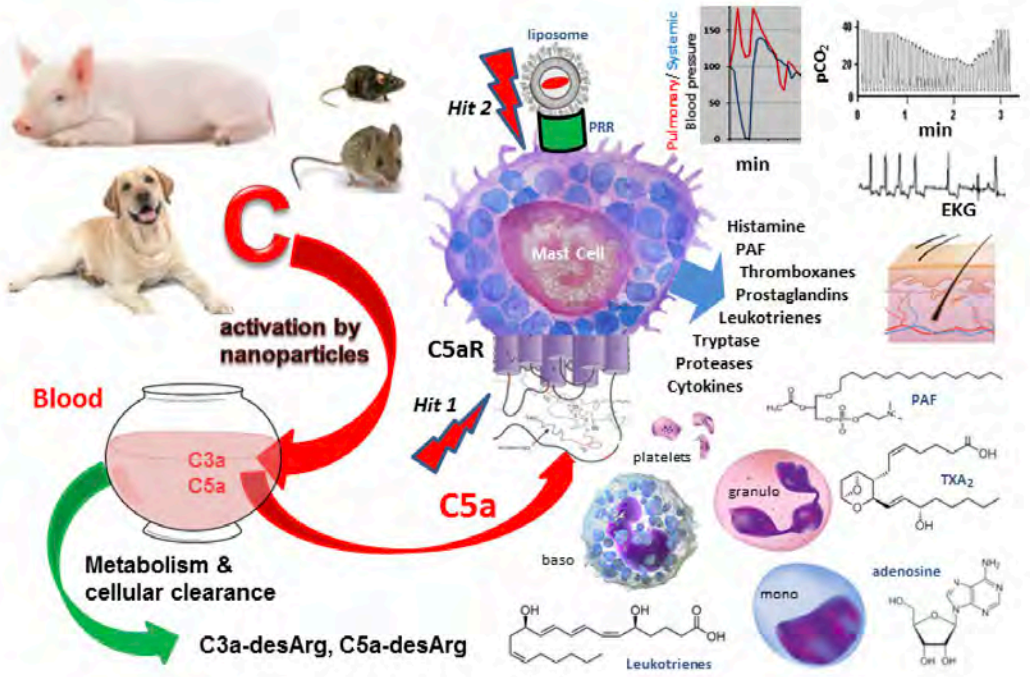


Dose dependence
- ***Pulmonary hypertension***
- ***Systemic hypotension***
- ***In domestic and miniature pigs***

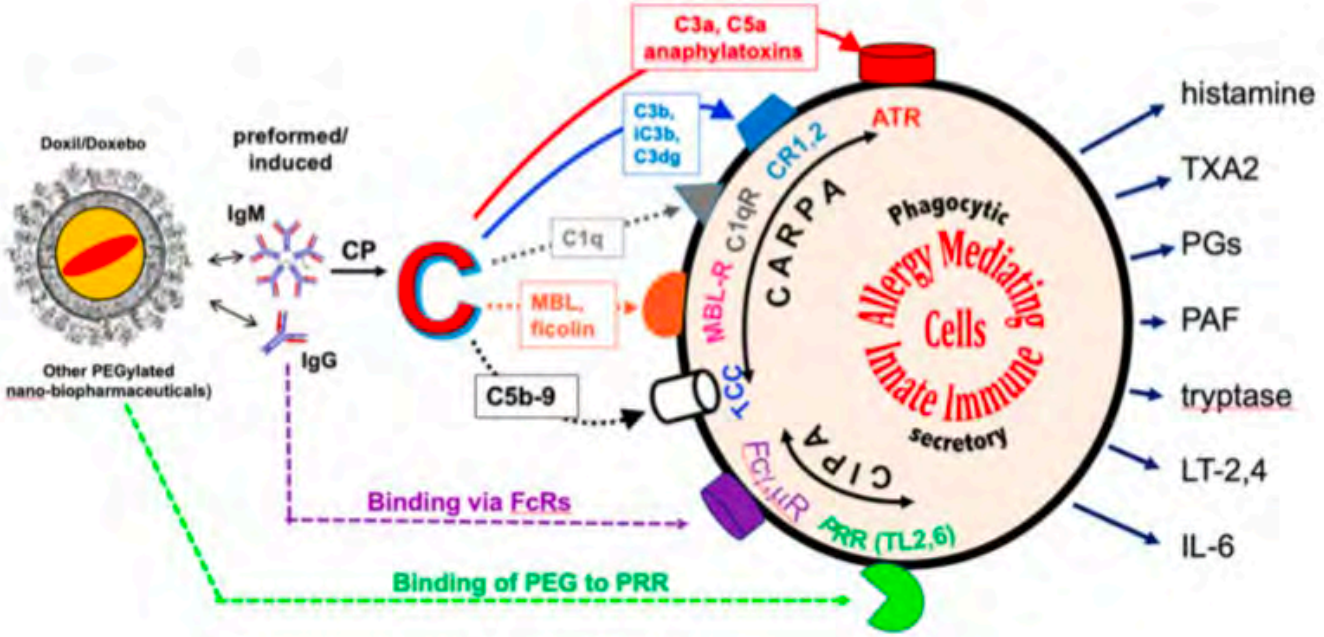
Advantages of adaptation of the CARPA model to minipigs

- **Standardizable gene and environmental variables**
- **Long-term studies avoiding weight gain**
- **Studies on old animals**
- **Regulatory-standard studies**

Mechanism



Anaphylatoxin buildup reaching release reaction threshold



Simultaneous engagements of AT and PR receptors

Double/multiple hit

Is the increased rate of rare anaphylaxis to mRNA vaccines explainable with CARPA?

The NEW ENGLAND JOURNAL of MEDICINE

REVIEW ARTICLE

Dan L. Longo, M.D., *Editor*

Maintaining Safety with SARS-CoV-2 Vaccines

Mariana C. Castells, M.D., Ph.D., and Elizabeth J. Phillips, M.D.

From Brigham and Women's Hospital, Boston (M.C.C.); and the Department of Medicine, Vanderbilt University Medical Center, Nashville (E.J.P.).

This article was published on December 30, 2020, at NEJM.org.

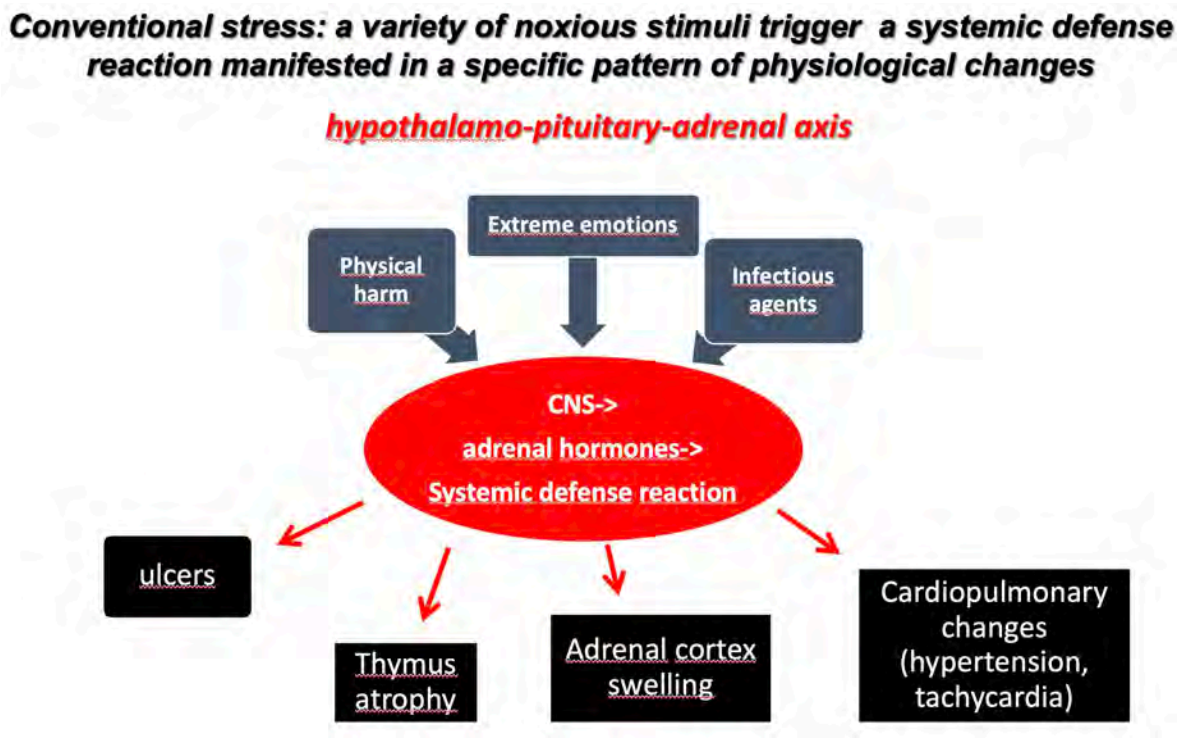
DOI: 10.1056/NEJMra2035343

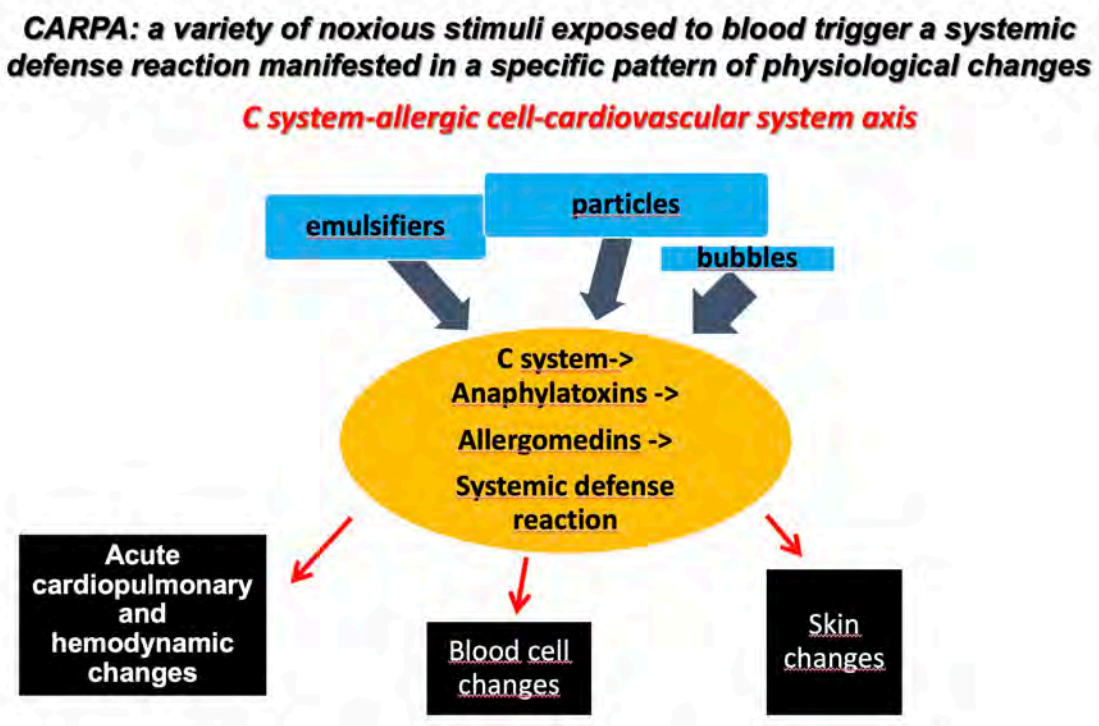
reported in the United States after vaccination of almost 2 million health care workers, and the incidence of anaphylaxis associated with the Pfizer SARS-CoV-2 mRNA vaccine appears to be approximately 10 times as high as the incidence reported with all previous vaccines, at approximately 1 in 100,000, as compared 1 in 1,000,000, the known and stable incidence of anaphylaxis associated with other vaccines. The EUA for the Moderna mRNA vaccine was issued on December 18,

Spectrum and kinetics of symptoms resemble CARPA

Is CARPA a stress reaction in blood?

Similarity between conventional stress and CARPA





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

Conclusions

- **Infusion reactions remain an unsolved problem for many therapeutic or diagnostic nanomedicines.**
- **Current erxperimental evidence supports the causal role of C activation.**
- **The porcine immune toxicity model is uniquely applicable for preclinical evaluation of the risk of acute hypersensitivty and long-term immunogenicity of NP-based drugs and agents.**
- **The model, complemented with in vitro C assays, enables the prediction of CARPA and elaboration of safe administration protocols**
- **Adaptation of the model to minipigs would expnad the utility of the model, in particular for regulatory purposoes.**

Budapest, Hungary

Contact: www.SeroScience.com

The ”nanomedicine” team at Semmelweis University & SeroScience

Acknowledgments

Bakos, T.
Csukás, D.
Dézsi, L.
Fülöp, T.
Kozma, G.
Mészáros, T.
Milosevits, G.
Őrfi, E.
Rosivall, L.
Szebeni, J.
Szénási, G.
Urbanics, R.
Velkei, M.