Accepted manuscript

Roadmap and Strategy for Overcoming Infusion Reactions to Nanomedicines

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Running Title: Infusion reactions to nanomedicines

Keywords: nanoparticles, infusion reactions, complement, macrophages, CARPA, anapylaxis, cytokine storm, allergy

Length of the text: 3,525 words

Length of the abstract: 151 words

Length of legends: 251 words

The number of references: 59

The number of Figures: 1 (the figure has two parts A and B)

The number of Tables: 4

Estimated final size of figures: one column

Estimated final size of tables: two pages

Abstract

Infusion reactions (IRs) are complex, immune-mediated side effects that mainly occur within minutes to hours of receiving a therapeutic dose of intravenously administered pharmaceutical products. These products are diverse and include both traditional (e.g., biologics and small molecules) and novel (e.g., nanotechnology-based) pharmaceuticals. Although IRs are not unique to nanomedicines, they represent a significant hurdle for the translation of nanotechnology-based drug products. This Perspective provides a big picture of the pharmaceutical field in general and nanomedicine and examines current understanding of mechanisms responsible for IRs to nanomedicines. We outline outstanding questions regarding IRs to nanomedicines, review currently available experimental evidence to provide some answers, and highlight the gaps. We review advantages and limitations of the in vitro tests and animal models presently employed for studying IRs to nanomedicines. Finally, we propose a roadmap to improve the current understanding of IRs to nanomedicines, and we recommend a strategy for overcoming the problem.

Immune-mediated adverse effects may occur in patients after a pharmaceutical product is administered to treat or diagnose a disease[1, 2, 3, 4]. When such reactions occur minutes to hours after systemic, intravenous product administration, they are often called infusion reactions (IRs), hypersensitivity, or anaphylaxis. However, there is no uniform terminology, and the use of these terms largely depends on the field of study (e.g., clinical oncology vs. immunology). The World Allergy Organization classifies hypersensitivity reactions (HSRs) as immediate (occurring within minutes to hours of

exposure) and delayed (requiring days before clinical manifestation)[2, 3]. According to the World Allergy Organization Anaphylaxis Guideline, the term "hypersensitivity" refers to reproducible symptoms occurring as the result of exposure to a defined stimulus; "allergy" is defined as an HSR initiated by a specific immunological mechanism; and "anaphylaxis" is classified as a severe, life-threatening, generalized or systemic HSR[5]. The European Society for Medical Oncology divides the reactions into anaphylactic and anaphylactoid and proposes the term "non-allergic anaphylaxis" instead of "anaphylactoid" due to a "non-immunological cause"[6]. The European Network for Drug Allergy's definition is independent of the underlying immunological mechanism and categorizes the HSRs as immediate and non-immediate. Moreover, differences exist in managing these reactions between European and American physicians[7]. The National Cancer Institute Common Terminology Criteria for Adverse Events distinguishes between allergy, anaphylaxis, and cytokine release syndrome (CRS) based on clinical manifestations[8]. The more commonly known Gell and Coombs classification is based on the underlying immunological mechanism and the time to manifestation, and includes four types: immediate, type I (developing within 15–30 minutes of exposure); type II (minutes to hours); type III (3–8 hours); and delayed, type IV (48–72 hours)[9].

While many immune-mediated adverse effects exist, here we focus on IRs. These reactions are unintended and occur at therapeutic doses of various products[4,], including biologics (such as recombinant proteins and antibodies)[10, 11], therapeutic nucleic acids, low-molecular-weight drugs[1, 12], and complex nanotechnology-based formulations[13, 14, 15] herein referred to as nanomedicines (Tables 1, 2). Despite the difference in underlying mechanisms, IRs may have overlapping clinical manifestations. Patients with acute IRs

experience various symptoms including, but not limited to, skin flushing or rash, chest and back pain, dyspnoea, wheezing, chills, fever, and rigor (Tables 1,2). These adverse effects require timely and accurate assessment, and proper management to avoid severe and potentially fatal consequences. Severe IRs are rare and occur in less than 5% of patients[4]. However, the incidence may increase when different drugs are used in combination (e.g., paclitaxel and carboplatin), when patients have a certain type of human leukocyte antigens (e.g., HLA-B*57:01 and abacavir), or when there is an underlying viral infection (e.g., Epstein-Barr virus infection and penicillin)[6, 13]. Despite decades of research suggesting that the incidence of IRs depends on both pharmacodynamics and pharmacogenetics, it is largely unknown why some patients develop these reactions while others do not. The lack of uniform terminology and classification of these reactions further complicates the issue. As such, acute IRs cause substantial stress among patients and their families as well as care providers and regulatory agencies[16]. Several world-leading health authorities (the World Health Organization, the United States Food and Drug Administration [17], and the European Medicines Agency) agree on the noxious nature of these adverse effects and the need for improved prevention and management[6]. These improvements largely depend on an understanding of mechanisms leading to the IRs. However, the molecular and cellular processes causing IRs are incompletely understood. Therefore, IRs in patients are currently managed by systemic administration of immunosuppressive, anti-pyretic and anti-inflammatory medications before the infusion, during administration, or both [1, 4, 6].

While not unique to any specific drug category, IRs present yet another hurdle in the translation of nanomedicines due to their complex nature and regulatory approval

process. This problem warrants thorough investigation and here, we examine the issue in the context of nanomedicines. We analyse the current understanding of the mechanisms underlying IRs as well as assays and models currently used to study these reactions at the preclinical stage. We identify gaps in the knowledge and propose a roadmap to fill them. We further suggest a strategy for overcoming translational barriers of nanomedicines caused by IRs.

Mechanisms of IRs in humans

According to Gell and Coombs, type I HSRs are mediated by IgE specific to at least one component of a drug product (Table 3)[9]. The main cellular contributors to this HSR type are mast cells, the activation of which leads to hay fever, allergic asthma, and/or anaphylactic shock[9, 13]. Type II HSR is cytotoxic hypersensitivity mediated by drug-specific antibodies (mainly IgG), the complement, and natural killer cells. Symptoms include pemphigus, nephritis, autoimmune haemolytic anaemia, and Goodpasture's syndrome[9, 13]. Type III HSRs are mediated by the immune complexes formed between a drug and an antibody (either IgM or IgG), and they involve complement activation. Clinical manifestations are serum sickness, fever, glomerulonephritis, and vasculitis[9, 13]. Type IV HSRs are mediated by T-helper cells and macrophages and manifest as erythema, induration, contact dermatitis, maculopapular rash, and granuloma[9, 13]. An immediate IgE-independent HSR with symptoms resembling a type I HSR is known as a complement activation-related pseudoallergy (CARPA)[18].

Type I–III reactions and CARPA are commonly recognized mechanisms associated with drug-mediated HSRs. Platinum-based formulations are notorious for type I HSRs[4, 6], while CARPA is the best-studied reaction to nanomaterials[18, 19]. CARPA has

been described for liposomal drugs (Doxil and Ambisome), micelles (Taxol and Taxotere), and modified dextran-coated iron oxides (Feraheme), all of which are approved for clinical use and are marketed with a black box warning of potentially life-threatening IRs. Properties of nanomaterials commonly associated with CARPA are summarized in Figure 1A.

Uncontrolled release of cytokines resulting from excessive proinflammatory stimuli, inadequate regulation of inflammation, or a combination thereof is responsible for severe cytokine release syndrome (CRS). The clinical manifestations of CRS include erythematous or pruritic rash, hypotension, fever, malaise, tachycardia, tachypnoea, generalized swelling, altered mental status, diffuse lymphadenopathy, and enlargement of the liver and spleen[20]. CRS created translation barriers for immunotherapies (e.g., CAR-T cells), biologics (e.g., TGN1412), and nanotechnology-formulated therapeutic nucleic acids (e.g., MRX34)[10, 21, 22, 23]. The consequences were severe and, in some cases, fatal[23]. It is important to mention that in some cases (e.g., CAR-T therapy), clinical CRS manifestations are not immediate and may take a week or more, which further complicates IR definition, diagnosis, and intervention. Moreover, some infections (e.g., influenza virus and bacteremia) may trigger cytokine release with symptoms indistinguishable from the drug-mediated CRS[24]. While various nanoformulations can induce the release of cytokines both in vitro and in vivo[25], the relevance of this mechanism to various types of nanomedicine-triggered IRs remains largely unknown.

The IRs to drugs are often heterogeneous and involve overlapping reactions and effector cells. For example, activation of the complement system, which occurs in CARPA, is also involved in the pathogenesis of type II and III HSRs. Conversely, the

macrophages may contribute to both type IV HSRs and CARPA. Cytokines that are produced by leukocytes are involved in CRS and can contribute to various types of HSRs, including pseudo allergy. An excellent demonstration of the complexity of frequently overlapping mechanisms that cause IRs to nanomedicines comes from the experiences with liposomes and lipid-based nanocarriers, which, according to a recent report by the U.S. Food and Drug Administration, dominate the current landscape of nanomedicine[26]. Various research groups worldwide have reported that lipid-based carriers are not immunologically inert[25, 27, 28]. Preferential clearance of these materials by macrophages, activation of pro-inflammatory cytokines, and the complement system are well-established[25, 27, 28]. However, the cause-effect relationship between the complement activation, cell uptake, and cytokine release is far from being understood. Despite the general acceptance that IRs involve multiple cellular and biochemical processes, the controversy over the leading cause of this toxicity creates significant hurdles and delays in the development of a unified strategy for predicting and overcoming the IRs to nanomedicines.

Controversy surrounding mechanisms of IRs

Activation of pulmonary intravascular macrophages (PIMs) by PEGylated liposomes was suggested as a key effector arm of CARPA in pigs[29]. The proposed double-hit scenario in this mechanism implies that both the complement and macrophage activation trigger the IRs symptoms [29]. Recently, a study of polystyrene beads in the same model concluded that PIMs activation is the leading cause of HSRs to nanomaterials and challenged the role of the complement[30, 31]. This mechanism caused robust debates[30, 32]. On the one hand, there is ample evidence that the pig model is sensitive to the detection

of nanomedicine-induced CARPA[32, 33]. On the other hand, PIMs are not present in the lungs of humans and animals commonly used in preclinical research (mice, rats, dogs, and nonhuman primates)[34, 35, 36]. In these animal species, the liver- and spleen-resident macrophages are primarily responsible for nanoparticle clearance. PIMs are induced in humans under certain pathological conditions, such as liver failure [34, 35]. In contrast, PIMs are common in the lungs of pigs, sheep, and horses not broadly recognized as preclinical models.[34, 35] Therefore, one area that requires close attention is the understanding of whether liver- and spleen-resident macrophages in humans play the same role as PIMs in pigs during IRs to nanomedicines. The hypothesis that hepatopulmonary macrophage migration triggers cardiorespiratory symptoms in humans exposed to nanomedicines[37] is attractive but requires thorough verification. The pivotal role of PIMs in nanoparticle-mediated IRs in the porcine model will benefit from confirmation by various research groups and with various types of nanomaterials. For example, a recent study demonstrated that during the infusion reaction triggered in pigs by carboxylated, hydrophobic, highly anionic polystyrene nanoparticles, complement activation-related opsonization coincided with the peak of pulmonary distress.[38] The relative contribution of the complement and PIMs, as well as the cause-effect relationship, may vary between different types of nanoparticles. Therefore, studies with clinically relevant well-characterized nanomedicines (e.g., liposomes, micelles, iron-oxides) are essential, while research-grade nanomaterials with poorly understood physicochemical properties (e.g., polystyrene beads) may not provide clinically relevant answer unless these particles are thoroughly characterized. The nature of a condition (e.g., cancer vs. inflammatory disorder) treated with a nanodrug should also be considered when analysing the

mechanism of IRs to the nanomedicine. Other recently proposed mechanisms, such as those involving platelets as an effector arm[39, 40], should also be thoroughly investigated in the context of nanoparticle physicochemical properties and interaction with other mechanisms.

While complement activation in CARPA is commonly verified by assessing the complement split products, there is no universal agreement regarding which macrophage activation markers are relevant to the IRs. Models relevant to the prediction of IR in humans are also being debated[30, 32, 33, 41, 42] . Therefore, there is currently a critical need to verify the biomarkers and models that are necessary for identifying the potential of nanomedicines to cause IRs in patients.

Biomarkers and models of infusion reactions

The current selection of biomarkers is not straightforward due to the controversy over both the definition of IRs[43] and the mechanism(s) responsible for them[30, 32]. A recent approach proposed for therapeutic antibody-mediated IRs based on the long usage of these products in the clinic could serve as a starting point for nanomedicines[44]. In this approach, clinical symptoms of IRs[12], also called Sampson criteria[44], are reviewed first to diagnose anaphylaxis[44]. Next, the assessment of drug-specific IgEs (a skin test and ELISA) and markers of mast cell degranulation (histamine and tryptase), are used to verify the anaphylactic nature of the reaction. Additionally, cytokines (e.g., IL-6, IL-8, IFNγ, TNFα, IL-2, and IL-10), complement split products (C3a, C5a, sC5b-9), and complement consumption (e.g., CH50) are used to verify IgE-independent pathogenesis of IRs.[44] Re-challenge or avoidance of the allergen is finally used to confirm the IR[44]. Because anaphylactoid reactions to proteins may also increase the levels of tryptase and

cytokines, these biomarkers are debated as suboptimal for diagnosing anaphylaxis to protein-based therapeutics.[18]

In the field of nanomedicine, assessing the complement split products (C3a, C5a, sC5b-9) and consumption (CH-50) is used to establish CARPA in both humans and animal models. However, earlier studies with nanomedicines indicated that CARPA response towards liposomes is accompanied by the release of many secondary messengers (e.g., thromboxane, leukotrienes, eicosanoids, histamines, cytokines, and tryptase).[18] In pigs, thromboxane A2 is recognized as the major mediator of pulmonary symptoms commonly seen during IRs. However, the use of this and other secondary messengers for predicting IRs to nanomedicines in humans remains unknown. While IgG and IgM have been implicated in type II and type III HSRs to proteins and low molecular weight drugs,[41] no data exist about such responses to nanomedicines. However, several studies described naturally existing IgM and IgG that can bind to various components commonly present in nanomedicines (cholesterol, phospholipids, and polyethylene glycol (PEG)).[45, 46] The accelerated blood clearance of PEGylated liposomes due to the anti-PEG IgM was reported in animals[47]. However, functional significance and relevance of these and other pre-existing antibodies to nanomedicine-triggered IRs in patients as well as their diagnostic utility require a thorough investigation.

Currently, identifying biomarkers that are relevant to IRs depends heavily on *in vitro* and *in vivo* models, both of which have advantages and limitations concerning their relevance to human patients (Table 4). Since there is limited information regarding nanomaterial-mediated type I–IV HSRs, our discussion will focus on models applicable

to the two established mechanisms (cytokines and CARPA) and the alternative mechanism (PIMs).

Rodent models reproduce the hypotension observed in humans during IRs. However, doses required to induce HSRs in mice and rats are several orders of magnitude higher than those needed to trigger reactions in humans.[42, 48] Therefore, rodent models are suitable for mechanistic studies but irrelevant for screening materials for reactogenicity.

The dog model reproduces some symptoms of human HSRs, such as hypotension, fainting, and other vegetative disturbance, at dose levels relevant to those in humans. However, studies of reactogenicity in dogs require large numbers of animals due to the high inter-animal variability in the response.[42] Therefore, screening for reactogenicity in the dog model may underestimate the toxicity, particularly if the number of tested animals is low.

The pig model allows reproduction of the cardiopulmonary distress typically observed in humans reacting to the infusion of nanomedicines[15]. However, it requires a better characterisation and understanding of the role of PIMs[34, 35]. Despite differences in the mechanism(s) underlying IRs, the pig model can be used to predict the reactogenicity of nanoparticles at low, clinically relevant doses using a reasonably low number of animals. Other animal models are not well-established to study nanomedicine-induced HSRs.

In vitro studies using patient serum or plasma are often considered for prediction of CARPA to nanomedicines. Likewise, cultures of human peripheral blood mononuclear cells can be used to estimate the risk of CRS induction. The correlation between in vitro screening and in vivo studies has been confirmed for CARPA[49] and CRS[25]. However,

current experience with these tests suggests that the positive response in complement or cytokine assays can predict the risk, but neither the incidence nor the magnitude, of IRs. This observation is consistent with the multicausality of IRs even when it is evident that complement activation or cytokines are primarily responsible. The utility of basophil activation, mast cell degranulation, leukocyte oxidative burst[50], and other common laboratory allergy tests[51] for nanomedicines requires thorough investigation.

Strategy and roadmap for addressing IRs to nanomedicines

Translational hurdles due to drug-mediated IRs are not unique to nanotechnology-formulated drug products. Therefore, one way to address the problem in nanomedicine is to leverage the knowledge and lessons learned from the clinical use of other drug products. For this to happen, the issue must be approached in a systematic way beginning with the identification of relevant biomarkers in patients, establishing appropriate models and understanding the mechanisms of IRs.

In the first step, reliable biomarkers need to be identified and assessed for correlation to clinical outcomes (Figure 1B). One way to do this is by using retrospective clinical trials to identify patients who are sensitive to IRs when administered with nanomedicines. Their sera could be retrieved and tested *in vitro*. However, obtaining viable cells from archived specimens of such patients could be problematic because common preservation techniques protect plasma proteins better than cells. The timeline between specimen collection and nanomedicine administration may also affect the assay outcome because blood composition is dynamic and reflects physiological status of the patient at the time of collection.

Another alternative is through prospective studies that enroll patients prescribed with nanomedicines and collect their fresh blood before and after administration of a nano-drug. Whole-blood samples collected before treatment could be exposed to a nanomedicine *in vitro*, and the various endpoints, including immune cell, complement, and clotting cascade activation markers, could be studied. However, prospective studies are potentially limited by patients' premedication with immunosuppressive drugs, which are classically used to prevent the incidence of IRs, or by a slow infusion rate, typically used to avoid anaphylaxis. Despite these limitations, some markers (e.g., complement split products or cytokines) can still be detected, even in the absence of clinical signs of HSRs. For example, in the case of Doxil and Taxol infused at high rates, HSR symptoms were detected in patients only when high levels (>5-fold above the baseline) of the terminal complex (sC5b-9) were detected in the blood.[52, 53] However, complement split products at physiologically significant levels (≥2-fold above the baseline) were detectable in the absence of clinical symptoms of HSRs[19].

The second step requires the establishment of *in vivo* and *in vitro* models and their relevance to human patients. One way to do this is by leveraging the advantages of existing models. To verify their relevance to humans one has to compare the underlying mechanism(s) between these models and patients. Although several mechanisms of IRs have been proposed for nanomedicines, CARPA is the most well-studied and understood [32]. The relevance of other mechanisms to nanomedicines remains unclear given the current lack of human data. This work, therefore, is critical and interdependent on biomarker selection.

The third step is mechanistic verification of the selected biomarkers. Here we propose the use of inhibitors in patients when approved drugs are available. For example, a clinically approved C5 inhibitory antibody, eculizumab (Soliris); a plasma-derived C1 inhibitor (C1INH); and a small-molecule inhibitor of factor D could be used to understand the contribution of the complement in humans[54]. Since it is not yet known what inhibitor would work best for preventing nanoparticle-induced complement activation in patients, nonclinical-grade inhibitors of C3 convertase (compstatin, APT070, or APL-2), soluble complement receptors (sCD35) and decay accelerating factor (CD55), chimeric receptors (CAB-2), or their animal counterparts, could be investigated in the animal models. For example, sCD35 was effective at inhibiting liposome-triggered IRs in the pig model of CARPA[55].

The contribution of other mechanisms (e.g., CRS, platelets, and direct macrophage activation) could be verified by inhibitors of cyclooxygenase (e.g., indomethacin) and cytokines (e.g., neutralizing antibodies). For example, indomethacin efficiently blocked liposome-mediated IRs in pigs, suggesting cooperation between macrophage- and complement-mediated mechanisms[55]. Special consideration should be given to the type of the nanomedicine tested (e.g., PEGylated liposome or dextran-coated iron oxides), and the category of human subjects enrolled in the clinical trial. For example, consequences of administering a complement inhibitor to cancer patients prescribed with Doxil are uncertain because the implications of complement in tumour growth are poorly understood. Likewise, inhibiting complement in chronic kidney disease patients prescribed with Feraheme may increase the risk of infections because the immune system in these patients is already weakened, and complement-mediated

protection from pathogens is further reduced by the inhibitor. The administration of empty carriers in combination with complement inhibitors can be significantly less dangerous and ethically justified in healthy volunteers.

Desensitization strategy, commonly used in the field of protein-based allergens, is not well investigated in the field of nanomedicine. The only known preclinical example involves injecting Doxebo (a placebo PEGylated liposome) to reduce the IRs to subsequently administered Doxil (a drug-loaded PEGylated liposome) in a pig model[29]. Clinical investigation of this and other desensitization strategies would further benefit the field.

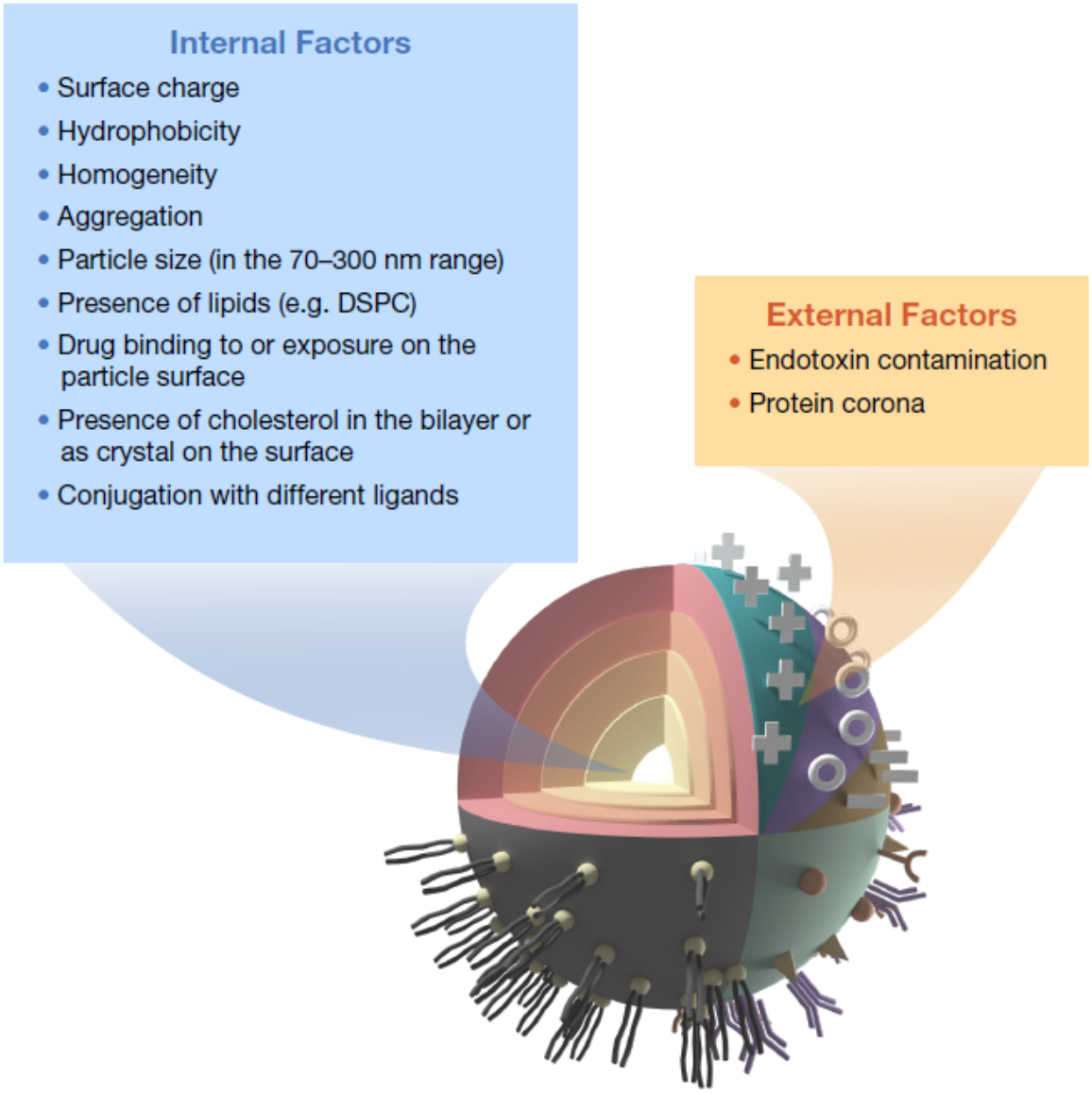
The current approach of slowing down the infusion rate is a powerful tool in reducing IRs to nanomedicines[19], and it would further benefit from an understanding of the underlying mechanism. The improved knowledge base will also allow researchers to unravel the complex relationship between IRs, other immune-mediated adverse effects, and long-term or tissue-specific toxicities similar to those described in rats with CARPA induced by a high dose of cholesterol-rich liposomes[56].

Conclusion

The foundation of the strategy to overcome IRs to nanomedicines comprises a mechanistic understanding of those IRs and the identification of the leading cause and relationship between various mechanisms, as well as critical attributes of the nanomedicines that are responsible for triggering IRs. The nanomedicine community must clarify the role of the complement, cytokines, macrophages, platelets, and other mechanisms in the context of the physicochemical attributes of the nanoparticles. In the

long run, this information can be used to understand the potential role of IRs in tissue-specific and long-term toxicities. Furthermore, it is necessary for the community to harmonize methods, models, and biomarkers for predicting IRs in patients. Finally, the improved knowledge should be used to combine existing strategies (which focus on management of the symptoms) with new ones (which focus on intervening at the root of the cause) to overcome translational barriers caused by IRs.

a


Internal Factors
• Surface charge
• Hydrophobicity
• Homogeneity
• Aggregation
• Particle size (in the 70–300 nm range)
• Presence of lipids (e.g. DSPC)
• Drug binding to or exposure on the particle surface
• Presence of cholesterol in the bilayer or as crystal on the surface
• Conjugation with different ligands
External Factors
• Endotoxin contamination
• Protein corona

b

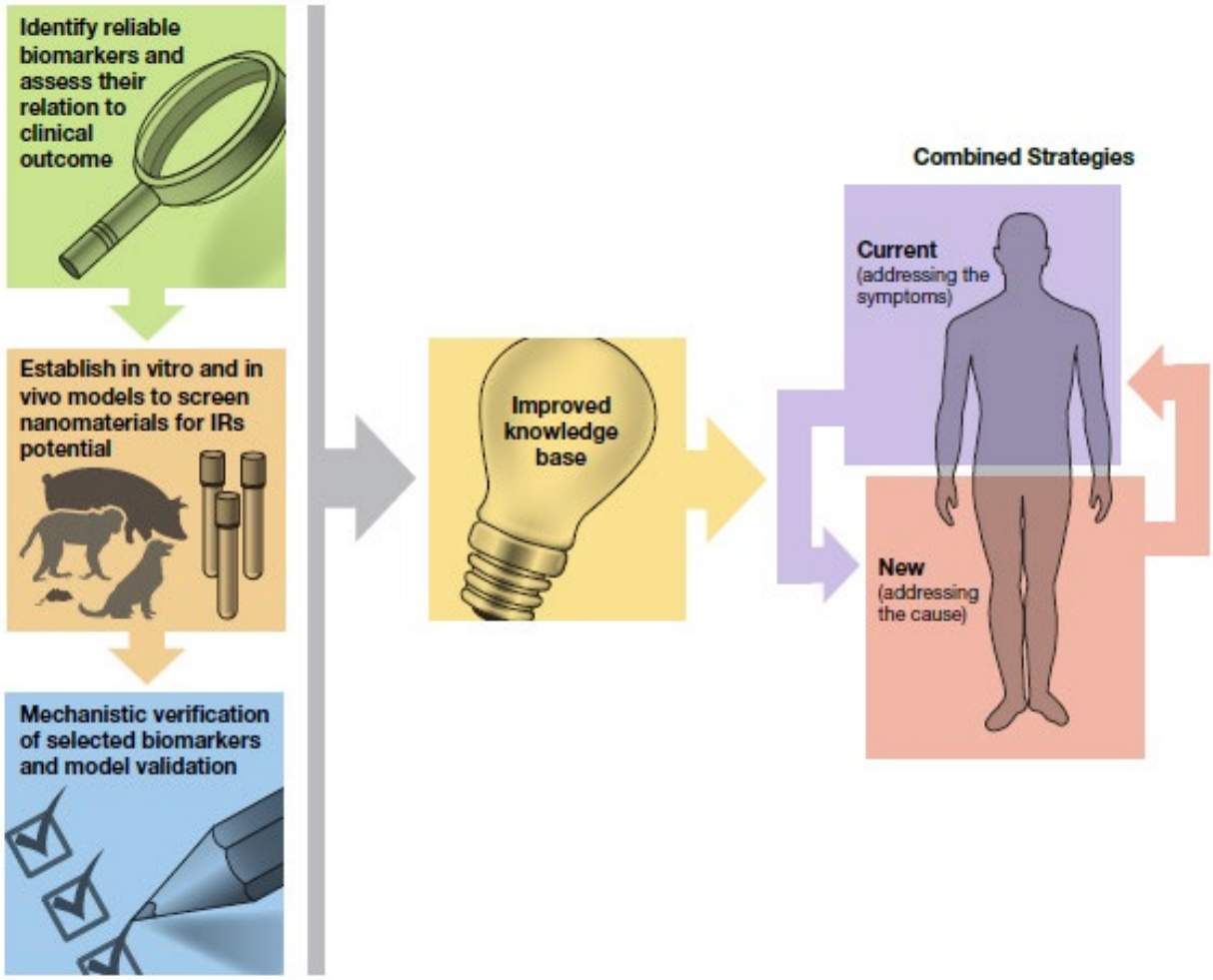


Figure 1. Strategy and roadmap to overcoming infusion reactions to nanomedicines. The strategy for overcoming nanomedicine-triggered IRs relies on a mechanistic understanding of those IRs, the identification of the leading cause and discovering the relationship between various mechanisms. **(a)** Some physicochemical attributes of nanomedicines that were linked to IRs. This list is incomplete as other potential attributes are not yet understood. The known internal properties can be fine-tuned to decrease the risk. External features cannot be controlled directly but can be addressed through the engineering of internal properties. **(b)** Identification of reliable biomarkers, corroboration

of a methodological framework and mechanistic verification serve to improve the current knowledge base of IRs to nanomedicines. This knowledge will enable improved healthcare by combining existing approaches for monitoring and managing the IRs with new ones, which are aimed at intervention at the root cause.

Table 1. Selected examples of nanotechnology-based drug products known to induce infusion reactions. The table was prepared based on numerous studies reviewed in references[18, 57, 58, 59]

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, dyspnoea, 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, dyspnoea, 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, dyspnoea, flushing, changes in blood pressure and heart rate, back pain
Onivyde (Merrimack Pharmaceuticals)	Irinotecan	metastatic pancreatic adenocarcinoma progressing after gemcitabine-based therapy	liposomes	rash, urticaria, periorbital oedema (pruritus)
Vyxeos (Jazz Pharmaceuticals)	daunorubicin and cytarabine	newly-diagnosed therapy-related acute myeloid leukemia (AML) and AML with myelodysplasia-related changes	liposomes	dyspnoea, headaches, chills, rash, nausea, vomiting, oedema

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, diarrhoea, dyspnoea, fever, headache, hypotension, nausea, rash, rhinitis, urticaria, vomiting

Taxol (Bristol-Myers Squibb)	paclitaxel	cancer	Cremophor EL® (8-20 nm)	acute respiratory distress, anaphylaxis, angioedoema, arrhythmias, bronchospasm, chills, dyspnoea, facial and upper thorax flushing, fever, rash, sudden death, tachycardia, urticaria, wheezing
Cyclosporine Injection, USP (Draxis Pharma, Inc.)	cyclosporine	Immuno-suppression		
Vumon Injection (Brystol-Myers Squibb)	teniposide	leukaemia		
Etoposide (Gensia Sicor Pharmaceuticals, Inc.)	podophyllotoxin	different cancers	Polysorbate 80 (8-16 nm)	apnoea, back pain, bronchospasm, chills, coughing, cyanosis, diaphoresis, dyspnoea, fever, flushing, facial swelling, hyper or hypotension, laryngospasm, loss of consciousness, rash, tachycardia, tightness in throat, tongue swelling, urticaria
Taxotere (Sanofi-Aventis)	docetaxel			back pain, bronchospasm, chest tightness, chills, dyspnoea, erythema, fatal anaphylaxis, fever, flushing, generalized rash, hypotension

Table 2. Selected examples of non-nanotechnology drug products known to induce infusion reactions. The table was prepared based on numerous studies reviewed in references[11, 57, 60].

Brand name (manufacturer)	mAb, type (target antigen)	Indication	Incidence	Symptoms
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_1k (CD52 on T and B cells)	B cell chronic lymphocytic leukemia (B-CLL)	4-7%	bronchospasm, chills, dyspnoea, emesis, fever, hypotension, nausea, pyrexia, rash, rigors, tachycardia, urticaria
Erbitux (Bristol-Myers Squibb, Eli Lilly)	cetuximab, chimeric IgG_1k (human EGFR)	metastatic colorectal cancer, head and neck cancer, squamous cell carcinomas	< 3% , fatal <0,1%	anaphylaxis, angioedoema, bronchospasm, cardiac arrest, chills, dizziness, dyspnoea, fever, hoarseness, hypotension, pruritus, rash, rigor, stridor, urticaria, wheezing
Herceptin (Genentech)	trastuzumab, humanized IgG_1k(human EGFR receptor 2, HER2/neu / erbB2)	metastatic breast and gastric cancer	<1%	asthenia, bronchospasm, chills, death within hours, dizziness, dyspnoea, further pulmonary complications, headache, hypotension, hypoxia, nausea, pain, rash, severe hypotension, vomiting
Mylotarg (Pfizer Inc./Wyeth Pharmaceuticals)	gemtuzumab ozogamicin. recombinant humanized IgG_4k	CD33 positive acute myeloid leukemia in first relapse	<8%	acute respiratory distress syndrome, anaphylaxis, dyspnoea, fatal anaphylaxis, hypotension, pulmonary edoema

	(CD33 on hematopoietic cells)			
Vectibix (Amgen)	panitumumab, recombinant humanized IgG_2k (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 leukaemia, rheumatoid arthritis, and non-Hodgkin's B-cell lymphoma	>80% severe: <10%	Acute respiratory distress syndrome (ARDS), bronchospasm, cardiogenic shock, flushing, hypotension, hypoxia, itching, myocardial infarction, pain (at the site of the tumour), pulmonary infiltrates, runny nose, swelling of the tongue or throat, ventricular fibrillation, vomiting

Table 3. Gell and Coombs classification of allergic reactions. Allergic reactions are separated into four types based on the underlying mechanism, time of symptom occurrence, mediators, and clinical manifestation. This summary is prepared based on reference [9].

	TYPE I	TYPE II	TYPE III	TYPE IV
Underlying mechanism	Immediate hypersensitivity or acute allergy	Antibody-mediated cytotoxic reaction	Immune-complex-mediated reaction	Delayed-type hypersensitivity
Mediators	IgE	Cytotoxic IgM and IgG antibodies	Immune complexes (mostly IgM)	Mainly T-helper cells and macrophages. No antibodies involved.
Immune response	Degranulation (histamine release) of mast cells and basophils and synthesis of new mediators (thromboxanes, prostaglandins, and leukotrienes)	Cytotoxic actions by natural killer (NK) cells, macrophages, neutrophils, and complement	Deposit of immune complexes in tissues. Inflammatory response involving complement activation, neutrophil degranulation, and platelet activation.	Cytotoxicity and accumulation of macrophages and T cells. Cytokine release and lymphocyte stimulation.
Time to develop	Usually from minutes (15–30 minutes) to a few hours. Late-onset reactions (18–24 hours) are uncommon.	From minutes to hours, but some clinical manifestations (thrombocytopenia, agranulocytosis, fever, anaemia) can be diagnosed after few days.	From 3–8 hours, but some clinical manifestations can develop even 9–11 days after exposure.	Several (2–14) days
Clinical symptoms	Urticaria, angioedema, asthma, rhinitis, conjunctivitis, cardio-respiratory anaphylactic shock, bronchospasm	Pemphigus, nephritis, autoimmune haemolytic anaemia, Goodpasture syndrome	Tissue injury. Several organs can be affected: lungs, joints, skin and kidneys. In addition, serum sickness, fever,	Most common: skin eruptions exposed to chemicals, cosmetics, drugs, and metals. Contact dermatitis,

			glomerulonephritis, and vasculitis are possible.	erythema, induration, maculopapular rash, and granuloma.

Table 4. Available Animal Models. Comparison of hemodynamic and other manifestations of HSRs in animal models. The summary is prepared based on reference [61]

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 labor intensive; not generally accepted for preclinical safety studies
Minipig	High	Reproduces clinical symptoms of human patients; consistent response between individual animals	Skills and labor intensive; not generally accepted for preclinical safety studies
Dog	High	Reproduces clinical symptoms of human patients; generally accepted for preclinical safety studies	High inter-animal 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

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Acknowledgments

The study was supported in part (M.A.D.) by federal funds from the National Cancer Institute, National Institutes of Health, under contract HHSN261200800001E, and by NIH grants CA194058 (D.S.) and EB022040 (D.S.). The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. J.S. acknowledges support by the European Union Seventh Framework Program grants NMP-2012-309820 (NanoAthero), NMP-2013-602923 (TheraGlio) and by the Applied Materials and Nanotechnology Center of Excellence at Miskolc University, Hungary. Y.B. acknowledges the partial support of The Barenholz Fund, which was established by the Hebrew University with royalties obtained from Y.B.'s inventions and is used to support research in the Barenholz Lab. A.G.F.'s work was funded by Xunta de Galicia (Grupo de referencia competitiva ED431C 2016041). The authors thank Ai Lin Chun of Science Story Lab for helpful critique.

Competing interest

J.S. is involved in SeroScience Ltd's CRO activity providing immune toxicology services. Y.B. is one of the inventors of two patents relevant to Doxil: (1) Barenholz, Y., and Haran, G. "Method of Amphipathic Drug Loading in Liposomes by pH Gradient," U.S. Patent 5,192,549, March 9, 1993, U.S. Patent 5,244,574, September 14, 1993; and

(2) Barenholz Y., and Haran, G. “Liposomes: Efficient Loading and Controlled Release of Amphipathic Molecules,” U.S. Patent 5,316,771, May 31, 1994. Both patents expired in March 2010. The Hebrew University received royalties from Doxil sales until the patent expiration. The Barenholz Fund, established with a portion of these royalties, is used to support research in Y.B.’s laboratory, including this study. The other authors do not have conflicts of interest related to the subject described in the manuscript.