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Opinion: Excipient Immunogenicity in Cancer Nanomedicine and the Challenge of Safe Repeat Dosing

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Opinion

ABSTRACT

Pharmaceutical excipients are traditionally selected to improve drug solubility, stability, drug duration by influencing release or circulation time, formulation loading capacity and delivery efficiency. In cancer nanomedicine, however, these formulation components can also influence immune recognition, complement activation, biodistribution, pharmacokinetics and treatment outcomes, particularly during repeated administration. For instance, there is increasing evidence that pre-existing or treatment-induced anti-PEG antibodies can accelerate carrier clearance, activate complement and alter systemic drug exposure for formulations containing Polyethylene Glycol (PEG) and PEG-lipids (that are central to liposome formation, lipid nanoparticles and polymer-based carriers). Other formulation components, including ethoxylated castor oil, polysorbates, poloxamers, ionizable lipids, helper phospholipids and cholesterol derivatives, may influence infusion-related reactions, cytokine release or blood compatibility in specific situations dependent on the formulation, excipient purity, excipient dose, administration route, and repeat-dosing conditions.

It is generally understood that excipients used in these more complex formulations/delivery systems are not inert and, crucially, actively influence the biological performance of the therapeutic product. While there are undoubted benefits of utilizing excipients in this manner, with decades of use in successful marketed products, the potential risks still need to be understood and put into context. This opinion article examines excipient-driven immune responses in cancer nanomedicine, with emphasis on anti-excipient antibodies, accelerated blood clearance, complement activation-related pseudoallergy and reduced repeat-dose performance. Clinical and formulation insights are drawn from PEGylated liposomal doxorubicin, solvent-based taxane formulations, albumin-bound paclitaxel and emerging lipid nanoparticle systems. Other topics addressed include: discussion of preclinical testing, clinical monitoring, formulation strategies and regulatory considerations for reducing immune-related variability. Recognizing excipients as context-dependent biological determinants, rather than universally inert or uniformly harmful components, is essential for designing cancer nanomedicines that remain safe, predictable and effective across repeated treatment cycles.

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INTRODUCTION

Nanomedicine has broadened the range of formulation strategies for drugs with poor solubility, including cytotoxic agents, nucleic acids, proteins, and combination therapies. Nanoscale delivery systems such as liposomes, lipid nanoparticles, polymeric micelles, and protein-based nanoparticles can enhance drug solubility, alter pharmacokinetic profiles, shield labile therapeutic agents, and minimize damage to healthy tissues. Despite these advantages, the clinical outcomes of nanomedicines have shown greater variability than anticipated from early preclinical studies. Factors such as biological barriers, irregular tumor vasculature, uptake by the mononuclear phagocyte system, and poor penetration into solid tumors can hinder consistent and effective delivery (1-2).

A potential contributor to this inconsistent performance is the formulation used to deliver the therapeutic and the various excipients that may be present. Components like surface polymers, surfactants, phospholipids, and stabilizers have, in the past, been regarded as "inactive" because they do not directly produce the intended therapeutic effect. However, in nanomedicine, this classification is increasingly problematic. These excipients form the primary interface between the nanocarrier and biological components such as blood, immune cells, plasma proteins, and the vascular lining. As such, they may influence the formation of the protein corona, may trigger complement activation, as well as affecting cellular internalization, shaping organ distribution and elimination pathways.

This issue is particularly relevant in oncology, where most nanomedicines are administered repeatedly over multiple cycles spanning weeks or months. An excipient that appears safe after a single dose may provoke antibody production, activate innate immune memory, or induce complement-driven responses that alter the pharmacological behavior of subsequent doses. As a result, traditional single-dose toxicity and pharmacokinetic assessments may not adequately predict performance after repeated administration.

Polyethylene glycol (PEG) is a well-documented example. PEGylation creates a hydrophilic surface layer that limits protein binding and extends circulation time, contributing to the success of stealth liposomes and several approved PEG-modified therapeutics (3-4). Nowadays PEG is no longer regarded as immunologically inert because anti-PEG antibodies have been found even in individuals never known to be exposed to PEGylated drugs, and their levels can rise following treatment. These immune responses have the potential to result in modified drug clearance, modified systemic exposure, and an increased risk of complement-related hypersensitivity reactions (5-6) Generally such responses lead to more rapid clearance, however this is not always the case. Although rare, there is the potential for anti-PEG antibodies to sustain circulatory exposure, rather than enhance clearance. Sustaining anti-peginesatide is one such example (5). Unlike many discussions that focus broadly on nanomedicine toxicity or PEG immunogenicity, this opinion article frames excipient immunogenicity as a repeat-dose formulation challenge in cancer therapy. This perspective is important because oncology nanomedicines are rarely administered as isolated single doses; they are usually dosed across multiple cycles, during which antibody formation, complement activation, altered clearance and changing patient immune status may influence both safety and therapeutic exposure. It is important to emphasize that excipient-related immune responses are not intrinsic or unavoidable properties of all materials within a chemical class. Immunological risk depends on excipient grade, purity, route of administration, dose, formulation architecture, impurity profile, patient factors and intended clinical use. Highly purified pharmaceutical grades of excipients such as ethoxylated castor oil, polyethylene glycol derivatives and polysorbates have been specifically developed to meet stringent quality requirements, and appropriate grade selection is necessary, especially in parenteral formulations to reduce avoidable immunological and blood compatibility concerns. Given that there will be increasing demand for using existing, or alternative, excipients to enable future therapeutics, the aim of this paper is to highlight the need for context-specific immunological evaluation when these materials are used in nanomedicine or repeated parenteral oncology dosing.

Approach to Literature Identification

A targeted narrative review was carried out using PubMed and Google Scholar as primary sources. Search terms and combinations included: "cancer nanomedicine," "pharmaceutical excipient," "PEG immunogenicity," "anti-PEG antibody,"

"accelerated blood clearance," "complement activation," "PEGylated liposomal doxorubicin," "ethoxylated / polyethoxylated caster oil," "polysorbate 80," "taxane hypersensitivity," "lipid nanoparticle immunogenicity," "repeat dosing," "stealth polymer," and "PEG alternative."

Emphasis was placed on original research exploring underlying mechanisms, clinical oncology findings, comprehensive reviews, formulation-related studies, and publications examining blood compatibility or preclinical immunotoxicity. Seminal older studies were included when they described foundational mechanisms still applicable today. Because this article is a narrative review, it does not aim to provide pooled prevalence estimates, meta-analysis, or quantitative comparison of hypersensitivity rates across formulations.

Excipients as Biological Drivers of Nanomedicine Performance

In traditional formulations, excipients typically provide more specific, and more straightforward, means for product design and development such as, enhancing solubility, adjusting viscosity, stabilizing pH, or preventing microbial growth. In nanomedicine, however, a more complex interplay of excipients is required for the core structure of the delivery system. For example, PEG-lipids govern surface hydration and nanoparticle stability, while ionizable lipids play a key role in nucleic acid encapsulation and facilitating endosomal escape. Phospholipids and cholesterol affect membrane integrity, leakage rates, protein binding, and activation of the complement system. Surfactants are essential for enabling intravenous delivery of poorly water-soluble anticancer agents (7).

As a result, excipient choice can significantly influence both the pharmacokinetics and pharmacodynamics of the formulation. Variations in particle size, surface charge, PEG density, lipid saturation, or degradation byproducts can all impact how the immune system recognizes the nanoparticle. Even small differences in excipient composition may alter complement activation and the patient's ability to tolerate infusion. Blood compatibility, therefore, emerges from the collective properties of the entire formulation, not just the active drug (8).

The term immunogenicity is interpreted broadly to include: the generation of antibodies targeting excipients, complement system activation, release of cytokines and chemokines, activation of mast cells or basophils, opsonization and uptake by phagocytic cells, innate inflammatory responses, immune-driven alterations in drug distribution, and hypersensitivity or infusion-related reactions. Not all such immune responses are detrimental. In some cancer vaccines, inflammatory lipids are intentionally included to boost antigen presentation. The concern arises when immune activation is uncontrolled, too strong, or inconsistent with the intended

Table 1. Potential immune reaction risks associated with excipients employed in nanomedicines for cancer

Excipient	Formulation role	Immune concern	Possible consequence during repeat dosing
PEG and PEG-lipids	Surface stabilization and prolonged circulation	Anti-PEG antibodies and complement activation	Accelerated clearance, reduced exposure, and infusion reactions
PEG-containing poloxamers	Surfactant and stabilizer	Induction or boosting of anti-PEG antibodies	Altered pharmacokinetics of later PEGylated products
Phospholipids and cholesterol nanoparticles	Structural components of liposomes and lipid	Complement activation and opsonization	Infusion reactions and increased phagocytic uptake
Ionizable lipids	Nucleic-acid encapsulation and endosomal escape	Innate inflammation and cytokine release	Reactogenicity and reduced repeat-dose tolerability
Ethoxylated/ polyethoxylated castor oil	Paclitaxel solubilization	Complement, mast-cell and basophil activation	Acute hypersensitivity and altered paclitaxel pharmacokinetics
Polysorbates	Docetaxel and supportive drug solubilization	Hypersensitivity and reactive degradation products	Infusion reactions and formulation instability
Albumin	Solvent-free drug carrier	Lower solvent-related hypersensitivity but possible protein-related variability	Improved tolerability with altered biodistribution

therapeutic goal. It is therefore important to understand that while there are mechanistic demonstrations of possible undesirable effects of some excipients under certain conditions, the findings need to be put into context with the clinical success of various nanomedicines, and cancer treatments, that have provided important health benefits over decades to a plethora of patients.

The major excipients used in cancer nanomedicine can influence immune recognition, complement activation, pharmacokinetics and treatment tolerability, particularly during repeated administration. Their principal formulation roles and potential immune consequences are summarized in Table 1.

PEG and PEG-lipids as Functional Formulation Components

PEG has become a widely used stealth agent due to its hydrophilic nature, molecular flexibility, and ease of attachment to proteins, lipids, and polymers. A PEG coating helps prevent particle aggregation, limits protein adsorption through steric hindrance, and extends circulation time in the bloodstream. Consequently, PEG-lipid conjugates are commonly incorporated into liposomes and lipid nanoparticles (9-10).

However, the biological behavior of PEG is influenced by multiple factors beyond its mere presence. Molecular weight, surface concentration, terminal functional groups, stability of chemical linkages, lipid anchor type, and rate of shedding all affect how PEG chains are arranged and whether they are accessible to immune recognition. At low densities, PEG molecules tend to adopt a mushroom conformation, whereas higher densities lead to a more extended brush configuration. These structural differences have been shown to impact circulation longevity, complement activation, and potential immunogenicity (11).

Based on these various properties, a balance is needed in PEG properties to enable a robust formulation with the desired clinical and safety performance. For example, a dense PEG layer enhances circulation time but may hinder cellular uptake and endosomal escape. PEG-lipids that shed quickly might improve intracellular delivery after administration but offer less protection during transit. Conversely, slowly dissociating variants maintain stability but increase prolonged exposure of repetitive PEG motifs, potentially raising immune recognition.

Recent analyses have moved beyond questioning whether PEG is immunogenic, focusing instead on identifying the specific conditions under which it triggers clinically relevant immune responses (12-14).

Pre-existing and Treatment-induced anti-PEG Antibodies

Anti-PEG antibodies can already be present in patients before they receive any PEGylated therapy. Exposure to everyday products containing PEG such as cosmetics, processed foods, household cleaners, and certain medications has been regarded as a contributing factor. While variations in detection methods make it difficult to determine exact prevalence, multiple studies confirm that both IgM and IgG anti-PEG antibodies exist in subsets of the general population (15).

Treatment itself can further elevate antibody levels. The immune response depends on characteristics of the PEGylated construct, including dose, administration route, dosing frequency, the type of payload, and how PEG is displayed on the nanoparticle surface. Free PEG tends to be less immunogenic than PEG arranged in repeating patterns on nanoparticles, as dense, repetitive epitopes can promote B-cell activation even without conventional T-cell involvement (10). Research suggests that splenic marginal-zone B cells and T-cell-independent pathways contribute to the production of anti-PEG IgM. Koide et al (16). showed that rapid clearance of PEGylated liposomes can occur via a T-cell-independent B-cell mechanism. Additionally, monoclonal anti-PEG antibodies have been found to recognize distinct PEG structures and exert different effects on nanoparticle clearance (17).

Clinically, the mere presence of anti-PEG antibodies does not predict outcomes. The antibody isotype, concentration (titer), binding strength (affinity), and ability to activate complement are all critical factors. For instance, some antibodies bind PEG without significantly affecting circulation, while others trigger opsonization, complement deposition, and phagocyte-mediated removal. Awareness of the potential risks is needed to ensure that during development any untoward clinical observations are not because of formulation components, as well as the therapeutic agent.

Accelerated Blood Clearance and Challenges with Repeated Dosing

Accelerated blood clearance refers to the phenomenon where a PEGylated carrier is rapidly eliminated from circulation after prior exposure. Early studies in rodents and rhesus monkeys found that repeating doses of PEGylated liposomes led to a dramatic reduction in circulation time upon the second administration (18). Anti-PEG IgM was later identified as a primary driver of this effect (19).

The proposed mechanism unfolds as follows: the initial dose presents repetitive PEG structures to the immune system, PEG-responsive B cells produce anti-PEG IgM, upon re-exposure, these antibodies bind to PEG on the nanoparticle surface, complement proteins deposit on the opsonized particles, liver and spleen phagocytes clear the particles from circulation and as a result, systemic drug levels and tumor accumulation decline.

The extent of this effect varies based on the initial dose strength, interval between doses, nature of the encapsulated drug, and nanoparticle characteristics. High-dose cytotoxic payloads may suppress antibody-producing cells, which helps explain why the phenomenon is not consistently observed across all treatments (20-21).

While anti-PEG IgM plays a central role, pre-existing IgG can also modulate complement activation and clearance kinetics. Mima et al. demonstrated that anti-PEG IgM contributes significantly to the accelerated clearance of PEG-conjugated proteins, indicating that this mechanism extends beyond liposomal systems (22).

In oncology, accelerated clearance can reduce systemic drug exposure and compromise tumor targeting. Hsieh et al. showed that pre-existing anti-PEG antibodies can impair both the pharmacokinetics and therapeutic performance of PEGylated liposomes (23). Miao et al. further demonstrated in animal models that prior exposure to PEG-containing poloxamer excipients could induce anti-PEG antibodies and weaken the antitumor activity of subsequently administered PEGylated liposomal doxorubicin (24). These findings have wider implications: a patient's response to a PEGylated nanomedicine may be influenced not only by previous use of the same drug but also by earlier exposure to unrelated products containing PEG.

Complement Activation and Infusion Reactions

Pseudoallergic reactions linked to complement activation termed Complement Activation-Related Pseudoallergy (CARPA), are hypersensitivity responses that do not involve IgE but instead arise from triggering the complement system. Fragments such as C3a and C5a can activate mast cells, basophils, macrophages, and cardiopulmonary pathways, leading to symptoms like flushing, shortness of breath, chest or back pain, blood pressure fluctuations, rapid heart rate, and occasionally severe cardiovascular or respiratory distress (25).

Unlike classic allergic reactions, CARPA can occur during initial exposure, is often influenced by infusion speed, and may diminish upon repeated administration due to tachyphylaxis. However, distinguishing CARPA from IgE-mediated allergy, reactions driven by anti-PEG antibodies, or direct mast cell activation is not always straightforward.

Nanocarriers such as liposomes and lipid-based micelles can trigger complement through classical, alternative, or lectin pathways, depending on their surface properties. Factors including particle size, curvature, surface charge, cholesterol levels, PEG layer structure, and contaminants influence this interaction (26).

A key clinical example is PEGylated liposomal doxorubicin. Chanan-Khan et al. found that complement activation correlated with hypersensitivity during first infusions. Later, Neun et al. showed certain anti-PEG antibodies could amplify complement activation by Doxil in laboratory settings (27-28).

Still, the link between complement activation and clinical symptoms is not absolute and some patients show complement activity without symptoms, while others experience reactions through non-complement mechanisms. Despite this, CARPA remains a valuable framework for understanding infusion-related adverse events that may occur with nanoparticles.

Slower infusion rates and premedication help reduce both the frequency and severity of these reactions. Strategies to induce tachyphylaxis include small initial doses or pretreatment with inert, drug-free vesicles resembling those in the marketed product, Doxil. In pigs, placebo vesicles reduced subsequent reactions to PEGylated liposomal doxorubicin. Doxebo, a drug-free version of this formulation, has shown promise in preclinical studies as a safe pretreatment that does not interfere with the efficacy of the active drug (29).

Clinical Implications for PEGylated Liposomal Doxorubicin

PEGylated liposomal doxorubicin exemplifies both the advantages and challenges of using active excipients that possess the potential to stimulate an immune response. Encapsulation limits systemic exposure to free doxorubicin and alters tissue distribution, while PEG coating extends circulation time and enhances tumor targeting. The same PEGylated surface can also interact with naturally occurring antibodies and activate complement and this duality leads to several clinical considerations: potential for acute reactions during infusion, variability in drug behavior between patients, changes in clearance after prior PEG exposure, reduced tumor delivery in later treatment cycles, uncertainty when switching between similar liposomal products, and limited ability to predict reactions based on standard allergy history.

Gabizon et al. highlighted that immune responses to PEGylated liposomes mediated by antibodies and complement poses potential risks to both patient safety and treatment effectiveness (30). These concerns extend to biosimilar or generic versions, because similar size of nanoparticles/vesicular carriers and drug loading do not ensure equivalent interactions with the immune system.

Ethoxylated Castor Oil and Lessons from Solvent-Based Paclitaxel Formulations

In the past, paclitaxel's poor aqueous solubility historically required formulation with ethoxylated castor oil and ethanol to enable intravenous administration. This formulation approach addressed a major solubility problem, but it also contributed to infusion-related reactions, altered paclitaxel pharmacokinetics and solvent-associated tolerability concerns (31). Importantly, these effects should not be interpreted as a general indictment of all ethoxylated castor oil grades or all surfactant-based excipients. The immunological and clinical behavior of such excipients depends strongly on pharmaceutical grade, purification level, impurity profile, route of administration, concentration and the final formulation environment.

In parenteral drug development, appropriate excipient-grade selection is essential. Highly purified pharmaceutical grades are developed to reduce impurities and improve suitability for injectable use. Therefore, adverse reactions historically associated with solvent-based paclitaxel formulations should be discussed as formulation- and grade-dependent events rather than as unavoidable properties of ethoxylated castor oil itself. This distinction is important because poorly specified or inappropriate grades may introduce avoidable immunological risk, whereas purified grades used under suitable quality specifications can reduce such concerns.

Ethoxylated castor oil-containing formulations can form micellar structures that influence the relationship between total plasma paclitaxel concentration and freely available drug. In addition, infusion-related reactions may involve complement activation, direct mast-cell or basophil activation, or other non-IgE mechanisms, particularly during early infusions (32). These observations stimulated the development of solvent-free formulations such as albumin-bound paclitaxel, which avoids this solubilizing vehicle and has demonstrated improved tolerability in some clinical settings (33).

The broader lesson from paclitaxel formulation history is that excipient identity, grade, purity and formulation context can materially influence clinical performance (34). For cancer nanomedicine, this reinforces the need to define excipients as critical formulation factors rather than treating them as interchangeable inactive ingredients.

Polysorbates and Docetaxel Formulations

Polysorbates are widely used non-ionic surfactants in pharmaceutical formulations, including some parenteral oncology products. Their clinical performance depends on grade, purity, storage conditions, concentration, degradation profile and formulation context. Highly purified pharmaceutical-grade polysorbates are available and are preferred for parenteral dosage forms because they reduce avoidable impurity-related and compatibility concerns (35).

In docetaxel formulations, polysorbate-containing vehicles have been associated with hypersensitivity reactions and infusion-site reactions, although the contribution of the excipient can be difficult to separate from the effects of the active drug, co-solvents, patient factors and infusion conditions. Polysorbates may undergo oxidative or hydrolytic degradation, generating peroxides, aldehydes or free fatty acids that can affect formulation stability and biological reactivity (36). For this reason, any formulation risk assessment should focus not only on the type of excipient but also on excipient grade, impurity limits, storage conditions, route of administration and cumulative exposure.

Alternative docetaxel formulations have been explored to reduce solvent-related toxicity and improve delivery. Clearly, polysorbate use in parenteral oncology products requires careful quality control, appropriate grade selection and evaluation of degradation products that may influence tolerability or stability (37).

Ionizable and Helper Lipids in Emerging Cancer Nanomedicine

Lipid nanoparticles (LNPs) are gaining prominence as delivery vehicles for messenger RNA, small interfering RNA, gene-editing tools, and cancer vaccines. These systems typically consist of four core components: an ionizable lipid, a helper phospholipid, cholesterol, and a PEG-conjugated lipid. Each plays a role in determining the particle's physical structure, stability, and biological behavior (38).

Ionizable lipids remain largely uncharged at physiological pH but gain a positive charge in acidic environments, such as within endosomes. This characteristic behavior facilitates efficient nucleic acid encapsulation and promotes escape from endosomal compartments, while minimizing the toxicity associated with permanently cationic lipids (38). However, ionizable lipids and their breakdown products can still trigger

innate immune responses, liver toxicity, or adverse effects in specific tissues.

LNPs themselves can act as immune stimulants. Ndeupen et al. found that an LNP formulation used in preclinical mRNA vaccine studies provoked strong inflammatory reactions (39). Lee et al. further analyzed how factors like LNP composition, the type of RNA payload, and activation of innate immune sensors shape both immunogenicity and performance upon repeated administration (40).

There is also evidence that LNP components can activate the complement system. Bakos et al. showed that lipids from mRNA-LNP vaccines could induce complement activation and stimulate pro-inflammatory cytokines under laboratory conditions (41). While vaccine exposure patterns differ from those seen in repeated intravenous cancer treatments, the underlying mechanisms are still pertinent especially for oncology LNPs delivered systemically.

In the context of cancer vaccines, some degree of immune activation may be advantageous. However, for applications involving gene silencing or protein replacement, such responses could interfere with therapeutic goals. Therefore, immunogenicity should be assessed based on the intended function of the therapy, rather than being broadly labeled as either beneficial or harmful. Clinical consequences are difficult to determine based on such findings, but they highlight factors that could be associated with adverse events after multiple doses of nanomedicines, and so should be considered.

Why Repeat Dosing Presents Unique Formulation Challenges

Repeated administration introduces three primary risks:

Pharmacokinetic loss

The development of antibodies against excipients, along with increased opsonization, can lead to faster clearance from circulation and reduced delivery to target tumors. As a result, a formulation that appears effective after the first dose may lose efficacy following immune sensitization (20-21).

Acute reactogenicity

Infusion-related reactions may arise due to complement activation or antibody-mediated responses. The likelihood of such events can depend on the dose level, infusion speed, and time intervals between doses (27-28)

Cross-product interactions

Immune responses triggered by one product containing PEG or surfactants might affect the safety or performance of another. This is significant in oncology, where patients often receive multiple therapeutics, supportive agents, imaging compounds, and vaccines (25), (45).

Consequently, risks associated with repeated dosing vary between individuals and depend on prior exposure history. A purely product-focused evaluation may overlook critical patient-specific factors, and their treatment regimen, such as considering any previous contact not only with the active ingredient but also with structurally similar excipients.

Preclinical Evaluation of Excipient Immunogenicity

Standard toxicology studies often fail to capture the full scope of immune responses elicited by nanomedicines. Differences across species in complement reactivity, antibody production, and mononuclear phagocyte function mean that conventional rodent models may not reliably predict acute cardiopulmonary reactions observed in humans.

Dobrovolskaia et al. advocated for dedicated immunotoxicity testing alongside traditional safety assessments (42). A comprehensive strategy should incorporate: markers of complement activation, including C3a, C5a, and soluble terminal complement complex, measurements of anti-excipient IgM and IgG before and after dosing, analysis of cytokine release, assessment of basophil and mast cell activation when applicable, tests for hemolysis and platelet activation, evaluation of phagocytic uptake, repeat-dose pharmacokinetic profiles, tissue distribution data, effects related to infusion rate, and identification of impurities and degradation byproducts. The correlation between *in vitro* and *in vivo* findings remains limited. Dobrovolskaia et al. stressed that assay results must be interpreted considering the formulation's physicochemical properties and anticipated clinical use (42).

The porcine model has proven highly sensitive to complement-driven cardiopulmonary reactions and has been used to study CARPA. Szebeni et al. highlighted its value in predicting infusion reactions, while Jackman et al. developed a quantifiable model for nanoparticle-induced CARPA (25), (43).

No single test can capture all potential immune reactions. A more robust approach combines physicochemical characterization, human blood-based assays, repeat-dose animal studies, and clinical monitoring to build a weight-of-evidence assessment (42-43).

Clinical Monitoring and Patient Stratification

Routine screening for anti-PEG antibodies is not currently part of standard oncology care. Its implementation is limited

by the lack of standardized detection methods, variability in antibody isotype and binding affinity, uncertain thresholds for clinical significance, possible assay interference from free PEG or other PEGylated substances, inconsistent correlation between antibody status and symptoms, and insufficient prospective clinical outcome data.

Nonetheless, more thorough evaluation may be warranted in certain high-risk scenarios. These include prior severe reactions to PEG-containing drugs, unexplained drops in drug exposure over treatment cycles, enrollment in trials involving highly PEG-dense nanoparticles, or sequential use of multiple PEGylated agents. Perkins et al. demonstrated that PEG displayed on liposomal surfaces can provoke stronger immune signals than free PEG in individuals with known PEG sensitivity (44). This underscores the importance of how an excipient is presented structurally not just its chemical identity in determining immune recognition.

Formulation Approaches to Reduce Immunogenicity

Optimizing PEG Design

The molecular weight, surface density, terminal functional group, and lipid anchor of PEG should be optimized collectively. Reducing surface-exposed PEG may limit antibody binding but risks compromising colloidal stability. PEG-lipids designed to shed quickly after administration can minimize prolonged exposure, though premature detachment may destabilize the particle.

Cleavable or Detachable Stealth Layers

Using environmentally responsive linkers allows PEG to dissociate either during circulation or within the tumor microenvironment. This strategy can be used to balance prolonged circulation with restored cellular interaction at the target site. However, precise control over the timing of cleavage, unintended shedding, and the biological impact of degradation products is essential for effective delivery performance.

Alternatives to PEG

Materials such as polysarcosine, poly(2-oxazoline), polyvinylpyrrolidone, polyzwitterions, and hydrophilic polysaccharides are being explored as PEG substitutes. These provide hydration and steric stabilization without triggering PEG-specific immune responses with such materials consequently having growing relevance of in drug delivery (45).

Polysarcosine is one of the better-studied alternatives. Son et al. reported that liposomes coated with polysarcosine elicited fewer antibodies and avoided the accelerated blood clearance commonly seen with PEGylated counterparts (46). Still, novelty does not guarantee immune inertness. Thorough evaluation through repeat-dose and cross-reactivity testing remains necessary.

Protein-based and Biomimetic Carriers

Systems using albumin demonstrate how replacing surfactants can enhance tolerability in cancer therapy. Other biomimetic strategies such as coating nanoparticles with cell membranes or endogenous proteins may help evade immune detection, though they introduce challenges related to manufacturing consistency and potential antigenicity **(**33**).**

Managing Impurities to Modify Immune Responses to Excipients

Contaminants such as peroxides, aldehydes, free fatty acids, residual solvents, endotoxin, and β-glucans can influence immune responses. Therefore, excipient specifications should consider limits for biologically active impurities, going beyond standard compendial tests for identity and purity **(**36**).**

In summary, several formulation and clinical strategies have been proposed to reduce excipient-related immune reactions and improve the safety of repeated cancer nanomedicine administration. The expected benefits and major limitations of these approaches are presented in Table 2.

Regulatory and Quality Considerations

Excipients in nanomedicines should be treated as critical components, as their molecular distribution, surface density, degradation behavior, impurities, and batch-to-batch consistency can significantly influence biological outcomes.

For PEG-lipids, key characteristics include: the molecular weight distribution of PEG, type of lipid anchor, stability of the linker, levels of free PEG, susceptibility of PEG-lipid to hydrolysis, surface density on nanoparticles, rate of shedding from the particle surface, particle size distribution, potential to activate complement, and ability to bind anti-PEG antibodies (12)**.**

In the case of surfactants like polysorbate 80, peroxide content and fatty acid degradation products are important quality indicators (37**)**. For ionizable lipids, evaluation should cover apparent pKa, metabolic byproducts, residual solvents, and potential to trigger innate immune responses.

Complement activation may also affect product comparability. As Szebeni et al. noted, two liposomal formulations with identical pharmacopoeial compositions might still differ

Table 2. Pros and cons for various excipient design strategies, and clinical testing approaches to mitigate excipient-related immune risk during repeated cancer nanomedicine dosing

Strategy	Expected benefit	Limitation
Reduced PEG density	Lower antibody accessibility	Possible loss of stability and circulation time
Cleavable PEG	Temporary stealth followed by improved cellular uptake	Risk of premature or incomplete cleavage
Rapid-shedding PEG-lipids	Reduced duration of PEG exposure	Lower *in-vivo* stability
Polysarcosine or polyoxazoline coating	Reduced anti-PEG recognition	Limited long-term clinical evidence
Zwitterionic surfaces	Strong hydration and reduced protein adsorption	Synthesis and scale-up challenges
Albumin-based carriers	Avoidance of synthetic surfactants	Protein-source and manufacturing variability
Controlled infusion rate	Reduced acute complement-mediated reactions	Does not prevent antibody-mediated clearance
Drug-free priming vesicles	Possible reduction of infusion reactions through tachyphylaxis	Additional formulation and dosing complexity
Pre-dose antibody testing	Identification of selected high-risk patients	Limited assay standardization
Repeat-dose pharmacokinetic monitoring	Detection of treatment-emergent clearance	Cost and limited routine availability

immunologically if their particle structure or excipient arrangement varies, impacting bioequivalence (47).

For more sophisticated delivery systems used to deliver certain biologics, the decision to employ certain excipients should go beyond a check that it is included on FDA's Inactive Ingredient Database. A substance previously used in conventional formulations cannot be presumed safe when presented at high density on a nanoparticle surface, where its biological interactions may differ substantially.

Knowledge Gaps Requiring Further Attention

Several knowledge gaps remain. First, the prevalence and clinical relevance of anti-excipient antibodies in cancer patients are still uncertain. Malignancy, chemotherapy and immunotherapy may independently alter antibody production, complement activity and inflammatory responses, making extrapolation from healthy populations difficult.

Second, prospective clinical data linking pre-existing anti-PEG antibodies with infusion reactions, pharmacokinetic changes, tumor response or survival outcomes remain limited. Third, most accelerated blood clearance studies have relied on healthy animal models, whereas responses in tumor-bearing subjects receiving combination therapy may differ substantially.

Cross-reactivity between PEG, PEG-containing poloxamers, polysorbates and other polyether-based molecules requires more precise characterization. Many PEG alternatives are still evaluated in short-term preclinical studies without adequate repeat-dose or long-term immunogenicity testing. Finally, regulatory criteria for determining when an excipient change has meaningful immunological consequences remain insufficiently defined.

Future Directions

A stronger development framework for cancer nanomedicines should consider monitoring both pre-existing and newly developed anti-excipient antibodies in selected clinical trials. Pharmacokinetic analysis should cover multiple treatment cycles, not just initial dosing, since repeated administration can affect drug clearance, distribution in the body, and overall therapeutic effectiveness. Clinically relevant human blood assays should be used to assess complement activation and cytokine release. At the same time, key excipient properties such as molecular weight, surface density, chemical linkage, and spatial arrangement should be consistently reported to enhance reproducibility and enable meaningful comparisons across studies.

Research studies should employ infusion rates that reflect clinical practice and, when possible, directly compare PEGylated and non-PEGylated versions of identical delivery

systems. Long-term tracking of drug levels in the bloodstream, tumor response, and immune markers will help clarify how immune reactions to excipients impact treatment outcomes over time.

Advancing the field will also require standardized reference materials for anti-PEG antibody testing, systematic investigation of interactions between different excipient-containing therapies, and the formal inclusion of excipient immunogenicity in quality-by-design approaches. Collectively, these steps offer potential opportunities to improve product safety, to provide more predictable performance with repeated dosing, and that may positively impact clinical translation of cancer nanomedicines.

Current advances in artificial intelligence and its ability to assimilate diverse data sets to learn and predict outcomes will also contribute to improving understanding in this area (48).

CONCLUSION

Excipients play an active role in determining the performance of cancer nanomedicines. Components such as PEG, PEG-lipids, surfactants, and structural lipids enhance formulation stability and delivery efficiency, but they also modulate immune responses including antibody development, complement activation, pharmacokinetics, and infusion-related tolerability.

These effects become evident during repeated administration. Anti-excipient antibodies can change a long-circulating formulation towards more rapid clearance. Complement activation may turn a stabilizing surface component into a trigger for acute immune reactions. Solubilizing agents also have the potential to alter the toxicity profile and distribution of anticancer drugs.

Technologies like PEGylation and surfactant-based formulation continue to offer important benefits to therapeutic activity and product performance. While there is considerable mechanistic evidence for a variety of immune responses can be elicited by a range of excipients used in nanomedicine and cancer therapy, there is very little definitive information to confirm that these lead to treatment failure. For many cancer therapies dose-limiting toxicities can be due to a multitude of issues such as myelosuppression cardiotoxicity, hepatotoxicity, GI toxicity, neuropathy, or the biological effects of the drug itself. Host-related factors such as hyper-or suppressed immune conditions can also influence individualized immune responses to therapies, and the excipients used in them. Nevertheless, when designing parenteral formulations for such treatments it is recommended that excipient selection be paired with tailored immunological evaluation to understand, and to mitigate, any potential risks. Repeated-dose studies, complement assessments, antibody monitoring, control of impurities, and consideration of clinical exposure history should all become more standard practice in formulation design for the more advanced delivery systems in this therapeutic area.

Recognizing the important active biological roles of functional excipients incorporated into nanomedicine formulations, and how they behave in the body, will be increasingly important for the creation of new products that perform consistently from the first dose and throughout the entire course of treatment.

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Declarations

Ethics approval and consent to participate.

Not applicable. This narrative review did not involve human participants, human data or animal subjects.

Competing interests

The authors declare no competing interests.

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