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DISCUSSION

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PEGylation technology: addressing concerns, moving forward

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ABSTRACT

PEGylation technology, that is grafting of poly(ethylene glycol)(PEG) to biologics, vaccines and nanopharmaceuticals, has become a cornerstone of modern medicines with over thirty products used in the clinic. PEGylation of therapeutic proteins, nucleic acids and nanopharmaceuticals improves their stability, pharmacokinetic and biodistribution. While PEGylated medicines are safe in the majority of patients, there are growing concerns about the emergence of anti-PEG antibodies and their impact on the therapeutic efficacy of PEGylated medicines as well as broader immune responses, particularly in complement activation and hypersensitivity reactions. These concerns are beginning to scrutinize the future viability of PEGylation technology in medicine design. Here, we outline these concerns, encourage more efforts into looking for comprehensive scientific evidence on the role of anti-PEG antibodies in hypersensitivity reactions, discuss alternatives to PEG and propose strategies for moving PEGylation technology forward.

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1. Introduction

In the dynamic world of drug delivery and biotechnology, few innovations have had as profound an impact as PEGylation: the covalent attachment of PEG to biomolecules, particulate drug delivery systems, cells, and implant and stent surfaces. Since the pioneering work of Frank Davis and coworkers on protein PEGylation (Abuchowski et al. 1977; Davis et al. 1979; Gabizon et al. 2003), PEGylation has become a cornerstone of pharmaceutical formulation, particularly in enhancing the stability and therapeutic performance of protein drugs and of various drug delivery systems. The ease of synthesis and characterization of PEGylated proteins, polymers and constructs further underscores their utility in a wide range of clinical products in the market and reviewed recently (Gao et al. 2024). Particularly, the PEGylation protein market is experiencing rapid growth, where the global PEGylation proteins market size is currently estimated at US$ 2.4 billion and is forecasted to reach over US$ 6 billion by 2034.

PEGylation has historically been praised for its ability to protect and prolong the circulation time of therapeutic molecules and nanomedicines (Davis et al. 1979; Gabizon et al. 2003; Gao et al. 2024). These properties are endowed by PEG flexibility (due to the absence of double and triple bonds) and its ability to form hydrogen bonds with many water molecules. For example, PEG_{2000} is a polymer consisting of 45 ethylene oxide units that can bind to 136 water molecules or about 3 water molecules per ethylene oxide unit, where the molecular weight of hydrated PEG_{2000} is more than double that of non-hydrated PEG (Tirosh et al. 1998). High hydration and flexibility make PEG a bulky molecule, allowing it to sterically stabilize (involving elastic and osmotic contributions) therapeutic proteins and nanoparticles and minimize their interaction with other molecules and cell surface receptors (Davis et al. 1979; Blume and Cevc 1990; Klibanov et al. 1990; Moghimi and Szebeni 2003). The density of PEG on the surface of proteins or nanoparticles influences the molecule's conformation. Thus, at a low surface density, PEG assumes a 'mushroom' conformation, while at high density it takes on a 'brush' conformation (Moghimi and Szebeni 2003; Garbuzenko et al. 2005). Today, it is widely recognized that the biological performance of a PEGylated construct (e.g., pharmacokinetics,

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This article has been corrected with minor changes. These changes do not impact the academic content of the article.

protein binding and macrophage sequestration) is dependent on the molecular weight of the PEG moiety and its surface density (reviewed by Haroon et al. 2022).

As the field has evolved, the discourse surrounding PEGylation has increased, with recent debates highlighting both its revolutionary potential and emerging concerns, notably PEG safety (Chen et al. 2021; Anchordoquy et al. 2024; Fu et al. 2024; Simberg and Moghimi 2024). One significant concern that has gained attention in recent years is the activation of the complement system (Simberg and Moghimi 2024), a critical component of the innate immune system, by PEGylated constructs. Complement activation and fixation is a double-edge sword; it offers a protective role against intruders through lysing and opsonization mechanisms, yet when uncontrolled, complement activation triggers proinflammatory reactions and organ damage (Haroon et al. 2023). Hamad et al. (2008) were the first to report complement activation on a time-scale of minutes by high concentrations of soluble PEG and in a PEG molecular weight-dependent manner, where high molecular weight PEGs (>10,000 Da) being most effective in activating human complement. Activation of the alternative pathway of complement by high PEG concentration is thought to arise through water activity and effective hydration and conformational changes of the third complement protein (C3), resulting in accelerated 'C3 tickover'. The extent of PEG hydration increases with PEG molecular mass. Therefore, increasing PEG size and concentration both increases proteins' effective hydration. This is due to PEG being excluded from the protein's surface as well as protein partitioning (C3 in this case) into hydrophobic PEG phase, which compete with the steric exclusion (Bhat and Timasheff 1992). With low molecular weight PEGs (<5,000 Da), the PEG phase is too short resulting in exclusion of partitioned C3 due to reduced available water content, which could explain the poor ability of short PEGs in activating alternative pathway, unless PEG concentration is exceedingly high. In relation to the latter, a single subretinal injection of PEG 400 Da (1 mg in 2 µL) in mice was shown to induce choroidal neovascularisation through local complement activation (increased levels of the C3 split products in retinal pigment epithelium and choroid, co-localised with C9 deposition), presumably through accelerated local C3 hydration and increased alternative pathways turnover (Lyzogubov et al. 2011). PEGs also activate human complement through the lectin pathway (Hamad et al. 2008).

It has widely been demonstrated that complement also recognizes PEGylated surfaces as foreign, including a wide range of PEGylated preclinical and clinical nanoparticle formulations (Vu et al. 2019; Moghimi et al. 2020; Li et al. 2024, Szebeni et al. 2018, 2022). Quantitatively, these nanoparticles display very small amount of low molecular weight PEG-conjugates as in PEG-phospholipids (PEG = 2000–5000 Da), but at comparable concentrations free PEG molecules as well as PEG-phospholipid micelles do not activate complement (Moghimi et al. 2006). These differences suggest that PEG conformation and density, PEG-conjugate linkage type and protein intercalation into the surface PEG cloud modulate complement activation by PEGylated nanoparticles (Moghimi et al. 2020). Unintended complement activation, however, is problematic as it can reduce the effectiveness of the drug delivery system (e.g., by inducing drug leakage from liposomes and promoting nanoparticle clearance by phagocytic cells through C3 opsonization) and could potentially lead to hypersensitivity through multifaceted mechanisms (Szebeni et al. 2018; Moghimi et al. 2023).

Another major issue is the increasing incidence of anti-PEG antibodies of IgM and IgG (and to some extent IgE) classes in the population. These antibodies can develop in response to repeated exposure to PEGylated substances, such as through cosmetics, food, or vaccines, including those for COVID-19 (Chen et al. 2021; Ju et al. 2022; Zhou et al. 2023; Fu et al. 2024). The presence of these antibodies is thought to pose a significant challenge, as they can bind to PEGylated therapeutics, potentially rendering them ineffective, trigger complement activation through multiple pathways, and promote clearance by phagocytic cells (Chen et al. 2021; Fu et al. 2024; Simberg and Moghimi 2024). These concerns have led to intense debate on the future viability of PEGylation, with some questioning whether the technology may become obsolete, particularly as anti-PEG antibodies become more prevalent. Here, we list these concerns and propose concerted strategies and a roadmap for moving the field forward.

2. Main

2.1. Emerging concerns and challenges

2.1.1. Hypersensitivity reactions

Infusion of PEGylated medicines in some individuals triggers acute allergic reactions with mucocutaneous (e.g., skin flushing or rash, urticaria), cardio-pulmonary (e.g., chest tightness, hypotension, bronchospasm, dyspnea, tachycardia, back pain, wheezing, angioedema), autonomic (e.g., dizziness, nausea, vomiting, sweating) and neuro-psychosomatic manifestations (Szebeni et al. 2018; Moghimi et al. 2023). These reactions evolve within minutes of infusion and not initiated or mediated by preexisting IgE antibodies. Some individuals may also experience delayed onset of symptoms, which could be related to cytokine release storm, but not investigated in detail. The aforementioned infusion reactions are not unique to PEGylated medicines and shared with non-PEGylated nanomedicines and therapeutic monoclonal antibodies (Szebeni et al. 2018; Moghimi 2018). There are suggestions that uncontrolled complement activation by PEGylated medicines (as well as non-PEGylated nanomedicines) and particularly the liberation of anaphylatoxins C3a and C5a are a causative factor (Szebeni et al. 2018). Recent studies in rats have also indicated that complement opsonization could be another causative factor (Li et al. 2024a), triggering adverse reactions, perhaps, through complement receptor-mediated pro-inflammatory signaling pathways. Available evidence from the porcine model strongly supports an effector role for pulmonary intravascular macrophages (PIMs) in hypersensitivity reactions to PEGylated medicines regardless of complement activation (Wibroe et al. 2017; reviewed recently, Moghimi et al. 2023). PIMs are absent in the lungs of humans

and other commonly used laboratory animals (mice, rats, rabbits, dogs), but induced PIMs in rats and humans have been reported under certain pathological conditions of the liver and lungs (Moghimi 2018). Thus, what remains to be unraveled is the role of other macrophages in contact with blood (e.g., Kupffer cells and splenic marginal zone and red-pulp macrophages), neutrophils, monocytes, dendritic cells, mast cells, platelets and possible interplay between some immune cells in human infusion related reactions (reviewed by Moghimi et al. 2023). Notwithstanding, anti-PEG antibodies could play important roles in triggering adverse reactions through Fc- and/or complement-receptor (e.g., by promoting C3 opsonization) signaling processes (Chen et al. 2023; Li et al. 2024a). Regardless of underlying mechanisms, in clinical practice and in most cases, the incidence and severity of hypersensitivity reactions by PEGylated medicines (e.g., Oncaspar, Onivyde, Onpattro, Palynziq, PEGylated liposomal doxorubicin products) is reduced with universal premedication with a cocktail of corticosteroids, H2 blockers and acetaminophen as well as by slowing down the infusion/injection rate (Szebeni et al. 2018; Moghimi et al. 2023). However, there are examples where universal premedication prior to PEG-medicine administration (e.g., PEGylated asparaginase) did not alter incidence or severity of hypersensitivity reactions (Menig et al. 2024). Followings are examples of PEGylated medicines withdrawn from the market or stopped at late-stage clinical trials due to safety concern: Omontys (peginesatide), Krystexxa (pegloticase) and Revolixys (pegnivacogin).

2.1.2. Prescreening for anti-PEG antibodies

As the prevalence of anti-PEG antibodies increases and considering the incidence of hypersensitivity reactions to PEGylated medicine, there is growing discussion about whether patients should be prescreened for these antibodies before receiving PEGylated therapies (Simberg and Moghimi 2024). Determining a 'safe' concentration of anti-PEG antibodies for different classes of PEGylated drugs and nanomedicines is an area requiring further research and clinical validation.

2.1.3. Differences in vaccine-induced anti-PEG antibodies

Interestingly, the Pfizer-BioNTech Comirnaty® vaccine induces fewer anti-PEG antibodies compared with the Moderna Spikevax® (Carreño et al. 2022; Ju et al. 2022). The reasons for this difference are not yet fully understood but could be related to differences in stability, dose, nanoparticle size distribution and surface characteristics. Indeed, these parameters modulate nanoparticle drainage into the local lymphatic system and uptake by lymph node phagocytic cells (Moghimi et al. 2006). However, it raises important questions about the design of LNP mRNA therapies, including their stability in biological fluids and how they can be optimized to minimize the induction of anti-PEG antibodies. Of note, the affinity of humanized anti-PEG IgG and IgM for Comirnaty is lower than for PEGylated liposomal products such as Doxil, which could be related to differences in surface PEG density and/or conformation (Bavli et al. 2023).

2.1.4. Impact on other PEGylated medicines

There is concern that the mRNA vaccines based on PEGylated LNPs might impact the efficacy or safety of other PEGylated medicines in patients, particularly if they lead to the generation of cross-reactive anti-PEG antibodies (Chen et al. 2021; Simberg and Moghimi 2024). This interaction needs to be carefully studied to ensure that the benefits of PEGylation are not compromised. For instance, PEGylated liposomes, particularly those with a low surface PEG density are susceptible to destabilization by anti-PEG antibodies through complement activation and insertion of the membrane attack complex into the liposome bilayer (Chen et al. 2020, 2024).

2.2. Moving forward: balancing innovation with safety

As the pharmaceutical industry continues to innovate, it is essential to balance the benefits of PEGylation with the potential risks. While PEGylation has undoubtedly revolutionized drug delivery, particularly in the field of nanomedicine, the aforementioned concerns about immune responses and the emergence of anti-PEG antibodies cannot be ignored. With the increasing recognition of anti-PEG antibodies and their potential to induce immune responses, it becomes crucial to develop strategies that can foresee and prevent adverse effects in patients.

2.2.1. Standardization of anti-PEG assays

As anti-PEG antibodies become a significant concern, the need for standardized assays to detect and quantify these antibodies is becoming increasingly important. Currently, there is a lack of uniformity in how anti-PEG antibodies are measured across different studies and clinical trials. This inconsistency makes it challenging to compare results and draw definitive conclusions about the prevalence and impact of anti-PEG antibodies. The standardization of anti-PEG assays would involve the development of validated protocols that can be universally adopted across laboratories (Chen et al. 2016, 2021; Li et al. 2024b). These assays should be sensitive, specific, and capable of distinguishing between different classes of anti-PEG antibodies, such as IgG, IgM and IgE. The inclusion of reference anti-PEG antibody standards can help compare between different laboratories and studies. Additionally, standardized assays would allow for more accurate screening of patients before administering PEGylated therapies, ensuring that those at risk of adverse reactions can be identified and managed appropriately. The pharmaceutical industry, in collaboration with regulatory bodies, must prioritize the development and implementation of these standardized assays. Doing so will not only improve patient safety but also facilitate the continued use of PEGylation in drug delivery.

2.2.2. Anti-PEG antibody specificity

Available information on the mode of anti-PEG antibody binding to PEG is limited to and derived from the crystal structures of anti-PEG antibody Fab fragments (Huckaby et al. 2020). This has identified an open ring-like structure in the Fab paratope that binds 16 monomer subunits in flexible PEG

chains. Understanding this mechanism (i.e. the extent of chain flexibility-rigidity) can potentially impact the PEGylation strategy and provide means for the development of safer PEGs (for instance by increasing macromolecular rigidity through PEG branching). However, not much is known about the mode of anti-PEG antibody binding to PEGylated surfaces composed of different PEG molecular weights and densities in full blood or lymph to account for the role of nonspecific protein binding/intercalation. This is important, particularly when differentiating between low versus high-affinity anti-PEG antibodies and consequences thereof, such as complement activation and interaction with Fc receptors. Nonspecific protein binding to PEGylated nanoparticles could either mask anti-PEG antibody binding or even promote the binding of such antibodies by influencing PEG conformation (Moghimi et al. 2023). Therefore, future work should focus in purifying anti-PEG antibodies, distinguishing between low- and high-affinity anti-PEG antibodies and assessing their mode of binding to PEGylated proteins and nanoparticles in the blood. This is important, since there are major differences between PEGylated proteins/enzymes (they usually carry PEGs >10,000 Da and the number of attached PEG molecules vary from one to tens, depending on the protein type) versus PEGylated nanoparticles (they usually display PEGs of 2000–5000 Da with variable surface density, depending on nanoparticle type) (Gao et al. 2024). Thus, differences in PEG sizes, hydration and conformational cloud could modulate the mode and the extent of anti-PEG antibody binding and responses thereof.

2.2.3. Strategies to predict PEG-associated toxicities

Predicting and mitigating PEG-associated toxicities is an area of growing interest within the pharmaceutical industry. One approach is the development of predictive assays that simulate human immune responses to PEGylated drugs in vitro. By understanding how PEGylated nanoparticles or drugs interact with human immune cells and proteins, researchers can identify potential risks before clinical trials begin. Additionally, advancements in computational modeling and machine learning can be leveraged to predict how variations in PEG structure, size and density might influence immune responses.

Toward such efforts, PEG-pairing of nanoparticles (surface functionalization with a combination of low and high molecular weight PEGs) dramatically reduces complement activation by altering PEG conformational attributes, which minimize statistical protein binding/intercalation (Pannuzzo et al. 2020). However, the impact of PEG-pairing on anti-PEG antibody binding has not been investigated. Notwithstanding, a recent study has shown that nanoparticle surface coverage with high-density brush-shaped PEG conjugates can reduce anti-PEG antibody binding (Xiao et al. 2025). In contrast to the abovementioned surface engineering initiatives, one study demonstrated the effectiveness of intravenous free PEG 40k Da administration (50 mg/kg) as a prophylaxis against anaphylaxis induced by PEGylated liposome in swine (Shen et al. 2024). Interestingly, free PEG dosing itself did not induce hypersensitivity, presumably due to its blood concentration levels being far below concentrations needed to over-activate complement (Moghimi et al. 2006; Hamad et al. 2008). However, the desensitization-induced mechanism(s) of free PEG administration is unclear, but this could be related to the modulation of Fcγ receptor signaling by PEG-antibody complexes in porcine pulmonary intravascular macrophages (Moghimi 2018; Moghimi et al. 2023). Of note, IgE antibodies against PEG have casually been implicated in rare hypersensitivity reactions to PEG imaging agents and lipid nanoparticle (LNP) mRNA vaccines (Zhou et al. 2023). Administration of free PEG molecules as a prophylaxis measure, however, could be detrimental in individuals with pre-existing anti-PEG IgE antibodies.

While research has predominantly focused on antibody- and complement-mediated responses, it is instrumental to address complement- and immunoglobulin-independent mechanisms by which PEGylated particles interact with cell receptors such as scavenger, Toll-like and lipoprotein receptors (Soenen et al. 2014; Asoudeh et al. 2024). For example, many PEGylated nanoparticles interact with apolipoproteins and lipoproteins and these associations could play important roles in cell uptake through lipoprotein receptors and modulate intracellular processes leading to desirable or undesirable effects (Li et al. 2022; Liu et al. 2023). Understanding the mode of interaction could evolve in developing PEG architectures or derivatives to modulate nanomedicine pharmacokinetics and improve broader safety. Another important issue that has rarely received attention is the intracellular fate of PEG (Moghimi and Szebeni 2003). Considering the notional free energy of hydrogen binding (Lloyd 1998), it is unlikely for PEGylated molecules to escape phagolysosomes. Thus, gradual PEG accumulation in lysosomes could alter organelle density, modify or modulate lysosomal transporters and activity of lysosomal enzymes and trigger untoward responses.

2.2.4. The lesser evil: do we need alternatives to PEG?

As the debate around PEGylation intensifies, a critical question emerges: do we genuinely need alternatives to PEG? Given the growing concerns about anti-PEG antibodies and immune reactions, some researchers argue for the development of new polymers that could replace PEG. However, the risks associated with the widespread presence of anti-PEG antibodies in the population are not yet fully understood. Although PEG antibodies are widespread, they are likely low affinity and present at low concentrations in most individuals (Chen et al. 2021). Similar to anti-PEG antibodies, anti-dextran antibodies are also prevalent in most individuals and have been associated with hypersensitivity reactions to intravenous iron therapy of dextran-stabilized iron(III)-oxyhydroxide/oxide nanoparticles (Fleming et al. 1992; Wang et al. 2015). While debate continues, recent studies (Vu et al. 2019; Li et al. 2024) suggest that the presence of natural antibodies recognizing foreign surfaces is a broader issue at least with respect to complement activation and opsonization, not unique to PEG or dextran.

Thus, is replacing PEG might be a case of 'the lesser evil'? PEG has established an impressive track record in terms of manufacturing, characterization, and chemistry. It has taken

decades for PEG to become a trusted pharmaceutical excipient, with regulatory bodies like the US Food and Drug Administration being well-versed in its toxicology and pharmacology profiles. Replacing PEG with a new polymer would not only be an uphill battle but could also introduce unforeseen challenges that could take decades to fully understand and mitigate. Other polymers are also likely to induce polymer-specific antibodies in similar applications in which PEG displays immunogenicity (as in the case of dextran). Moreover, alternatives may not be suitable for all applications. PEG's unique properties, such as its ability to enhance drug solubility and extend circulation time, are not easily replicated. While it is important to explore and innovate, abandoning PEG entirely could be counterproductive. Instead, optimizing PEGylation (e.g. as in PEG-pairing initiatives and functionalization with high-density brush-shaped PEG conjugates) to minimize its drawbacks might be a more pragmatic approach, balancing the need for innovation with the realities of regulatory and clinical practice. Thus, rather than abandoning PEG, the focus should be on looking for further scientific evidence on the role of anti-PEG antibodies in hypersensitivity reactions.

2.2.5. What alternatives to PEG are available?

Exploring alternatives, such as tagging drug carriers (or proteins) to albumin, Fc fragments, polysarcosine, polyglycerol derivatives, polyvinylpyrrolidone and polyoxazolines has been proposed (Yang et al. 2016; Hoang Thi et al. 2020; Berger et al. 2023; Gao et al. 2024; Kang et al. 2024; Lorson et al. 2018). Likewise, 'zwitterionic polymers' (polymers having a pair of oppositely charged groups in their repeating units) also offer a strong hydration effect through ionic solvation and drawing attention as an alternative to PEGylation to minimize protein adsorption and enhance protein stability (Estephan et al. 2011). However, not all these alternatives may be suitable for all applications, particularly in the case of nanoparticles, which have large surface areas and specific requirements that PEG has successfully met. Other complications include grafting chemistries, accumulation in cells and even immunogenicity upon frequent administration. Furthermore, species difference in immune responses must be considered and responses in preclinical animal models may not correspond to and reflect human responses (discussed in section 2.2.6).

Notwithstanding, among the aforementioned PEG alternatives, polyoxazolines are attracting increasing attention as an alternative to PEGylation in pharmaceutical development (Bludau et al. 2017; Lorson et al. 2018; Golba et al. 2025). A potential advantage of polyoxazolines over PEG and other alternatives is the vast diversity of monomers that can be employed during polymerization, allowing for a wide variation of polymer properties (Mahand et al. 2022). Polyoxazolines are synthesized by living cationic ring-opening polymerization, which provides direct control of polymer chain length and low dispersity (Đ = Mw/Mn ~ 1.05 to 1.3). Polyoxazolines exhibit thermal stability, do not undergo peroxidation and are miscible in a wide variety of solvents. The controlled process allows for the quantitative introduction of terminal functional groups and various pendant side groups via functional monomers and polymer analog reactions, making the manufacturing of end-functionalised end-functionalized and telechelic polymers technically facile. Additionally, block, gradient, and random copolymerization of different 2-oxazoline monomers are possible. As to their biological evaluation, substituting PEG-lipid with poly(2-ethyl-2-oxazoline)-lipid in LNPs has been reported to decrease IgM formation against the respective polymer and improve gene expression in the liver after repeated dosing in mice (Sanchez et al. 2024). Poly(2-methyl-2-oxazoline), a polymer more hydrophilic than both PEG and poly(2-ethyl-2-oxazoline), was also employed in polyplexes for nucleic acids delivery (He et al. 2015; Yamaleyeva et al. 2023) and has shown considerably less plasma protein binding than similar PEG-based construct. Today, some poly(2-ethyl-2-oxazoline)-conjugated drugs are commercially being developed (Nasdaq.com 2024).

There are also developments with block copolymers of poly(2-oxazolines) and more recently poly(2-oxazolines)-pol y(2-oxazines) have emerged as a novel polymeric micelle platform for drug delivery, uniquely capable of carrying high loads of water-insoluble drugs with improved safety (He et al. 2016; Lübtow et al. 2017; Wan et al. 2018; Alves et al. 2019; Hwang et al. 2020, 2021; Zahoranová and Luxenhofer 2021; Lim et al. 2023). This capability enhances the solubility, stability, efficacy, and safety of multiple drugs, allowing for 10 to 100 times higher drug loads than other solubilization methods, with drug loadings often reaching 40 to 50% by weight (Alves et al. 2019).

Of note, polyvinylpyrrolidone was used as an effective plasma substitute given to >500,000 human subjects with excellent safety records over years (Hecht et al. 1943; Ravin et al. 1952). Thus, pyrrolidonation could potentially serve as a prominent alternative strategy to PEGylation. In support of this notion, pyrrolidonation of poly(amido amine) dendrimers has, at least, overcome complement activation in human plasma (Wu et al. 2021).

2.2.6. Relevance of the toxicities observed in animal models

Animal models have been instrumental in understanding the safety and efficacy of PEGylated and broader non-biological complex drugs. However, the relevance of toxicities observed in these models to human patients is a topic of ongoing debate (Moghimi 2018; Moghimi and Simberg 2018; Li et al. 2021). While animal studies can provide valuable insights, they often do not fully replicate the complexities of the human immune system. For instance, the prevalence and behavior of anti-PEG antibodies can differ significantly between species, leading to potential discrepancies in how PEGylated drugs are tolerated.

Furthermore, there are species differences in the extent and pathways of complement activation by PEGylated (and non-PEGylated) nanomedicines and associated responses (Tavano et al. 2018; Li et al. 2021, 2024a; Moghimi and Simberg 2022). A notable example is the difference in complement activation and complement opsonization of polyoxazoline-coated nanoparticles between mice, pigs and

humans (Tavano et al. 2018, 2025). Polyoxazolinated nanoparticles overcome complement activation in mice sera, where the lack of C3 opsonization contributes to their poor recognition and uptake by primary murine macrophages (Tavano et al. 2018). This is in line with the long-circulating properties of intravenously injected polyoxazoline-coated/grafted nanoparticles in mice (Zalipsky et al. 1996; Bludau et al. 2017). However, in both human and porcine sera polyoxazolinated particles robustly trigger complement activation and this promotes their uptake by primary human and porcine macrophages, respectively (Tavano et al. 2018, 2025). Furthermore, the porcine pattern-recognition molecule ficolin 2, through its S2 binding site recognizes polyoxazolines and this directly promotes nanoparticle uptake exclusively by monocytes. In human sera, ficolin opsonization of polyoxazolinated nanoparticles is isoform-dependent, showing inter-individual variability (Tavano et al. 2025). These observations suggest that unlike murine, polyoxazolinated nanoparticles may not exhibit prolonged circulation times in swine and human subjects. Of note, in a different study drug-loaded poly(2-oxazoline)-poly(2-oxazine) micelles did not trigger complement activation in citrated human plasma (He et al. 2016). The reason for this is not clear, but it may be related to the 'soft' nature of these micelles or their small size, or to the plasma preparation and handling (including the anticoagulant type) (Moghimi and Simberg 2022).

Other examples include pigs and sheep. Unlike normal human lungs, pigs and sheep have intravascular pulmonary macrophages that rapidly respond to many types of intravenously injected PEGylated and non-PEGylated nanoparticles by releasing proinflammatory mediators that cause vasoconstriction, bronchoconstriction and pulmonary hypertension (Moghimi and Simberg 2018).

Moreover, the doses and formulations used in animal studies may not always correlate with those used in human clinical trials. This raises questions about the translatability of animal data to real-world clinical outcomes. As such, while animal models remain a critical component of preclinical testing, their limitations must be acknowledged, and their findings must be interpreted with caution. To bridge this gap, there is a need for more sophisticated models that better mimic human physiology and immune responses. Additionally, integrating data from animal studies with human cell-based assays and clinical data can provide a more comprehensive understanding of the potential risks associated with PEGylation.

3. Conclusions

PEGylation has played a pivotal role in advancing protein drug products and drug delivery systems, offering numerous benefits that have improved patient outcomes. However, as the understanding of its limitations grows, the pharmaceutical industry must address these challenges to ensure the continued success and safety of PEGylated drugs. Longitudinal studies that monitor patients for signs of immune sensitization to PEG over time, however, can provide valuable insights into the long-term safety of PEGylated therapies. These strategies, combined with ongoing research into the mechanisms of PEG-associated toxicities, will be essential in ensuring the safe and effective use of PEG in future drug formulations. Through ongoing innovations in immunogenomics, anti-PEG antibody assays/diagnostics, machine learning, and artificial intelligence-powered approaches in materials design and engineering, as well as exploiting orthogonal effects of currently available complement inhibitors, it is possible to overcome these hurdles and continue to harness the full potential of PEGylation technology in the future of medicine. These approaches could introduce and implement better risk assessment strategies in treatment and patient selection and pave the path toward stratified therapies with PEGylated medicines.

Authors' contributions

CRediT: **Dmitri Simberg**: Conceptualization, Formal analysis, Writing – original draft, Writing: review & editing; **Yechezkel Barenholz**: Conceptualization, Formal analysis, Writing – review & editing; **Steve R. Roffler**: Conceptualization, Formal analysis, Writing – review & editing; **Katharina Landfester**: Conceptualization, Formal analysis, Writing – review & editing; **Alexander V. Kabanov**: Conceptualization, Formal analysis, Writing – review & editing; **Seyed M. Moghimi**: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing.

Disclosure statement

D.S. is a member of the editorial board of Drug Delivery. S.M.M. is the Editor-in-Chief of Drug Delivery. A.V.K. holds patents and patent applications involving polyoxazolines and other polymers. He is also a co-founder and shareholder of Softkemo Pharma Corp. and DelAQUA Pharmaceuticals Inc., both of which are involved in the development of PEG or polyoxazoline-based pharmaceuticals. The authors declare that they have no other competing interests.

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Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

References

Abuchowski A, McCoy JR, Palczuk NC, van Es T, Davis FF. 1977. Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. J Biol Chem. 252(11):3582–3586.

Alves VM, Hwang D, Muratov E, Sokolsky-Papkov M, Varlamova E, Vinod N, Lim C, Andrade CH, Tropsha A, Kabanov A, et al. 2019. Cheminformatics-driven discovery of polymeric micelle formulations

for poorly soluble drugs. Sci Adv. 5(6):eaav9784. doi: 10.1126/sciadv.aav9784.

Anchordoquy T, Artzi N, Balyasnikova IV, Barenholz Y, La-Beck NM, Brenner JS, Chan WCW, Decuzzi P, Exner AA, Gabizon A, et al. 2024. Mechanisms and barriers in nanomedicine: progress in the field and future directions. ACS Nano. 18(22):13983–13999. doi: 10.1021/acsnano.4c00182.

Asoudeh M, Nguyen N, Raith M, Denman DS, Anozie UC, Mokhtarnejad M, Khomami B, Skotty KM, Isaac S, Gebhart T, et al. 2024. PEGylated nanoparticles interact with macrophages independently of immune response factors and trigger a non-phagocytic, low-inflammatory response. J Control Rel. 366:282–296. doi: 10.1016/j.jconrel.2023.12.019.

Bavli Y, Chen B-M, Gross G, Hershko A, Turjeman K, Roffler S, Barenholz Y. 2023. Anti-PEG antibodies before and after a first dose of Comirnaty® (mRNA-LNP-based SARS-CoV-2 vaccine). J Control Rel. 354:316–322. doi: 10.1016/j.jconrel.2022.12.039.

Berger M, Toussaint F, Djemaa SB, Laloy J, Pendeville H, Evrard B, Jerôme C, Lechanteur A, Mottet D, Debuigne A, et al. 2023. Poly(vinyl pyrrolidone) derivatives as PEG alternatives for stealth, non-toxic and less immunogenic siRNA-containing lipoplex delivery. J Control Rel. 361:87–101. doi: 10.1016/j.jconrel.2023.07.031.

Bhat R, Timasheff SN. 1992. Steric exclusion is the principle source of the preferential hydration of proteins in the presence of polyethylene glycols. Protein Sci. 1(9):1133–1143. doi: 10.1002/pro.5560010907.

Bludau H, Czapar AE, Pitek AS, Shukla S, Jordan R, Steinmetz NF. 2017. POxylation as an alternative stealth coating for biomedical applications. Eur Polym J. 88:679–688. doi: 10.1016/j.eurpolymj.2016.10.041.

Blume G, Cevc G. 1990. Liposomes for sustained drug release in vivo. Biochim Biophys Acta. 1029(1):91–97. doi: 10.1016/0005-2736(90)90440-y.

Carreño JM, Singh G, Tcheou J, Srivastava K, Gleason C, Muramatsu H, Desai P, Aberg JA, Miller RL, Study Group P, et al. 2022. mRNA-1273 but not BNT162B2 induces antibodies against poly(ethylene glycol (PEG) contained in mRNA-based vaccine formulations. Vaccine. 40(42):6114–6124. doi: 10.1016/j.vaccine.2022.08.024.

Chen B-M, Chen E, Lin Y-C, Tran TTM, Turjeman K, Yang S-H, Cheng T-L, Barenholz Y, Roffler SR. 2024. Liposomes with low levels of grafted poly(ethylene glycol) remain susceptible to destabilization by anti-poly(ethylene glycol) antibodies. ACS Nano. 18(33):22122–22138. doi: 10.1021/acsnano.4c05409.

Chen B-M, Cheng TL, Roffler SR. 2021. Polyethylene glycol immunogenicity: theoretical, clinical, and practical aspects of anti-polyethylene glycol antibodies. ACS Nano. 15(9):14022–14048. doi: 10.1021/acsnano.1c05922.

Chen B-M, Su Y-C, Chang C-J, Burnouf P-A, Chuang K-H, Chen C-H, Cheng T-L, Chen Y-T, Wu J-Y, Roffler SR, et al. 2016. Measurement of pre-existing IgG and IgM antibodies against polyethylene glycol in healthy individuals. Anal Chem. 88(21):10661–10666. doi: 10.1021/acs.analchem.6b03109.

Chen E, Chen B-M, Su Y-C, Chang Y-C, Cheng T-L, Barenholz Y, Roffler SR. 2020. Premature drug release from polyethylene glycol (PEG)-coated liposomal doxorubicin via formation of the membrane attack complex. ACS Nano. 14(7):7808–7822. doi: 10.1021/acsnano.9b07218.

Chen W-A, Chang D-Y, Chen B-M, Lin Y-C, Barenholz Y, Roffler SR. 2023. Antibodies against poly(ethylene glycol) activate innate immune cells and induce hypersensitivity reactions to PEGylated nanomedicines. ACS Nano. 17(6):5757–5772. doi: 10.1021/acsnano.2c12193.

Davis FF, van Es T, Palczuk NC. 1979. Non-immunogenic polypeptides. US Patent. US:4179337.

Estephan ZG, Schlenoff PS, Schlenoff JB. 2011. Zwitteration as an alternative to PEGylation. Langmuir. 27(11):6794–6800. doi: 10.1021/la200227b.

Fleming LW, Stewart WK, Parratt D. 1992. Dextran antibodies, complement conversion and circulating immune complexes after intravenous iron dextran therapy in dialysed patients. Nephrol Dial Transplant. 7(1):35–39.

Fu J, Wu E, Li G, Wang B, Zhan C. 2024. Anti-PEG antibodies: current situation and countermeasures. Nano Today. 55:102163. doi: 10.1016/j.nantod.2024.102163.

Gabizon A, Shmeeda H, Barenholz Y. 2003. Pharmacokinetics of pegylated liposomal doxorubicin: Review of animal and human studies. Clin Pharmacokinet. 42(5):419–436. doi: 10.2165/00003088-200342050-00002.

Gao Y, Joshi M, Zhao Z, Mitragotri S. 2024. PEGylated therapeutics in the clinic. Bioeng Transl Med. 9(1):e10600. doi: 10.1002/btm2.10600.

Garbuzenko O, Barenholz Y, Priev A. 2005. Effect of grafted PEG on liposome size and on compressibility and packing of lipid bilayer. Chem Phys Lipids. 135(2):117–129. doi: 10.1016/j.chemphyslip.2005.02.003.

Golba B, Zhong Z, Romio M, Almey R, Deforce D, Dhaenens M, Sanders NN, Benetti EM, De Geest BG. 2025. Cyclic poly(2-methyl-2-oxazoline)-lipid conjugates are good alternatives to poly(ethylene glycol)-lipids for lipid nanoparticle mRNA formulation. Biomacromolecules. 26(3):1816–1825. doi: 10.1021/acs.biomac.4c01587.

Hamad I, Hunter AC, Szebeni J, Moghimi SM. 2008. Poly(ethylene glycol) s generate complement activation products in human serum through increased alternative pathway turnover and a MASP-2-dependent process. Mol Immunol. 46(2):225–232. doi: 10.1016/j.molimm.2008.08.276.

Haroon HB, Dhillon E, Farhangrazi ZS, Trohopoulos PN, Simberg D, Moghimi SM. 2023. Activation of the complement system by nanoparticles and strategies for complement inhibition. Eur J Pharm Biopharm. 193:227–240. doi: 10.1016/j.ejpb.2023.11.006.

Haroon HB, Hunter AC, Farhangrazi ZS, Moghimi SM. 2022. A brief history of long circulating nanoparticles. Adv Drug Deliv Rev. 188:114396. doi: 10.1016/j.addr.2022.114396.

He Z, Miao L, Jordan R, S, Manickam D, Luxenhofer R, Kabanov AV. 2015. A low protein binding cationic poly(2-oxazoline) as non-viral vector. Macromolecular Biosci. 15:1004–1020.

He Z, Wan X, Schulz A, Bludau H, Dobrovolskaia MA, Stern ST, Montgomery SA, Yuan H, Li Z, Alakhova D, et al. 2016. A high capacity polymeric micelle of paclitaxel: Implication of high dose drug therapy to safety and in vivo anti-cancer activity. Biomaterials. 101:296–309. doi: 10.1016/j.biomaterials.2016.06.002.

Hecht G, Weese, H, Periston. 1943. Ein neuer Blutflüssigkeitsersatz. Munch Med Wschr. 90:11–16.

Hoang Thi TT, Pilkington EH, Nguyen DH, Lee JS, Park KD, Truong NP. 2020. The importance of poly(ethylene glycol) alternatives for overcoming PEG immunogenicity in drug delivery and bioconjugation. Polymers (Basel). 12(2):298. doi: 10.3390/polym12020298.

Huckaby JT, Jacobs TM, Li Z, Perna RJ, Wang A, Nicely NI, Lai SK. 2020. Structure of an anti-PEG antibody reveals an open ring that captures highly flexible PEG polymers. Commun Chem. 3(1):124. doi: 10.1038/s42004-020-00369-y.

Hwang D, Ramsey JD, Kabanov AV. 2020. Polymeric micelles for the delivery of poorly soluble drugs: from nanoformulation to clinical approval. Adv Drug Deliv Rev. 156:80–118. doi: 10.1016/j.addr.2020.09.009.

Hwang D, Vinod N, Skoczen SL, Ramsey JD, Snapp KS, Montgomery SA, Wang M, Lim C, Frank JE, Sokolsky-Papkov M, et al. 2021. Bioequivalence assessment of high-capacity polymeric micelle nanoformulation of paclitaxel and Abraxane® in rodent and non-human primate models using a stable isotope tracer assay. Biomaterials. 278:121140. doi: 10.1016/j.biomaterials.2021.121140.

Ju Y, Lee WS, Pilkington EH, Kelly HG, Li S, Selva KJ, Wragg KM, Subbarao K, Nguyen THO, Rowntree LC, et al. 2022. Anti-PEG antibodies in humans by SARS-CoV-2 lipid nanoparticle mRNA vaccine. ACS Nano. 16(8):11769–11780. doi: 10.1021/acsnano.2c04543.

Kang DD, Hou X, Wang L, Xue Y, Li H, Zhong Y, Wang S, Deng B, McComb DW, Dong Y, et al. 2024. Engineering LNPs with polysarcosine lipids for mRNA delivery. Bioact Mater. 37:86–93. doi: 10.1016/j.bioactmat.2024.03.017.

Klibanov AI, Maruyama K, Torchilin VP, Huang L. 1990. Amphiphatic polyethyleneglycol effectively prolong the circulation time of liposomes. FEBS Lett. 268:235–237.

Li M, Jin X, Liu T, Fan F, Gao F, Chai S, Yang L. 2022. Nanoparticle elasticity affects systemic circulation lifetime by modulating adsorption of apolipoprotein A-I in corona formation. Nat Commun. 13(1):4137. doi: 10.1038/s41467-022-31882-4.

Li Y, Ettah U, Jacques S, Gaikwad H, Monte A, Dylla L, Guntupalli S, Moghimi SM, Simberg D. 2024b. Optimized enzyme-linked immunosorbent assay for anti-PEG antibody detection in healthy donors and patients treated with PEGylated liposomal doxorubicin. Mol Pharm. 21(6):3053–3060. doi: 10.1021/acs.molpharmaceut.4c00278.

Li Y, Jacques S, Gaikwad H, Wang G, Banda NK, Holers VM, Scheinman RI, Tomlinson S, Moghimi SM, Simberg D, et al. 2024a. Inhibition of acute complement responses towards bolus-injected nanoparticles using targeted short-circulating regulatory proteins. Nat Nanotechnol. 19(2):246–254. doi: 10.1038/s41565-023-01514-z.

Li Y, Saba L, Scheinman RI, Banda NK, Holers M, Monte A, Dylla L, Moghimi SM, Simberg D. 2024b. Nanoparticle-binding immunoglobulins predict variable complement responses in healthy and diseased cohorts. ACS Nano. 18(42):28649–28658. doi: 10.1021/acsnano.4c05087.

Li Y, Wang G, Griffin L, Banda NK, Saba LM, Groman EV, Scheinman R, Moghimi SM, Simberg D. 2021. Complement opsonisation of nanoparticles: differences between humans and preclinical species. J Control Rel. 338:548–556. doi: 10.1016/j.jconrel.2021.08.048.

Lim C, Hwang D, Yazdimamaghani M, Atkins HM, Hyun H, Shin Y, Ramsey JD, Rädler PD, Mott KR, Perou CM, et al. 2023. High-dose paclitaxel and its combination with CSF1R inhibitor in polymeric micelles for chemoimmunotherapy of triple negative breast cancer. Nano Today. 51:101884. doi: 10.1016/j.nantod.2023.101884.

Liu K, Nilsson R, Lázaro-Ibáñez E, Duàn H, Miliotis T, Strimfors M, Lerche M, Salgado Ribeiro AR, Ulander J, Lindén D, et al. 2023. Multiomics analysis of naturally efficacious lipid nanoparticle coronas reveals high-density lipoprotein is necessary for their function. Nat Commun. 14(1):4007. doi: 10.1038/s41467-023-39768-9.

Lloyd JB. 1998. Intestinal permeability to polyethyleneglycol and sugars: a re-evaluation. Clin Sci (Lond). 95(1):107–110. doi: 10.1042/cs0950107.

Lorson T, Lübtow MM, Wegener E, Haider MS, Borova S, Nahm D, Jordan R, Sokolski-Papkov M, Kabanov AV, Luxenhofer R, et al. 2018. Poly(2-oxazoline)s based biomaterials: a comprehensive and critical update. Biomaterials. 178:204–280. doi: 10.1016/j.biomaterials.2018.05.022.

Lübtow MM, Hahn L, Haider MS, Luxenhofer R. 2017. Drug specificity, synergy and antagonism in ultrahigh capacity poly(2-oxazoline)/poly(2-oxazine) based formulations. J Am Chem Soc. 139(32):10980–10983. doi: 10.1021/jacs.7b05376.

Lyzogubov VV, Tytarenko RG, Liu J, Bora NS, Bora PS. 2011. Polyethylene glycol (PEG)-induced mouse model of choroidal neovascularization. J Biol Chem. 286(18):16229–16237. doi: 10.1074/jbc.M110.204701.

Mahand SN, et al. 2022. Polyoxazoline: a review article from polymerization to smart behaviors and biomedical applications. Eur Polymer J. 178:111484.

Menig S, Dinh A, Angus J, Tucker S, Leger KJ, Rushing T, Orgel E. 2024. Lack of benefit from premedication for pegylated asparaginase during pediatric acute lymphoblastic leukemia/lymphoma therapy: a side-by-side comparison. Pediatr Blood Cancer. 71(1):e30716. doi: 10.1002/pbc.30716.

Moghimi SM, Hamad I, Andresen TL, Jørgensen K, Szebeni J. 2006. Methylation of the phosphate oxygen moiety of phospholipid-methoxy(polyethylene glycol) conjugate prevents PEGylated liposome-mediated complement activation and anaphylatoxin production. FASEB J. 20(14):2591–2593. doi: 10.1096/fj.06-6186fje.

Moghimi SM, Haroon HB, Yaghmur A, Hunter AC, Papini E, Farhangrazi ZS, Simberg D, Trohopoulos PN. 2023. Perspectives on complement and phagocytic cell responses to nanoparticles: from fundamentals to adverse reactions. J Control Rel. 356:115–129. doi: 10.1016/j.jconrel.2023.02.022.

Moghimi SM, Simberg D, Papini E, Farhangrazi ZS. 2020. Complement activation by drug carriers and particulate pharmaceuticals: principles, challenges and opportunities. Adv Drug Deliv Rev. 157:83–95. doi: 10.1016/j.addr.2020.04.012.

Moghimi SM, Simberg D. 2018. Translational gap in animal models of human infusion reactions to nanomedicines. Nanomedicine (Lond). 13(9):973–975. doi: 10.2217/nnm-2018-0064.

Moghimi SM, Simberg D. 2022. Critical issues and pitfalls in serum and plasma handling for complement analysis in nanomedicine and bionanotechnology. Nano Today. 44:101479. doi: 10.1016/j.nantod.2022.101479.

Moghimi SM, Szebeni J. 2003. Stealth liposomes and long circulating nanoparticles: critical issues in pharmacokinetics, opsonisation and protein-binding properties. Prog Lipid Res. 42(6):463–478. doi: 10.1016/s0163-7827(03)00033-x.

Moghimi SM. 2006. The effect of methoxy-PEG chain length and molecular architecture on lymph node targeting of immuno-PEG liposomes. Biomaterials. 27(1):136–144. doi: 10.1016/j.biomaterials.2005.05.082.

Moghimi SM. 2018. Nanomedicine safety in preclinical and clinical development: focus on idiosyncratic injection/infusion reactions. Drug Discov Today. 23(5):1034–1042. doi: 10.1016/j.drudis.2017.11.006.

Nasdaq.com. 2024. Serina therapeutics announces completion of merger with AgeX therapeutics. https://www.nasdaq.com/press-release/serina-therapeutics-announces-completion-of-merger-with-agex-therapeutics-2024-03-26.

Pannuzzo M, Esposito S, Wu L-P, Key J, Aryal S, Celia C, di Marzio L, Moghimi SM, Decuzzi P. 2020. Overcoming nanoparticle-mediated complement activation by surface PEG pairing. Nano Lett. 20(6):4312–4321. doi: 10.1021/acs.nanolett.0c01011.

Ravin HA, Seligman AM, Fine J. 1952. Polyvinyl pyrrolidone as a plasma expander – studies on its excretion, distribution and metabolism. N Engl J Med. 247(24):921–929. doi: 10.1056/NEJM195212112472403.

Sanchez AJDS, Loughrey D, Echeverri ES, Huayamares SG, Radmand A, Paunovska K, Hatit M, Tiegreen KE, Santangelo PJ, Dahlman JE, et al. 2024. Substituting poly(ethylene glycol) lipids with poly(2-ethyl-2-oxazoline) lipids improves lipid nanoparticle repeat dosing. Adv Healthc Mater. 13(17):e2304033. doi: 10.1002/adhm.202304033.

Shen L, Li Z, Ma A, Cruz-Teran C, Talkington A, Shipley ST, Lai SK. 2024. Free PEG suppresses anaphylaxis to PEGylated nanomedicine in swine. ACS Nano. 18(12):8733–8744. doi: 10.1021/acsnano.3c11165.

Simberg D, Moghimi SM. 2024. Anti-poly(ethylene glycol)(PEG) antibodies: from where are we coming and where are we going. J Nanotheranostics. 5(3):99–103. doi: 10.3390/jnt5030007.

Soenen SJ, Manshian BB, Abdelmonem AM, Montenegro J, Tan S, Balcaen L, Vanhaecke F, Brisson AR, Parak WJ, De Smedt SC, et al. 2014. The cellular interactions of PEGylated gold nanoparticles: effect of PEGylation on cellular uptake and cytotoxicity. Part Syst Charact. 31(7):794–800. doi: 10.1002/ppsc.201300357.

Szebeni J, Simberg D, González-Fernández Á, Barenholz Y, Dobrovolskaia MA. 2018. Roadmap and strategy for overcoming infusion reactions to nanomedicines. Nat Nanotechnol. 13(12):1100–1108. doi: 10.1038/s41565-018-0273-1.

Szebeni J, Storm G, Ljubimova JY, Castells M, Phillips EJ, Turjeman K, Barenholz Y, Crommelin DJA, Dobrovolskaia MA. 2022. Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines. Nat Nanotechnol. 17(4):337–346. doi: 10.1038/s41565-022-01071-x.

Tavano R, Gabrielli L, Lubian E, Fedeli C, Visentin S, Polverino De Laureto P, Arrigoni G, Geffner-Smith A, Chen F, Simberg D, et al. 2018. C1q-mediated complement activation and C3 opsonization trigger recognition of stealth poly(2-methyl-2-oxazoline)-coated silica nanoparticles by human phagocytes. ACS Nano. 12(6):5834–5847. doi: 10.1021/acsnano.8b01806.

Tavano R, Morillas-Becerril L, Geffner-Smith A, Ronzani G, Gervasutti R, Arrigoni G, Battisti I, Morbidelli M, Polverino de Laureto P, Palazzi L, et al. 2025. Species differences in opsonisation and phagocyte recognition of preclinical poly-2-alkyl-2-oxazoline-coated nanoparticles. Nat Commun. 16(1):2642. doi: 10.1038/s41467-025-57648-2.

Tirosh O, Barenholz Y, Katzhendler J, Priev A. 1998. Hydration of polyethylene glycol-grafted liposomes. Biophys J. 74(3):1371–1379. doi: 10.1016/S0006-3495(98)77849-X.

Vu VP, Gifford GB, Chen F, Benasutti H, Wang G, Groman EV, Scheinman R, Saba L, Moghimi SM, Simberg D, et al. 2019. Immunoglobulin deposition on biomolecule corona determines complement opsonisation efficiency of preclinical and clinical nanoparticles. Nat Nanotechnol. 14(3):260–268. doi: 10.1038/s41565-018-0344-3.

Wan X, Min Y, Bludau H, Keith A, Sheiko SS, Jordan R, Wang AZ, Sokolsky-Papkov M, Kabanov AV. 2018. Drug combination synergy in

worm-like polymeric micelles improves treatment outcome for small cell and non-small cell lung cancer. ACS Nano. 12(3):2426–2439. doi: 10.1021/acsnano.7b07878.

Wang C, Graham DJ, Kane RC, Xie D, Wernecke M, Levenson M, MaCurdy TE, Houstoun M, Ryan Q, Wong S, et al. 2015. Comparative risk of anaphylactic reactions associated with intravenous iron products. JAMA. 314(19):2062–2068. doi: 10.1001/jama.2015.15572.

Wibroe PP, Anselmo AC, Nilsson PH, Sarode A, Gupta V, Urbanics R, Szebeni J, Hunter AC, Mitragotri S, Mollnes TE, et al. 2017. Bypassing adverse injection reactions to nanoparticles through shape modification and attachment to erythrocytes. Nat Nanotechnol. 12(6):589–594. doi: 10.1038/nnano.2017.47.

Wu L-P, Ficker M, Christensen JB, Simberg D, Trohopoulos PN, Moghimi SM. 2021. Dendrimer end-terminal motif-dependent evasion of human complement and complement activation through IgM hitchhiking. Nat Commun. 12(1):4858. doi: 10.1038/s41467-021-24960-6.

Xiao Y, Lian X, Sun Y, Sung Y-C, Vaidya A, Chen Z, Gupta A, Chatterjee S, Zheng L, Guerrero E, et al. 2025. High-density brush-shaped polymer lipids reduce anti-PEG antibody binding for repeated administration of mRNA therapeutics. Nat Mater. doi: 10.1038/s41563-024-02116-3.

Yamaleyeva DN, Makita N, Hwang D, Haney MJ, Jordan R, Kabanov AV. 2023. Poly(2-oxazoline)-based polyplexes as a PEG-free plasmid DNA delivery platform. Macromolecular Biosci. 23(11):2300177.

Yang B, Lim SI, Kim JC, Tae G, Kwon I. 2016. Site-specific albumination as an alternative to PEGylation for the enhanced serum half-life in vivo. Biomacromolecules. 17(5):1811–1817. doi: 10.1021/acs.biomac.6b00238.

Zahoranová A, Luxenhofer R. 2021. Poly(2-oxazoline)- and poly(2-oxazine)-based self-aseemblies, polyplexes, and drug nanoformulations – an update. Adv Healthcare Mater. 10:2001382.

Zalipsky S, Hansen CB, Oaks JM, Allen TM. 1996. Evaluation of blood clearance rates and biodistribution of poly(2-oxazoline)-grafted liposomes. J Pharm Sci. 85(2):133–137. doi: 10.1021/js9504043.

Zhou Z-H, Cortese MM, Fang J-L, Wood R, Hummell DS, Risma KA, Norton AE, KuKuruga M, Kirshner S, Rabin RL, et al. 2023. Evaluation of association of anti-PEG antibodies with anaphylaxis after mRNA COVID-19 vaccination. Vaccine. 41(28):4183–4189. doi: 10.1016/j.vaccine.2023.05.029.