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REVIEW ARTICLE

Adverse Impacts of PEGylated Protein Therapeutics: A Targeted Literature Review

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Abstract

The beneficial effects of polyethylene glycol (PEG)-conjugated therapeutics, such as increased half-life, solubility, stability, and decreased immunogenicity, have been well described. There have been concerns, however, about adverse outcomes with their use, but understanding of those adverse outcomes is still relatively limited. The present study aimed to characterize adverse outcomes associated with PEGylation of protein-based therapeutics on immunogenicity, pharmacologic properties, and safety. A targeted review of English language articles published from 1990 to September 29, 2023, was conducted. Of the 29 studies included in this review, 18 reported adverse safety outcomes such as hematologic complications, hepatic toxicity, injection site reactions, arthralgia, nausea, infections, grade 3 or 4 adverse events (AEs), and AE-related discontinuations and dose modifications. Fifteen studies reported immunogenicity-related outcomes, such as the prevalence of pre-existing antibodies to PEG, treatment-emergent antibody response, and hypersensitivity reactions to PEGylated drugs. Seven studies reported pharmacological outcomes such as increased clearance and reduced activity in response to PEGylated drugs. This review aims to contribute to a balanced view of PEGylated therapies by summarizing the adverse outcomes or lack of benefit associated with PEGylated therapeutics reported in the literature. We identified several studies characterizing adverse outcomes, pharmacological effects, and immunogenicity associated with the use of PEGylated therapeutics. Our findings suggest that using PEGylated therapeutics may require careful monitoring for adverse safety outcomes, including screening and monitoring for pre-existing antibodies and those induced in response to PEGylated therapy, as well as monitoring and adjusting the dosing of PEGylated therapeutics.

1 Introduction

Proteins have great therapeutic potential, offering targeted mechanisms of action due to their varied functional roles, including acting as catalysts, signaling molecules, transporters, scaffolds, and receptors [1]. Nonetheless, potential drawbacks include immunogenicity, solubility, and stability, as well as their propensity to aggregate, denature, and degrade [2]. To help mitigate these limitations, some structural and chemical modifications made to proteins include site-specific mutagenesis, antibody–drug conjugation, fusion to other proteins, post-translational modifications such as glycosylation, methylation, phosphorylation, and attachment of polyethylene glycol (PEG) chains [3]. Since the approval of the first PEGylated protein therapeutic in 1990, PEG has been increasingly used

Key Points

Contrary to the belief that PEG is non-immunogenic, this targeted literature review of PEGylated therapeutics reports evidence of pre-existing and treatment-induced anti-PEG antibodies resulting in increased drug clearance and decreased activity of PEGylated drugs.

The findings of our review highlight the need for monitoring of PEGylated therapeutics for adverse safety outcomes, sensitive detection assays for quantification of antibodies, and dose adjustments for optimal treatment outcomes.

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to modify the properties of biomolecules, with the aims of increasing drug half-life, enhancing drug solubility and stability, and decreasing immunogenicity [4–6]. The rationale behind PEGylation involves the formation of a hydrophilic shell; the shell and its bound water cover the immunogenic epitopes and protect the PEGylated proteins and enzymes from the immune system, thereby decreasing the production of neutralizing antibodies and other adverse immune reactions [7]. Another benefit is that PEG conjugation increases the size of the biomolecule, resulting in decreased renal filtration and increased half-life [5]. PEGylation also alters the binding kinetics between the drug and its receptor with a slower rate of attachment and dissociation and a decreased binding affinity to the protein clearance receptor, resulting in extended circulation and a delayed clearance, which may enable less frequent dosing compared to the non-PEGylated parent compound [4]. Lastly, PEG is a water-soluble polymer that enhances the solubility of conjugated therapeutics [6].

The clinical and pharmacologic properties of PEGylated compounds have been extensively investigated across many indications, including hepatitis, hemophilia, and various cancers, and the beneficial effects of PEGylation have been well described [8–14]. Despite the benefits of treatment with PEGylated therapeutics, covalent modification of a protein may have unanticipated or undesirable effects, leading to concerns about the potential for adverse patient outcomes. While PEG was once thought to be non-immunogenic, several recent studies report the existence of both pre-existing antibodies against PEG, likely due to its ubiquitous presence in cosmetics and personal care products, as well as the development of anti-PEG antibodies in response to PEGylated therapeutics [15–17]. It has been proposed that the presence of anti-PEG antibodies may contribute to a multitude of adverse outcomes observed with PEGylated drugs, such as hypersensitivity reactions, accelerated drug clearance, and decreased drug activity [15, 18–20].

The objective of the present literature review was to identify and synthesize evidence characterizing adverse outcomes associated with PEGylation of protein-based therapeutics on immunogenicity, pharmacologic properties including pharmacokinetics, and safety, to provide a balanced perspective of the positive and negative impacts of PEGylation of protein-based therapeutics and increase awareness of the potential risks associated with PEGylation.

2 Methods

2.1 Literature Search Methods

Literature searches were conducted in OvidSP (http://ovidsp.ovid.com/) to identify peer-reviewed studies of interest from 1990 to 29 September 2023, in Embase, MEDLINE, and MEDLINE In Progress using pre-specified search strategies, which included a combination of indexing terms (Medical Subject Headings in MEDLINE and Emtree in Embase), as well as free-text keywords as recommended by the Cochrane Collaboration [21]. Searches were restricted to studies assessing humans, published in English, and with no geographic limits. Additional gray literature searches were conducted, which included indexed conference abstracts from meetings of interest between 2021 and 2022, and the initial search was supplemented with a citation-chasing/snowballing approach to find additional articles. The search strategy with the search terms can be found in the Supplementary Information.

2.2 Screening Process

The records resulting from the searches were imported to EndNote X9, where duplicates were removed. Following deduplication, the study selection process was conducted using Nested Knowledge screening software to ensure accurate record-keeping in two systematic steps: title and abstract screening and full-text screening. Each record was assessed against the eligibility criteria during study selection using the Population, Interventions and Comparisons, Outcomes, and Study Design (PICOS) framework (Table 1). In the first screening stage, the artificial intelligence (AI) reprioritization capability was enabled in Nested Knowledge to allow for the shuffling of references in the background to present the ones most likely to be included. The articles were screened at the title/abstract level using the PICOS criteria; the reasons for exclusion are presented in Table 2. Based on the outcomes of interest, the most relevant articles were selected and retrieved for full-text screening.

2.3 Data Extraction

A data extraction table was generated in Microsoft Excel® to record top-level data elements such as citation information, study characteristics (design, location, and objective), patient characteristics (population, sample size, indication, and intervention/comparator), and outcomes of interest [antibodies, adverse events (AEs), pharmacokinetics (PK), and hypersensitivity].

Table 1 PICOS inclusion/exclusion criteria

Criteria	Inclusion	Exclusion
Population	Patients treated with any PEGylated protein-based therapy	Studies not evaluating patients with PEGylated protein-based therapy
Interventions	Any or none	NA
Comparators	Any or none	NA
Outcomes	• Safety o Treatment-related AEs o Any grade AEs o SAEs o Immune-mediated AEs o Discontinuation due to any cause o Discontinuation due to AEs • Immunogenicity (including the formation of antidrug antibodies) • Pharmacology o Pharmacokinetics o Pharmacodynamics	Other outcomes not of interest
Study design	• Clinical trials • Observational studies • Systematic reviews • Narrative reviews (for citation chasing)	• Full-text articles not published in English • Conference abstract published prior to 2021 • Editorial, erratum, trial protocol, guideline, case report, clinical trial with results not reported, etc. • In vitro, ex vivo, animal, etc.

AE adverse events, *NA* not applicable, *PEG* polyethylene glycol, *PICOS* population, intervention, comparison, outcomes, and study design, *SAE* serious adverse event

Table 2 Reasons for exclusion

Reason for exclusion	Description
Non-English language	Full-text article not published in English
Conference abstract published prior to 2021	Conference abstract published prior to 2021
Publication type not of interest	Editorial, erratum, trial protocol, guideline, narrative review, etc.
Study design not of interest	In vitro, ex vivo, animal, etc.
Population not of interest	Studies not evaluating patients with PEGylated protein-based therapeutics
Outcomes not of interest	No reported outcomes of interest

3 Results

3.1 Overview of Selected Publications

Searches conducted on 29 September 2023 identified 9942 records. After deduplication and title/abstract screening, 178 articles were selected for full-text retrieval and were reviewed, and 29 were included. Of the 29 studies, 18 reported on adverse outcomes, including hypersensitivity ($n=2$), 7 reported on pharmacology, and 15 reported on antibodies, of which 6 reported only on anti-PEG antibodies and 9 reported on both anti-PEG antibodies and antibodies against the PEGylated drug. A summary of the study characteristics, patient characteristics, and outcomes is presented in Table 3. Most studies were observational ($n=16$) or prospective clinical trials ($n=10$). The most common disease areas were acute lymphoblastic leukemia (ALL; $n=7$) and hepatitis C virus (HCV; $n=5$) (Fig. 1); the most common

study locations were USA ($n=8$) and Germany ($n=6$), and most studies were conducted in adult populations ($n=17$).

3.2 Safety Outcomes

3.2.1 Adverse Events (AEs)

3.2.1.1 Hematologic Complications In six studies of patients with HCV or cancer, hematologic AEs were associated with a PEGylated versus non-PEGylated product (Table 3) [22–27]. A study of adolescents and adults with newly diagnosed acute lymphoblastic leukemia (ALL) reported a longer duration of coagulation dysfunction ($p=0.002$) and agranulocytosis ($p<0.01$) in patients treated with PEGylated asparaginase (PEG–ASP) compared with *E. coli* asparaginase (L-ASP), which the authors attributed to the longer half-life and decreased immunogenicity of PEG-ASP compared with L-ASP [22]. In a multicenter, open-label, randomized phase 3 study of adults with melanoma, the rates of granulocytopenia ($p<0.0001$) and leukocytopenia ($p=0.0001$) were 3 and 5 times higher, respectively, in patients receiving PEGylated interferon (PEG-IFN) versus IFN [25]. The authors suggested that, in addition to differences in PK, the higher rates of AEs may be due to differences in dosing between PEG-IFN (100 µg per week) and IFN [3 million units (MU) 3× per week], which could have resulted in a higher overall dose in the PEG-IFN arm [25]. Leukopenia also occurred in more than twice as many patients receiving PEG-IFN compared with IFN [56% versus 23.5%; $p=$not reported (NR)] in another phase 3 study in adult patients with melanoma [23]. An observational study of adult patients with hepatitis C virus (HCV) reported neutropenia in a significantly higher proportion of patients treated with PEG-IFN + ribavirin versus IFN + ribavirin (48% versus 9%; $p=0.0009$) [27]. Two Cochrane systematic reviews of patients with HCV also reported that those treated with PEG-IFN + ribavirin had more than twice the risk of developing neutropenia [risk ratio (RR): 2.15; 95% CI 1.76, 2.61; $p<0.0001$ [24], and RR: 2.25; 95% CI 1.58, 3.21; $p=$NR [26]] compared with those treated with IFN + ribavirin. The Cochrane reviews also reported that patients receiving PEG-IFN + ribavirin had more than twice the risk of developing thrombocytopenia (RR: 2.28; 95% CI 1.14, 4.54; $p=$NR [26], and RR: 2.63; 95% CI 1.68, 4.11; $p<0.0001$ [24]) than patients receiving IFN + ribavirin.

3.2.1.2 Hepatic Toxicity In four studies of patients with diabetes or cancer, hepatic toxicity was significantly associated with a PEGylated versus non-PEGylated product [23, 28–30]. In one of the phase 3 trials of patients with melanoma, rates of liver enzyme elevation were at least twice as high in the PEG-IFN arm compared with the IFN arm [alanine transaminase (ALT): 33.0% versus 16.5%, $p=$NR; aspartate transaminase (AST): 19.1% versus 9.4%, $p=$NR] [23]. In adults with high-risk, Philadelphia chromosome-negative ALL, the rates of grade 3 or 4 hepatic toxicity were higher in patients receiving PEG-ASP compared with L-ASP, both during induction therapy ($p=0.055$) and consolidation therapy ($p=0.009$). The authors attributed the differences in AE rates in the consolidation period to differences in the duration of activity between PEG-ASP and L-ASP [30]. In two randomized phase 3 trials in adults with type 1 diabetes (T1D), a significantly higher proportion of patients receiving basal insulin peglispro (BIL) had an ALT elevation ≥ 3× upper limit of normal (ULN) versus those receiving insulin glargine (GL), and ALT levels were significantly higher at 26, 52, and 78 weeks (all $p<0.001$) in patients receiving BIL versus GL. The authors noted consistent findings in other studies of PEGylated drugs, but did not propose an explanation for these observations [28, 29].

3.2.1.3 Other AEs *Injection site reactions (ISRs):* In four studies of patients with HCV or diabetes, ISRs were associated with a PEGylated versus non-PEGylated product [24, 26, 28, 29]. Two Cochrane reviews reported a higher risk of ISRs in patients with HCV receiving PEG-IFN + ribavirin compared with IFN + ribavirin (RR: 1.71; 95% CI 1.50, 1.93; $p<0.0001$ [24] and 2.56; 95% CI 1.06, 6.22; $p=$NR [26]). A significantly higher proportion of ISRs was also reported in two randomized phase 3 trials in adults with T1D receiving BIL compared with those receiving GL ($p<0.001$ for both trials), possibly due to factors affecting injection site location and the slow absorption of BIL through the lymphatic system [28, 29].

Bone and/or joint pain: Three studies of cancer or HCV reported bone pain or arthralgia [24, 26, 31]. In patients with breast cancer, bone pain occurred in a higher proportion of patients with PEGylated granulocyte colony-stimulating factor (PEG-G-CSF) compared with G-CSF ($p=0.09$) [31]. Cochrane reviews of patients with HCV also reported a higher proportion of patients with arthralgia in patients receiving PEGylated therapy versus non-PEGylated therapy (30% versus 24%; RR: 1.19; 95% CI 1.05, 1.35; $p=0.01$) [24, 26].

Gastrointestinal AEs, including nausea and vomiting: In two studies of patients with breast cancer and melanoma, gastrointestinal effects ($p=0.005$) and nausea or vomiting ($p=0.003$) occurred in a significantly higher proportion of patients treated with PEGylated versus non-PEGylated therapy [25, 31]. Grob et al. suggested that dosing differences between the PEG-IFN arm and the IFN arm, which possibly corresponded to a higher dose in the PEG-IFN arm, may have contributed to higher rates of toxicities with the PEGylated treatment [25]. The Cochrane review of patients with chronic HCV reported a significantly greater risk of

Table 3 Overview of the 29 studies and their outcomes

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Hillarp, 2023 [42]	Observational; Norway; NA	Adult; 83	Hemophilia A	PEGylated rFVIII; 10 kDa PEG	Abs directed against PEG and PEGylated rFVIII in 4 (5%) patients	NR	NR	NR
Pezeshkpoor, 2023 [45]	Observational; Germany; NA	Adult; 46	Hemophilia A	40PEG-BDD-FVIII; 40 kDa PEG	Abs directed against PEG and PEGylated FVIII in 2 (4%) patients	NR	NR	NR
Albrecht, 2022 [32]	Observational; Germany; NR	NR; 27	CTCL	PEG-IFNα-2a versus non-pegylated-IFNα-2a; NR	NR	Ophthalmological side effects: RR: 20.3; 95% CI 2.7, 150.4; $p = 0.0035$	NR	NR
Siebel, 2022 [39]	Observational; Germany; NA	Pediatric; 1,444	ALL	PEG-ASP; NR	NR	NR	Pre-existing anti-PEG Abs increased initial clearance of PEG-ASP by 41.4%	NR
Khalil, 2022 [37]	Observational; Germany; CTCAE	Pediatric and adult; 947	ALL	PEG-ASP; NR	• Prevalence of anti-PEG Abs o Prior to the first administration of PEG-ASP: 13.9% (IgG); 29.1% (IgM) o Following administration of PEG-ASP: 4.2% (IgG); 4.5% (IgM)	NR	• Pre-existing anti-PEG Abs reduced PEG-ASP activity levels in a concentration-dependent manner o Anti-PEG IgG: OR 2.06; 95% CI 1.44, 2.96 o Anti-PEG IgM: OR 1.65; 95% CI 1.27, 2.15 o $p < 0.001$	Pre-existing anti-PEG IgG was significantly associated with first-exposure hypersensitivity reactions ($p < 0.01$)

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Kloos, 2020 [43]	Observational; Netherlands; CTCAE v4.03	Pediatric; 18	ALL	PEG-ASP; 20 kDa PEG	• Anti-PEG-ASP Abs o Induction: 92% IgG; 67% IgM o Intensification: 100% IgG; 83% IgM • Anti-ASP Abs o Induction: 17% IgG; 0% IgM o Intensification: 83% IgG; 33% IgM • Anti-PEG Abs o Induction: 100% IgG; 75% IgM o Intensification: 100% IgG; 50% IgM •Anti-SS-linker Abs o Induction: 50% IgG; 42% IgM o Intensification: 17% IgG; 17% IgM	NR	NR	NR
Liu, 2019 [33]	Clinical trial; USA; CTCAE v3.0	Pediatric; 598	ALL	PEG-ASP versus L-ASP; NR	• 618 (11.5%) of 5369 samples positive for anti-PEG-ASP o 96 (15.5%) of 618 positive for both anti-PEG and anti-L-ASP o 495 (80.1%) of 618 positive for anti-PEG alone o 9 (1.5%) of 618 positive for anti-L-ASP alone	• Allergic reaction rate o L-ASP: 169 (41%) of 410 patients o PEG-ASP: 81 (13.5%) of 598 patients o $p = 1.4 \times 10^{-23}$ • Severe (grade 3 or 4) allergic reaction rate o L-ASP: 24 (5.9%) of 410 patients o PEG-ASP: 58 (9.7%) of 598 patients o $p = 0.028$	Increased drug clearance with anti-PEG-ASP ($p = 5.0 \times 10^{-6}$)	• Silent hypersensitivity: o L-ASP: 89 (22%) of 410 patients o PEG-ASP: 107 (18%) of 598 patients o $p = 0.13$

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Ribera, 2018 [30]	Clinical trial; Spain; CTCAE v4.0	Adult; 126	ALL	PEG-ASP versus L-ASP; NR	NR	Hepatic (grade 3 or 4) toxicity • Induction therapy o L-ASP: 18/85 (21%) o PEG-ASP: 13/34 (38%) o $p=0.055$ • Consolidation therapy o L-ASP: 8/254 (3%) o PEG-ASP: 9/82 (11%) o $p=0.009$	NR	NR
Ashrafi, 2018 [31]	Clinical trial; Iran; NR	Adult; 24	Breast cancer	PEG-G-CSF versus G-CSF; NR	NR	Compared to G-CSF, patients treated with PEG-G-CSF had higher bone pain: 20.8% versus 0%; $p=0.09$ GI effects: 29.2% versus 0%; $p=0.005$	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Liang, 2018 [22]	Observational; China; CTCAE v4.03	Pediatric and adult; 122	ALL	PEG-ASP versus L-ASP; NR	NR	• Coagulation dysfunction o PEG-ASP: 9.80 ± 5.51 days o L-ASP: 6.80 ± 4.21 days o $p = 0.002$ • Agranulocytosis o PEG-ASP: 18.89 ± 8.79 days o L-ASP: 12.03 ± 8.34 days o $p < 0.01$ • Grade 4 or 5 infections o PEG-ASP: 22.73% o L-ASP: 7.25% o $p = 0.018$	NR	NR
Yang, 2016 [17]	Observational; USA; NR	Adult; 377	NA	NA; NR	Anti-PEG Abs: • Contemporary specimens: 72% o IgG 18%; IgM 25%; both IgG and IgM 30% • Specimens from 1970 to 1999: 56% o IgG 20%; IgM 19%; both IgG and IgM 16%	NR	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Garg, 2016 [29]	Clinical trial; Multi-center; MedDRA v16	Adult; 455	T1D	BIL versus GL; NR	•Anti-BIL treatment-emergent Ab response o BIL: 40.3% o GL: 27.5% o $p = 0.002$	• ALT elevation ≥ 3× ULN o BIL: $n = 13$, 4.5% o GL: $n = 1$, 0.7% o $p = 0.041$ •ALT at 26 weeks (IU/L) o BIL: 29.4 ± 0.9 o GL: 21.0 ± 1.3 o $p < 0.001$ •ALT at 78 weeks (IU/L) o BIL: 28.3 ± 1.0 o GL: 21.3 ± 1.3 o $p < 0.001$ •ISR o BIL: 24.8% o GL: 0% o $p < 0.001$	NR	NR
Eigentler, 2016 [23]	Clinical trial; Germany; CTCAE	Adult; 909	Melanoma	PEG-IFN versus IFN; Branched 40 kDa mPEG	NR	• Did not receive full dose and duration of treatment: o PEG-IFN: 26.2% o IFN: 13.3% • Rate of leukopenia o PEG-IFN: 56% o IFN: 23.5% • Rate of elevation of ALT o PEG-IFN: 33.0% o IFN: 16.5% • Rate of elevation of AST o PEG-IFN: 56% o IFN: 23.5%	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Bergenstal, 2016 [28]	Clinical trial; Multi-center; MedDRA	Adult; 1,114	T1D	BIL versus GL; NR	• Anti-BIL treatment-emergent Ab response o BIL: 38.4% o GL: 23.2% o $p < 0.001$	At 52 weeks, • ALT (IU/L) o BIL: 29.9 ± 0.8 o GL: 23.4 ± 0.9 o $p < 0.001$ • ALT ≥ 3× ULN o BIL: 4.8% o GL: 2.0% o $p = 0.021$ • Serum triglycerides (mmol/L) o BIL: 1.18 ± 0.02 o GL: 0.99 ± 0.03 o $p < 0.001$ •ISR o BIL: 13.3% o GL: 0.2% o $p < 0.001$	NR	NR
Lubich, 2016 [38]	Observational; Austria/USA; NR	Pediatric and adult; 1020	NA	NA; • Linear: 0.2 kDa, 2 kDa, 20 kDa • Branched: 20 kDa	Prevalence of anti-PEG Abs • Flow cytometry: 23% (95% CI 20, 27) o IgG: 13% (95% CI 11, 15) o IgM: 15% (95% CI 12, 17) • ELISA: 24% (95% CI 21, 28) o IgG: 14% (95% CI 11, 17) o IgM: 12% (95% CI 10, 15)	NR	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Hershfield, 2014 [41]	Clinical trial; USA; HAQ	Adult; 30	Gout	Pegloticase; 10 kDa mPEG	• PR: 3/17 developed Abs; 5.2% of 115 samples tested positive • TR: 12/12 developed Abs; 47/62 (75.8%) samples tested positive	• IR: 13/30 (43%) patients o IR among Ab positive patients: 8/13 (62%) experienced reaction during 10/41 (24%) infusions o IR among Ab-negative patients: 5/17 (29%) during 11/81 (13%) infusions	• C_{max} ($t=2$ h) mU/mL o PR: 30.9 ± 2.9 o TR: 16.9 ± 5.4 o $p=0.0009$ • C_{min} ($t=21$ days) mU/mL o PR: 9.9 ± 1.4 o TR: 0.4 ± 0.4 o $p<0.0001$ • $AUC_{2\ h-21\ days}$, $mU*mL^{-1}*day$ o PR: 397 ± 38 o TR: 105 ± 65 o $p<0.0001$	NR
Hauser, 2014 [24]	SR/MA; NA; NR	NR; 5938	HCV	PEG-IFN + ribavirin versus IFN + ribavirin; NR	NR	Increased risk with PEG-IFN + ribavirin for: • Neutropenia: 15.1% versus 7.1%; RR 2.15; 95% CI 1.76, 2.61; $p<0.0001$ • Thrombocytopenia: 5.8% versus 2.1%; RR 2.63; 95% CI 1.68, 4.11; $p<0.0001$ • Arthralgia: 29.7% versus 23.6%; RR 1.19; 95% CI 1.05, 1.35; $p=0.01$ • ISR: 53.7% versus 28.7%; RR 1.71; 95% CI 1.50, 1.93; $p<0.0001$ • Nausea: 34.0% versus 28.6%; RR 1.13; 95% CI 1.01, 1.26; $p=0.03$	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Grob, 2013 [25]	Clinical trial; Multi-center; CTCAE v2.0	Adult; 898	Melanoma	PEG-IFN versus IFN; NR	NR	Compared with IFN, PEG-IFN was associated with higher rates of: • Grade 3 or 4 AEs: 47.3% versus 25.2%; $p < 0.0001$ • Leukopenia: 5.3% versus 0.9%; $p = 0.0001$ • Granulocytopenia: 15.2% versus 4.5%; $p < 0.0001$ • Fatigue: 16.9% versus 8.7%; $p = 0.0003$ • Weight loss: 5.1% versus 2.5%; $p = 0.04$ • Nausea/vomiting: 1.8% versus 0%; $p = 0.003$ • Elevated SGPT: 5.1% versus 1.8%; $p = 0.007$ • Discontinuation due to AEs: 19.4% versus 12.8%; $p =$ NR	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Simin, 2007 [26]	SR/MA; NA; NR	NR; 4811	HCV	PEG-IFN + ribavirin versus IFN + ribavirin; NR	NR	Compared with IFN, PEG-IFN significantly increased the risk of: • Neutropenia: 15% versus 6%; RR: 2.25; 95% CI 1.58, 3.21 • Thrombocytopenia: 6% versus 2%; RR: 2.28; 95% CI 1.14, 4.54 • Arthralgia: 30% versus 24%; RR: 1.19; 95% CI 1.05, 1.35 • ISR: 55% versus 30%; RR: 2.56; 95% CI 1.06, 6.22 • Dermatological symptoms: 36% versus 20%; RR: 1.78; 95% CI 1.15, 2.73	NR	NR
Ganson, 2006 [19]	Clinical trial; USA; NR	Adult; 13	Gout	PEG-uricase; 10 kDa mPEG	Abs were directed against anti-PEG rather than anti-uricase	Ab-positive ($n=3$) subjects had ISR 8–9 days after injection	• Ab-negative subjects ($n=8$): plasma uricase measurable at 21 days postinjection (half-life: 10.5–19.9 days) • Ab-positive subjects ($n=5$): plasma uricase undetectable 10 days postinjection	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Toniutto, 2005 [34]	Observational; Italy; NR	Adult; 24	HCV	PEG-IFN + ribavirin versus IFN + ribavirin; NR	NR	• Total dose reduction due to AEs: o PEG-IFN: 11/12 (92%) o IFN: 6/12 (50%) o $p < 0.05$ • Dose reduction due to anemia and leukopenia: o PEG-IFN: 9/12 (75%) o IFN: 4/12 (33%) o $p < 0.05$	NR	NR
Puoti, 2004 [27]	Observational; Italy; NR	Adult; 255	HCV	PEG-IFN + ribavirin versus IFN + ribavirin; NR	NR	• Rates of infection: o PEG-IFN + ribavirin: 26/152 = 17.1% o IFN + ribavirin: 5/103 = 4.9% • Neutropenia: o PEG-IFN + ribavirin: 48% o IFN + ribavirin: 9% o $p = 0.0009$ • HR with the use of PEG-IFN: o For all infections (95% CI): 4.6 (1.7, 12); $p = 0.0019$ o For respiratory infections (95% CI): 1.49 (0.35, 6.3); p = NR o For non-respiratory infections (95% CI): 9.2 (2.1, 39.3); $p = 0.003$	NR	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Crisafulli, 2023 [47]	Analysis of the Italian National Spontaneous ADR Reporting System; Italy; MedDRA v23.0	NR; NR	NA	PEGylated versus non-PEGylated medicinal products; NA	NR	NR	NR	• Higher frequency among PEGylated versus non-PEGylated products: 11.7% versus 9.4%, $p < 0.0001$ • Reporting rate per 100,000 (95% CI) o PEG-IFNα-2b: 5.3 (3.9, 7.2) versus IFNα-2b: 0.3 (0.1, 1.6); RRR (95% CI): 18.2 (2.5, 132.6) o PEG-IFNβ-1a: 35.7 (27.4, 46.6) versus IFNβ-1a: 9.5 (8.3, 10.9); RRR (95% CI): 3.8 (2.8, 5.1) o PEG-IFNα-2a: 3.9 (3.2, 4.9) versus IFNα-2a: 0.2 (0, 1.1); RRR (95% CI): 20.0 (2.8, 143.5) • Median time to onset of reactions (IQR): 10 days (0, 61) versus 36 days (3, 216)

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Chen, 2016 [35]	Observational; Taiwan; NA	Adult; 1504	NA	NA; NA	• Anti-PEG Ab prevalence in healthy donors: 44.3% o IgG: 25.7% o IgM: 27.1% • Anti-PEG Abs more common in females versus males: o IgG: 28.3% versus 23.0%; $p=0.018$ o IgM: 32.0% versus 22.2%; $p<0.0001$ • Prevalence of anti-PEG IgG higher in younger (up to 60% for 20-year-olds) versus older (20% for age > 50 years) • Anti-PEG IgG concentration is negatively associated with age in females ($p=0.0073$) and males ($p=0.026$)	NR	NR	NR
Armstrong, 2007 [40]	Observational; USA; NA	Pediatric; 44	ALL	PEG-ASP versus L-ASP; NA	• Of the 15 sera from PEG-ASP-treated patients with undetectable ASP activity, anti-PEG Ab was detected in 9 by serology and 12 by flow cytometry • Anti-PEG Ab was detected in 1 PEG-ASP-treated patient with lower ASP activity (123 U/L)	NR	• Presence of anti-PEG was very closely associated with rapid clearance of PEG-ASP • ASP activity by serology: o Anti-PEG positive, mean: < 5 U/L o Anti-PEG negative, mean: 353 U/L o $p=7.7\times10^{-5}$ • ASP activity by flow cytometry: o Anti-PEG positive, mean: 12 U/L o Anti-PEG negative, mean: 438 U/L o $p=3.6\times10^{-5}$	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Kraus, 2005 [61]	Observational; Germany; HADS	Adult; 98	HCV	PEG-IFN + ribavirin versus IFN + ribavirin; NR	NR	• Patients receiving PEG-IFN showed more slowly increasing HADS depression scores (not statistically significant) • More cases of clinically relevant depression with PEG-IFN during therapy (40% versus 33.3%; $p = 0.494$)	NR	NR
Fang, 2021 [36]	Observational; USA; NA	Adult; 300	NA	NA; NA	• Anti-PEG Ab prevalence in healthy donors: 65% of plasma samples from 300 healthy donors o IgG: 46.3% o IgM: 44.0% • Higher prevalence in females than males: o IgG: 53.4% versus 41.8% o IgM: 47.4% versus 41.8% • Highest prevalence in 18- to 24-year-olds • No correlation between anti-PEG IgG and IgM concentrations	NR	NR	NR
Sundy, 2007 [46]	Clinical trial; USA; NR	Adult; 24	Gout	PEG-uricase; 10 kDa mPEG	9/24 patients developed anti-PEG-uricase	NR	Rapid clearance of PEG-uricase in Ab positive subjects: 11.0 ± 6.0 days (range: 4, 21) versus 16.1 ± 5.9 days (range: 4, 22); $p = 0.06$	NR

Table 3 (continued)

Author, year	Study characteristics	Patient characteristics			Outcome extraction details			
	Study design; study location; toxicity evaluation system	Population description; Sample size	Indication	Intervention/ comparator; PEG MW, kDa	Antibodies	Adverse reaction	PK	Hypersensitivity
Myler, 2015 [44]	Observational; USA; NR	NR; 173	• HBV • HCV	• PEG-IFNα; 20kDa PEG • PEG-IFNλ; 40 kDa branched PEG	• Anti-PEG Ab: present in 6% of PEG-IFNλ versus 9% of PEG-IFNα treated subjects • Anti-IFN AB: present in 60% of PEG-IFNλ versus 33% of PEG-IFNα treated subjects • Pre-existing Ab: anti-PEG 6% versus anti-IFN 8%	NR	NR	NR

Abs antibodies, *ADR* adverse drug reaction, *ALL* acute lymphoblastic leukemia, *ALT* alanine transaminase, *ASP* asparaginase, *AUC* area under the concentration curve, *BDD* B-domain deleted, *BIL* basal insulin peglispro, *CI* confidence interval, *CTCAE* Common Terminology Criteria for Adverse Events, *CTCL* cutaneous T-cell lymphoma, *DET* data extraction template, *ELISA* enzyme-linked immunosorbent assay, *G-CSG* granulocyte colony stimulating factor, GI gastrointestinal, *GL* insulin glargine, *HADS* hospital anxiety and depression scale, *HAQ* Health Assessment Questionnaire, *HBV* hepatitis B virus, *HCV* hepatitis C virus, *HR* hazard ratio, *IFN* interferon, *IgG* immunoglobulin G, *IgM* immunoglobulin M, *IQR* interquartile range, *IR* infusion reaction, *ISR* injection site reaction, *kDa* kilo Dalton, *L-ASP E.coli* asparaginase, *MA* meta-analysis, *MedDRA* Medical Dictionary for Regulatory Activities, *mPEG* monomethoxyPEG, *MW* molecular weight, *NA* not applicable, *NR* not reported, *OR* odds ratio, *PEG* polyethylene glycol, *PK* pharmacokinetics, *PR* persistent responders, *rFVIII* recombinant factor VIII, *RR* relative risk, *RRR* reporting rate ratio, *SGPT* serum glutamic pyruvic transaminase, *SR* systematic review, *SS* disulfide bond, *T1D* type 1 diabetes, *TR* transient responders, *ULN* upper limits of normal

nausea with PEG-IFN + ribavirin versus IFN + ribavirin (RR: 1.13; 95% CI 1.01, 1.26; $p = 0.03$) [24].

Infections: A study of patients with HCV reported higher rates of infection with PEG-IFN + ribavirin vs. IFN + ribavirin, as well as an increased risk of all infections [hazard ratio (HR): 4.6; 95% CI 1.7, 12.0; $p = 0.0019$], non-respiratory infections (HR: 9.2; 95% CI 2.1, 39.3; $p = 0.003$), and respiratory infections (HR: 1.49; 95% CI 0.35, 6.3; $p =$ NR) [27].

In a study of patients with cutaneous T-cell lymphoma, a significantly higher risk of ophthalmological side effects was observed with PEG-IFN compared with IFN ($p = 0.0035$) [32]. In the clinical trial of patients with T1D, BIL resulted in significantly increased serum triglycerides compared with GL ($p < 0.001$), possibly because of the increased fatty acids in the liver resulting from lipolysis of adipocyte triglycerides due to the decreased peripheral insulin activity of BIL [28]. In the Cochrane review of patients with HCV, dermatologic symptoms were reported in a higher proportion of patients treated with PEG-IFN + ribavirin versus IFN + ribavirin (36% versus 20%; RR: 1.78; 95% CI 1.15, 2.73) [26]. In the clinical trial of patients with melanoma, a significantly higher proportion of patients treated with PEG-IFN versus IFN reported fatigue ($p = 0.0003$) and weight loss ($p = 0.04$), which the authors theorize may be due to dosing differences and differences in PK between the two treatment arms [25].

3.2.2 Severe Adverse Events (SAEs)

Four studies reported significant associations between PEGylated therapeutics and severe AEs. The rate of grade 3 or 4 AEs in patients with melanoma receiving PEG-IFN was almost twice that of patients receiving IFN ($p < 0.0001$) [25], while the rate of grade 4 or 5 infections was more than three times higher among those treated with PEG-ASP versus L-ASP ($p = 0.018$) [22]. Grade 3 or 4 allergic reactions were also significantly more frequent in patients with ALL treated with PEG-ASP versus L-ASP ($p = 0.028$) [33], as were rates of grade 3 or 4 hepatic toxicity during consolidation therapy ($p = 0.009$), possibly because of the differences in the duration of PEG-ASP and L-ASP activity during the consolidation period [30].

3.2.3 AE-Related Discontinuations and Dose Modifications

Three studies reported higher rates of AE-related dose reductions or discontinuations with PEGylated versus non-PEGylated therapeutics. In one study of patients with melanoma, more patients treated with PEG-IFN discontinued treatment due to an AE versus patients treated with IFN (19.4% versus 12.8%; $p =$ NR) during months 0–18 of treatment, which the authors suggested could be because of differences in PK as well as dosing differences between the PEG-IFN arm and the IFN arm [25]. The intended 36-month treatment in the PEG-IFN arm was not possible due to high rates of discontinuation, resulting in a median treatment duration of 19.2 months [25]. Approximately half of the PEG-IFN treatment interruptions were AE-related, with a large proportion of reasons for discontinuations remaining unclear, suggesting difficulty coping with treatment, possibly due to loss of motivation resulting from decreased quality of life (QoL) [25]. In another study of patients with melanoma, a significantly greater proportion of patients treated with PEG-IFN did not receive the full dosage and duration of treatment due to AEs (26% vs. 13%; $p < 0.001$) [23]. In a study of patients with HCV, almost twice as many patients treated with PEG-IFN required a dose reduction due to AEs compared with those treated with IFN ($p < 0.05$). A similar pattern was observed for dose reductions due to anemia and leukopenia specifically, with more than twice as many patients in the PEG-IFN group experiencing a reduction versus the IFN group ($p < 0.05$), possibly due to the increased half-life of PEG-IFN [34].

3.3 Immunogenicity

Table 3 presents an overview of the immunogenicity findings of this review. Fifteen studies reported on antibodies to different components of the PEGylated drug, of which six studies reported only on anti-PEG antibodies [17, 35–39] and nine studies differentiated antibodies specific to PEG, the PEGylated drug, and the native drug or linker molecule (Table 4) [19, 33, 40–46].

3.3.1 Prevalence of Pre-existing Antibodies to PEG

Four studies reported on the prevalence of pre-existing anti-PEG antibodies in the healthy population, which ranged from 23% to 72%, and no study found any correlation between the concentrations of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies [17, 35, 36, 38]. The prevalence of anti-PEG antibodies was higher in females than males for both IgG ($p = 0.018$) and IgM ($p < 0.0001$) [35, 36]. Both studies reported a higher prevalence of anti-PEG antibodies in the younger population, with one study reporting an anti-PEG IgG prevalence of up to 60% for 20-year-olds versus 20% for age > 50 years [35, 36]. A negative correlation for anti-PEG IgG concentration was reported with age in females ($p = 0.0073$) and males ($p = 0.026$) [35].

3.3.2 Frequency and Prevalence of Antibody Types

One clinical trial of pediatric patients with ALL reported on the frequency of differentiated antibodies specific to PEG,

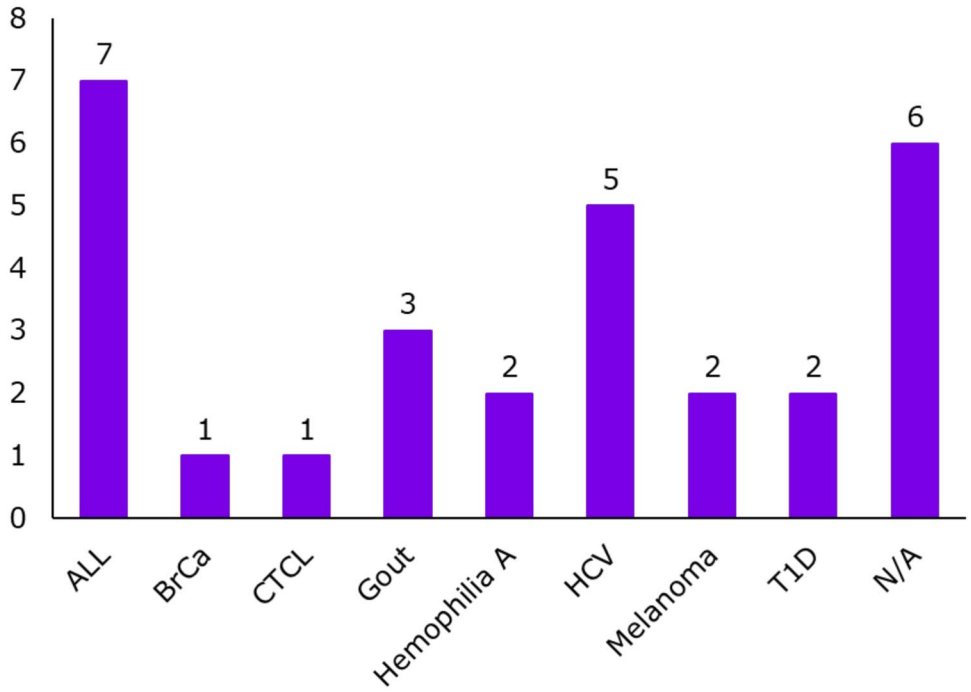

Fig. 1 Included studies by disease area. *ALL* acute lymphoblastic leukemia, *BrCa* breast cancer, *CTCL* cutaneous T-cell lymphoma, *HCV* hepatitis C virus, *N/A* not available, *T1D* type 1 diabetes

the PEGylated drug, and the native drug. Of the 11.5% of samples that tested positive for anti-PEG-ASP in the study, 80.1% were positive for anti-PEG alone, 15.5% were positive for both anti-PEG and anti-L-ASP, and 1.5% were positive for anti-L-ASP alone [33]. In another study of pediatric patients with ALL, the prevalence of pre-existing anti-PEG antibodies was higher prior to first administration of PEG-ASP (IgG 13.9%; IgM 29.1%) and decreased following administration of PEG-ASP (IgG 4.2%; IgM 4.5%) [37].

3.3.3 Treatment-Emergent Antibody Response

Two clinical trials of patients with T1D reported a significantly higher proportion of patients experiencing a treatment-emergent antibody response with the PEGylated product BIL versus non-PEGylated GL ($p = 0.002$ and $p < 0.001$) [28, 29].

3.3.4 Hypersensitivity Reactions

In an observational study of pediatric and adult patients with ALL, pre-existing anti-PEG IgG was significantly associated with first-exposure hypersensitivity reactions ($p < 0.01$) [37]. An analysis of the Italian National Spontaneous Adverse Drug Reaction Reporting System from 2001 to 2021 reported a higher frequency of hypersensitivity reactions for PEGylated versus non-PEGylated therapeutics ($p < 0.0001$) [47].

3.3.5 Increased Drug Clearance

In a study of pediatric patients with ALL, pre-existing anti-PEG antibodies increased initial clearance of PEG-ASP by 41.4% [39]. In another study, anti-PEG-ASP was associated with significantly faster drug clearance ($p = 5.0 \times 10^{-6}$) [33]. In another observational study of pediatric patients with ALL, the presence of anti-PEG was very closely associated with rapid clearance of PEG-ASP, and mean ASP activity in all nine of the anti-PEG-positive sera was below the limit of detection (< 5 U/L) [40]. In adult patients with refractory gout, PEG-uricase was detected in plasma at 11.0 ± 6.0 (mean ± standard deviation; SD) days in antibody-positive patients and 16.1 ± 5.9 days in antibody-negative patients ($p = 0.06$) [46].

3.3.6 Antibody-Driven Variations in Drug Activity

In an observational study of pediatric and adult patients with ALL, pre-existing IgG and IgM anti-PEG antibodies reduced PEG-ASP activity in a concentration-dependent manner ($p < 0.001$) [37]. Another observational study in pediatric patients with ALL showed significantly lower ASP activity in the sera of anti-PEG-positive patients compared with anti-PEG-negative patients using serology ($p = 7.7 \times 10^{-5}$) as well as flow cytometry ($p = 3.6 \times 10^{-5}$) [40]. In a clinical trial of adult patients with refractory gout, anti-pegloticase antibody was associated with a rapid decrease in plasma uricase activity as indicated by decreased maximum concentration

(C_{max}) ($p = 0.0009$), C_{min} ($p < 0.0001$), and area under the concentration curve (AUC, $p < 0.0001$) [41]. In another clinical trial of adult patients with gout, the half-life of plasma uricase in antibody-negative patients was 10.5–19.9 days compared with being undetectable after 10 days in antibody-positive patients [19].

4 Discussion

While there is a large body of evidence documenting the favorable clinical outcomes of PEGylated protein therapeutics, the potential for adverse outcomes with their use is of concern. To our knowledge, a thorough review of the literature describing such outcomes has not been published to date. In an effort to contribute to a balanced view of PEGylated therapies, the present literature review identified and synthesized evidence describing adverse impacts or lack of benefit associated with PEGylation of protein-based therapeutics.

The number of PEGylated therapeutics has steadily increased since the first US Food and Drug Administration (FDA) approval in 1990 to 38 FDA-approved PEGylated therapeutics as of 2023, with hematology and oncology dominating the approved indications [4]. Despite the increase in the number of FDA-approved PEGylated therapeutics, the FDA has also noted several safety concerns related to their use. The FDA has issued warnings for hypersensitivity reactions, severe allergic reactions related to antibodies against PEG, decreased drug activity due to neutralizing antibodies, cardiovascular events, infusion-related reactions, anaphylaxis, and liver injury for PEGylated drugs such as damoctocog alfa pegol, pegloticase, PEG-IFNα-2a, pegvisomant, and certolizumab pegol [48, 49]. Numerous FDA-approved medications carry warnings about hypersensitivity reactions to intravenous (IV) medicines compounded with PEG castor oil, and the FDA's Center for Drug Evaluation and Research has raised concerns about the renal safety of the dosage of PEG 400 in bivalirudin, a direct thrombin inhibitor [50, 51]. Oxycodegol, a PEGylated form of oxycodone, did not receive FDA approval due to hepatic toxicity and the risk of abuse [4]. Hypersensitivity reactions and fatalities led to a manufacturer recall of peginesatide, approved for treating anemia associated with chronic kidney disease [4]. It is also worth noting that PEGylation does not always result in less frequent dosing, as demonstrated by the clinical trial publication on adult patients with gout, in which the half-life of plasma uricase was shorter in patients with antibodies to PEG-uricase [19]. Another example is pegunigalsidase alfa, a recently approved PEGylated drug indicated for long-term enzyme replacement therapy in adult patients with Fabry disease. The patients who developed antibodies to pegunigalsidase alpha had lower plasma pegunigalsidase alpha concentrations [52]. The approved dosing frequency for pegunigalsidase alpha is identical to other non-PEGylated enzyme replacement therapies for Fabry disease [53, 54].

The clinical implications of this review's findings include screening and monitoring for pre-existing antibodies and those induced in response to PEGylated therapy as well as monitoring and adjusting the dosing of PEGylated therapeutics. As anti-PEG antibodies can be both pre-existing and drug induced, screening for antibodies before and during the administration of PEGylated therapeutics may be useful [17, 35, 42]. This has become critical after a large proportion of the population has received mRNA vaccines containing PEGylated lipid nanoparticles for coronavirus disease 2019 (COVID-19); the vaccinated population has been found to have higher levels of anti-PEG antibodies than the unvaccinated population [16]. Furthermore, pre-existing anti-PEG IgG and

Table 4 Immunogenicity: studies reporting on antibody types detected in humans

Study	Type of antibody		
	Anti-PEG	Anti-PEGylated drug	Other
Hillarp, 2023 [42]	✓	✓ (anti-PEG-rFVIII)	✗
Pezeshkpoor, 2023 [45]	✓	✓ (anti-PEG-FVIII)	✓ (anti-FVIII)
Kloos, 2020 [43]	✓	✓ (anti-PEG-ASP)	✓ (anti-SS-linker)
Liu, 2019 [33]	✓	✓ (anti-PEG-ASP)	✓ (anti-ASP)
Hershfield, 2014 [41]	✓	✓ (anti-pegloticase)	✗
Ganson, 2006 [19]	✓	✓ (anti-PEG-uricase)	✗
Armstrong, 2007 [40]	✓	✗	✓ (anti-ASP)
Sundy, 2007 [46]	✓	✓ (anti-PEG-uricase)	✗
Myler, 2016 [44]	✓	✓	✓ (anti-IFN)

ASP asparaginase, *FVIII* factor VIII, *IFN* interferon, *PEG* polyethylene glycol, *rFVIII* recombinant factor VIII, *RR* relative risk, *SS* disulfide bond

IgM antibodies significantly increased following the vaccination [16]. One study reported that anti-PEG supercarriers, people with extremely high levels of anti-PEG antibodies, have an increased risk of hypersensitivity and anaphylactic reactions following vaccination with PEG-containing mRNA COVID-19 vaccines [55]. However, the intricate dynamics between the PEG-antibody complex, the variety of antibodies generated against PEGylated drugs, the lack of sensitive tests, and the differing immunological mechanisms driving the antibody response pose challenges for screening and monitoring. Firstly, PEG is a synthetic linear polymer that can vary in size depending on the number of repeating units of ethylene oxide. PEG's affinity, specificity, and molecular weight determine the interaction between PEG and its antibodies [56]. Notably, one study included in this review reported a difference in inhibition kinetics for PEGylated factor VIII (FVIII) depending on its molecular weight. In this study, anti-PEG antibodies inhibited FVIII activity by 90% to 100% when conjugated to 40 and 60 kDa PEG, compared with 60% when conjugated to 20 kDa PEG, putatively due to fewer antibody binding sites on the smaller PEG molecule, suggesting that inhibition induced by anti-PEG antibodies could be dependent on the molecular weight of PEG [45]. Secondly, in contrast to a non-PEGylated drug where antibodies are only specific to the native drug molecule, antibodies to the PEGylated drug can be specific to the PEG moiety, the native drug, and the linker molecule [33, 40, 43–45]. Thirdly, the current prevalence of anti-PEG antibodies may be underestimated due to low test sensitivity. There is an unmet need to develop assays that represent the natural diversity of anti-PEG antibodies in humans and are sensitive enough to detect low-affinity antibodies, increases in the titer of pre-existing antibodies, and antibodies induced by PEG conjugation to the therapeutic product [56]. Lastly, the immunological mechanism driving the anti-PEG antibody response depends on the nature of the PEG molecule. PEG in a PEGylated protein therapeutic acts as a hapten, inducing anti-PEG antibodies in a T-cell-dependent manner. In contrast, PEG polymers, such as those in cosmetics or personal care products, drive the antibody response in a T-cell-independent manner [38]. It is unclear if the differences in the mechanisms of antibody production determine the outcome of antibody-associated hypersensitivity or silent inactivation.

The pharmacologic outcomes of the present review also emphasize the importance of monitoring the dosing of PEGylated therapeutics and adjusting or creating individualized dosing as required. Fixed doses of PEGylated therapeutics have shown considerable intra- and interpatient variability [57, 58]. Dose increments may be necessary in some patients due to neutralizing antibodies generated in response to PEGylated therapeutics that have the potential to alter the pharmacologic response by increasing drug clearance or reducing drug activity, thereby decreasing the efficacy [19, 33, 37, 40, 41, 46]. Dose reductions or therapy withdrawal may also be necessary in some patients due to PEGylated treatment-emergent AEs [25, 34]. A recent US study in adult patients with ALL demonstrated that therapeutic drug monitoring and individualized dosing can improve the tolerability of the PEG-ASP regimen [59]. However, another study in pediatric patients with ALL found similar PEG-ASP-related toxicity among patients who received individualized PEG-ASP dosing based on activity levels and those treated with a fixed-dose protocol [57]. Another recent study in pediatric and adult patients with ALL highlighted the need for creating pharmacokinetic models that can predict drug inactivation, which can be countered by either dose increment or switching to alternative non-PEGylated drugs to optimize treatment outcomes [60].

There are some limitations to the present review. The scope of this review was to identify and characterize adverse outcomes associated with the PEGylation of protein-based therapeutics; thus, the large body of literature documenting favorable clinical outcomes with PEGylated therapeutics was not included. Although our search did result in some efficacy findings related to the presence of anti-PEG antibodies, a thorough comparison of the efficacy of PEGylated versus non-PEGylated therapeutics was also beyond the scope of this review. Additionally, although this review identified many publications analyzing the safety of PEGylated therapeutics, many studies did not include a non-PEGylated comparator and, therefore, were excluded as they did not establish a causal relationship between the adverse outcomes and PEGylation, which resulted in a relatively small sample size of 29 studies. Finally, most of the studies in this review compared AEs between PEGylated and non-PEGylated drugs without determining the cause of observed differences between the two treatment types.

5 Conclusion

This literature review identified evidence of adverse outcomes associated with the use of PEGylated therapeutics, including several AEs and SAEs. Furthermore, despite the long-held belief that PEG is non-immunogenic, this review identified

evidence of pre-existing anti-PEG antibodies as well as those induced by treatment with PEGylated therapeutics, resulting in increased drug clearance and decreased activity. Overall, our findings suggest that the use of PEGylated therapeutics may require monitoring for adverse safety outcomes, sensitive detection assays for quantification of antibodies, and dose adjustments for optimal treatment outcomes.

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Declarations

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Conflicts of Interest C.L. is employed by Sanofi and may own Sanofi stock or stock options. During the course of the research, M.M. was employed by Sanofi and was a shareholder. Y.K. and V.P. are employed by Evidera, a part of Thermo Fisher Scientific which provides consulting and other research services to pharmaceutical, medical device, and related organizations. In their salaried positions, they work with a variety of companies and organizations and are precluded from receiving payment or honoraria directly from these organizations for services rendered. Evidera received funding from Sanofi to participate in the development of this manuscript. C.W. has received personal fees from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, CSL-Vifor, Eli Lilly and Company, GlaxoSmithKline, MSD, Novo Nordisk, and Sanofi outside the submitted work.

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Informed Consent Not applicable

Availability of Data and Material Not applicable

Code Availability Not applicable

Author Contributions C.L. and M.M. conceived the overall concept, contributed to the writing of the manuscript, provided expert review, and supervised manuscript development. V.P. conceived of the literature review design, conducted the search, and participated in the screening and data extraction. Y.K. did the majority of the writing and participated in the screening and data extraction. C.W. provided expert opinion. All authors read and approved the final manuscript.

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