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BRIEF REPORT

Physician Awareness of Immune Responses to Polyethylene Glycol-Drug Conjugates

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Antibodies against polyethylene glycol (PEG) can critically jeopardize the efficacy and safety of PEGylated therapeutics. For some PEG-drugs, a sizeable fraction of patients develop anti-PEG antibodies (APA), leading to reduced efficacy and potential adverse events. We surveyed physicians from several specialties to assess their awareness of APA. Overall, 83% of the physicians surveyed indicated that they have recently prescribed PEGylated drugs. Although 91% of respondents were aware of antidrug antibodies in general, only 22% were aware of APA responses. Further, there was limited awareness (35%) of PEG's inclusion in prescribed PEGylated therapeutics. These findings bring to light a need for improved awareness of APA, potentially via targeted education of physicians who prescribe specific PEGylated therapeutics that could induce or are otherwise affected by APA. Finally, it will be critical to quantitate the extent of knowledge transfer from the research community to clinicians, especially on topics of patient safety.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Patients can produce antibodies that recognize polyethylene glycol (PEG), a polymer increasingly used in medicine. Anti-PEG antibodies (APA) cause accelerated clearance and SAEs for some (but not all) PEGylated drugs.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ We assessed physician awareness (i) that immunological responses can be elicited directly against PEG, and (ii) that the PEGylated drugs they prescribe contain PEG.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ Only 22% of physicians who prescribe PEGylated therapeutics are aware of APA. Only 35% of physicians who prescribe PEGylated drugs know that PEG is a part of that drug compound.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ These findings underline a need for improved awareness of APA. Physicians who prescribe PEGylated therapeutics should undergo targeted education. Given the low levels of awareness, it may be important to quantitate the efficacy of knowledge transfer from the research community to clinicians, especially on topics of patient safety. Finally, as the use of PEG in medicine becomes more prevalent, providers should closely monitor potential polypharmacy issues.

Prolonged systemic circulation is paramount to the effectiveness of many biologics and nanomedicines. A popular strategy to extend circulation kinetics involves conjugating polyethylene glycol (PEG) to the active pharmaceutical ingredient (API), thereby minimizing attachment by proteins and opsonins and reducing the immunogenicity of the underlying API.[1,2] PEGylation has become a mainstay in biopharmaceutical production: a search of www.clinicaltrials.gov for studies of interventions with the keyword "PEG" revealed that there are currently 75 active (finished recruiting) trials involving a PEGylated therapeutic, and an additional 219 open studies (not yet recruiting, recruiting, or available for expanded access). Unmodified PEG alone is also under development for intravenous administration as a low-volume resuscitation solution for hemorrhagic shock.[3,4]

Surprisingly, animal and human studies have shown that some PEG-modified therapeutics can induce antibodies that specifically bind PEG, leading to rapid elimination, loss of efficacy, and a marked increase in risk of serious adverse events.[1,5–8] For example, in 2016 a phase III study (NCT01848106) of a PEGylated RNA aptamer (pegnivacogin) for inhibition of coagulation factor IXa was halted following serious adverse events (SAEs) linked to anti-PEG antibody (APA) responses.[7,9] In that study, the subjects who

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experienced SAEs all possessed very high titers of preexisting APA relative to the other subjects. In a clinical study of pegloticase, 45% (9/20) of patients developed high titer APA within days and became nonresponsive to the treatment.[6] APA-positive patients receiving pegloticase also had an increased rate of infusion reactions,[5,6,10] although the precise mechanism of APA involvement remains unclear.[11] Research on PEG-asparaginase revealed that about one-third of patients had poor response to the therapy.[8] Unsurprisingly, in these patients the rapid clearance of pegaspargase was associated with the presence of APA.[8] In addition to efficacy and safety concerns, APA have important implications for the management of specific diseases. For example, pegloticase is the last-line therapy for treatment-refractory tophaceous gout.

A unique aspect of APA, compared with most other antidrug antibodies, is the potential for preexisting APA, i.e., the presence of circulating APA in patients who have not been previously treated with the specific PEGylated drug. We recently found that ~70% of the general population possess detectable levels of preexisting APA, including ~7% and ~1% with concentrations of IgG and IgM exceeding 500ng/mL, respectively.[12] These results underscore the potential for APA to impact the efficacy and safety of PEGylated drugs in a large number of patients. Given the diversity of PEG-modified therapies—from proteins to nanoparticles to ultrasound contrast agents, the implications of PEG-specific immunity span a broad spectrum of the clinical landscape.[2,13]

Despite the important consequences of APA, physicians' awareness of this emerging issue has not previously been investigated. It is clear that for some PEGylated therapeutics, PEG sensitization status can be the key difference between a successful treatment or an adverse outcome.[6–8,14] Thus, for physicians prescribing these treatments, their awareness of APA, as well as their awareness of which prescribed drugs actually contain PEG, can be of important clinical value, particularly since PEGylated drugs are increasingly being developed and prescribed. Here, we sought to determine to what extent physicians who routinely prescribe PEGylated drugs are aware (i) that immunological responses can be elicited directly against PEG, and (ii) that the PEGylated drugs they prescribe contain PEG.

METHODS

In this study, physicians completed a 5-min pen-and-paper survey assessing their frequency of prescribing specific PEGylated drugs and their awareness of antidrug and/or anti-PEG antibody responses. Participants representing specialties likely to prescribe PEGylated drugs (including hematology, oncology, allergy, rheumatology, nephrology, and internal medicine) were included from four large academic medical institutions. Participants were explicitly asked not to guess on question items if they did not know an answer. For each question assessing physician knowledge, an option of "I don't know" was provided. Group proportions were compared using a two-tailed Fisher's exact test of independence and adjusted for multiple comparisons. The response rate was 83% (80 responses out of 96 offered, **Table 1**). The list of PEGylated therapeutics on the survey included: pegaspargase, pegfilgrastim, PEGylated liposomal doxorubicin, peginterferon alfa-2a, peginterferon alfa-2b, PEGylated epoetin beta, peginesatide, naloxegol, pegloticase, plegridy, certolizumab pegol, pegaptanib, pegvisomant, and pegademase bovine. The study was approved by the Institutional Review Board at the University of North Carolina at Chapel Hill (Study #15-2797).

Table 1 Demographics of physician respondents

	Percent of physicians (*n*)
Response rate	
Participated	83% (80/96)
Did not participate	17% (16/96)
Specialty	
Onc & Hem	31% (25/80)
Nephrology	34% (27/80)
Allergy & Rheum	15% (12/80)
IM & others	20% (16/80)
Frequency of prescribing PEGylated drugs[a]	
Never	20% (14/71)
Weekly	15% (11/71)
Monthly	27% (19/71)
Yearly	38% (27/71)
Time in practice	
≤10 years	54% (43/80)
>10 years	46% (37/80)
Total	100% (80/80)

[a]Denominator <80 because nine respondents prescribed PEGylated drugs but did not indicate their frequency of prescription.

RESULTS

Overall, 83% (66/80) of the physicians in this study prescribed PEGylated drugs (**Table 2**). The average participant's time spent in clinical practice was 14 years. Although 91% (99% confidence interval (CI): 82–99%) of respondents were aware of antidrug antibodies, only 22% (99% CI: 10–34%) were aware of the potential for APA responses. Exacerbating the matter, there was almost equally limited awareness (35%) of PEG's inclusion in prescribed PEGylated therapeutics across all specialties represented (**Table 2**), where "awareness" was generously defined as knowing that at least half of the PEGylated drugs you prescribe contain PEG. Only nine respondents of the 80 surveyed (11%) correctly identified all their prescribed PEGylated drugs as containing PEG.

Contrary to expectations, the physicians who indicated that they routinely prescribe PEGylated therapeutics were no more aware of the potential for anti-PEG responses than those who do not (Fisher's $P = 1.00$). Further, there was no difference observed between physicians who prescribe PEGylated drugs most frequently (on a weekly basis), compared with any other group (Fisher's $P = 0.38$). There was no apparent shared characteristic among the 22% of physicians who were found to be aware of APA.

Table 2 Physician awareness of anti-PEG antibodies and the presence of PEG in drugs

	Percent prescribed ≥1 PEGylated drug (*n*)	Percent aware that PEG is part of the drug (*n*)[a]	Percent aware of antidrug Abs (*n*)	Percent aware of anti-PEG Abs (*n*)	
Specialty					
Allergy & Rheum	83% (10/12)	80% (8/10)	90% (9/10)	10% (1/10)	N.S. ($P = 0.34$)[b]
Nephrology	89% (24/27)	13% (3/24)	81% (22/27)	15% (4/27)	
Onc & Hem	100% (25/25)	40% (10/25)	96% (24/25)	36% (9/25)	
IM & others	44% (7/16)	29% (2/7)	100% (14/14)	20% (3/15)	
Frequency of prescribing PEG-drugs					
Never	0% (0/14)	N/A	90% (9/10)	18% (2/11)	N.S. ($P = 1.00$)
Weekly	100% (11/11)	27% (3/11)	91% (10/11)	18% (2/11)	
Monthly	100% (19/19)	21% (4/19)	89% (17/19)	16% (3/19)	
Yearly	100% (27/27)	48% (13/27)	89% (24/27)	37% (10/27)	
Physicians who prescribed:					
pegaspargase[c]	100% (15/15)	47% (7/15)	93% (14/15)	27% (4/15)[c]	N.S. ($P = 0.78$)
pegloticase[c]	100% (21/21)	33% (7/21)	95% (20/21)	10% (2/21)[c]	
pegfilgrastim	100% (42/42)	40% (17/42)	95% (40/42)	29% (12/42)	
peg-liposomal doxorubicin	100% (24/24)	42% (10/24)	96% (23/24)	33% (8/24)	
peg-epoetin beta	100% (40/40)	25% (10/40)	88% (35/40)	15% (6/40)	
Time in practice					
≤10 years	84% (36/43)	33% (12/36)	93% (37/40)	20% (8/40)	N.S. ($P = 0.78$)
>10 years	81% (30/37)	37% (11/30)	89% (32/36)	24% (9/37)	
Total	83% (66/80)	35% (23/66)	91% (69/76)	22% (17/77)	

[a]Of those who prescribed PEGylated drugs, how many were aware that those drugs contained PEG? Knowing that at least half of their PEGylated drugs contained PEG was classified as "aware."
[b]N.S. denotes "No significance."
[c]Efficacy of these drugs has been shown to be significantly impacted by the presence of anti-PEG antibodies.[6,8] Unmarked drugs are not necessarily unaffected by anti-PEG antibodies; further investigation is warranted.

DISCUSSION

Given the demonstrated risks associated with APA, including first-exposure allergic reactions, we believe that physicians' awareness of both (i) the inclusion of PEG in the drugs they prescribe, and (ii) PEG's immunogenicity, will be essential for the safe and effective use of PEGylated medicines. Interestingly, despite the inclusion of the letters "peg" in the names of many PEG-modified therapeutics, most physicians surveyed were not aware that the drugs contained the PEG polymer. This underscores the potential need for a clearer indication of the inclusion of PEG in packet inserts, and possibly targeted education campaigns.

While our understanding of the anti-PEG response is still developing, we view APA as a new class of antidrug antibodies, with possibly more complex considerations due to the number of PEG-modified therapies currently in use or under development. Unlike traditional antidrug antibodies that occur only after use of a specific drug, APA stimulated or induced by one PEGylated drug (e.g., PEG-protein conjugate) can potentially render a second PEGylated drug that otherwise shares few structural similarities (e.g., PEG-liposome) nonefficacious or even unsafe. Polypharmacy associated with the induction of APA by different PEGylated drugs will inevitably become more prevalent as the number of approved PEGylated drugs increases and their use becomes correspondingly more widespread.

The prevalence of preexisting APA was estimated by Armstrong to be 27–28% among normal healthy subjects, with a predominantly IgG response (~19%, 5%, and 3% of the total individuals possessing IgG only, IgM only, and both IgM and IgG APA, respectively).[15] The prevalence of preexisting APA has also been reported in both healthy donors and untreated controls of clinical trials, on the order of ~19% and 16% of treatment-naïve gout and phenylketonuria patients, respectively.[6,16] More recently, we found detectable levels of APA in nearly 70% of the general population, likely due to improved sensitivity of detection.[12] The variance is possibly attributed to differences in the sensitivities of the APA-detection assays used across studies. There currently lacks a standardized assay protocol for quantification of APA, a shortcoming that must be rectified for diagnosis of APA.

At present, it is unclear why only a handful of PEGylated drugs appear to suffer from anti-PEG antibody responses, while most others are seemingly unaffected. Despite the widespread prevalence of detectable levels of preexisting APA, only two of the PEG-conjugated drugs included in the survey have documented evidence of a link between adverse clinical outcomes and APA to date (pegloticase and pegaspargase). If the negative effects of APA were primarily due to the presence of preexisting APA, we might expect that all PEGylated drugs would be similarly impacted. Since this is not the case, we are left with the hypothesis that PEGylated therapeutics susceptible to APA likely induce a relatively stronger humoral response compared with other PEGylated drugs, eventually resulting in adequate APA titers to impact subsequent dosing. In this scenario, measuring preexisting APA concentrations could still be of clinical value, since studies of PEGylated drugs have suggested that baseline APA titers might serve as a predictor of adverse effects.[6,7]

In humans, all negative APA-related clinical outcomes have been associated with drugs that are covalently modified with PEG. Rodent studies have shown, however, that the repeated administration of PEGylated liposomes causes the generation of APA and subsequent accelerated blood clearance (ABC) of PEG particles.[17] Notably, this has not been observed with PEGylated liposomal doxorubicin (PLD), due to PLD-mediated cytotoxicity after uptake by APA-secreting B cells. It is likely that PEGylated liposomes carrying nontoxic small molecule drugs would be impacted by APA.

Since patients with higher titers of preexisting APA may be at higher risk of adverse events,[7] we believe that it will be critical to measure baseline and mid-treatment concentrations of APA in participants of clinical trials for new PEGylated compounds to assess the possibility of a link between adverse events and antibody concentrations. Specifically, to identify individuals at elevated risk of adverse APA responses, we propose that the recommended clinical practice should track not only the prior use of a specific PEGylated drug but, more broadly, the recent use of other PEG-conjugated or PEG-liposomal therapies. As we continue to deepen our understanding of the mechanisms underlying the immunogenicity of PEG, it will be important to ensure that physicians who prescribe PEGylated therapeutics are aware of the documented patterns of antibody-related adverse outcomes observed for some of the PEGylated products that they employ. Given the low levels of awareness seen in this study, it will be critical to quantitate the efficacy of knowledge transfer from the research community to clinicians in other topic areas, especially on matters that are key to patient safety.

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1. Yang, Q. & Lai, S.K. Anti-PEG immunity: Emergence, characteristics, and unaddressed questions. *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.* **7**, 655–677 (2015).
2. Harris, J.M. & Chess, R.B. Effect of pegylation on pharmaceuticals. *Nat. Rev. Drug Discov.* **2**, 214–221 (2003).
3. Plant, V. *et al.* Low-volume resuscitation for hemorrhagic shock: understanding the mechanism of PEG-20k. *J. Pharmacol. Exp. Ther.* **361**, 334–340 (2017).
4. Parrish, D. *et al.* New low-volume resuscitation solutions containing PEG-20k. *J. Trauma Acute Care Surg.* **79**, 22–29 (2015).
5. Ganson, N.J. *et al.* Control of hyperuricemia in subjects with refractory gout, and induction of antibody against poly(ethylene glycol) (PEG), in a phase I trial of subcutaneous PEGylated urate oxidase. *Arthritis Res. Ther.* **8**, R12 (2006).
6. Hershfield, M.S. *et al.* Induced and pre-existing anti-polyethylene glycol antibody in a trial of every 3-week dosing of pegloticase for refractory gout, including in organ transplant recipients. *Arthritis Res. Ther.* **16**, R63 (2014).
7. Ganson, N.J. *et al.* Pre-existing anti-polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. *J. Allergy Clin. Immunol.* **137**, 1610–1613.e7 (2016).
8. Armstrong, J.K. *et al.* Antibody against poly(ethylene glycol) adversely affects PEG-asparaginase therapy in acute lymphoblastic leukemia patients. *Cancer* **110**, 103–111 (2007).
9. Povsic, T.J. *et al.* Pre-existing anti-PEG antibodies are associated with severe immediate allergic reactions to pegnivacogin, a PEGylated aptamer. *J. Allergy Clin. Immunol.* **138**, 1712–1715 (2016).
10. Sundy, J.S. *et al.* Efficacy and tolerability of Pegloticase for the treatment of chronic gout in patients refractory to conventional treatment. *JAMA* **306**, 711–720 (2011).
11. Verhoef, J.J. *et al.* Potential induction of anti-PEG antibodies and complement activation toward PEGylated therapeutics. *Drug Discov. Today* **19**, 1945–1952 (2014).
12. Yang, Q. *et al.* Analysis of pre-existing IgG and IgM antibodies against polyethylene glycol (PEG) in the general population. *Anal. Chem.* **88**, 11804–11812 (2016).
13. Jokerst, J.V. *et al.* Nanoparticle PEGylation for imaging and therapy. *Nanomedicine (Lond)* **6**, 715–728 (2011).
14. Lipsky, P.E. *et al.* Pegloticase immunogenicity: the relationship between efficacy and antibody development in patients treated for refractory chronic gout. *Arthritis Res. Ther.* **16**, R60 (2014).
15. Armstrong, J.K. The occurrence, induction, specificity and potential effect of antibodies against poly(ethylene glycol). In *PEGylated Protein Drugs: Basic Science and Clinical Applications* (ed. Veronese, F.M.) 147–168 (Birkhäuser Basel, Basel, Switzerland; 2009).
16. Longo, N. *et al.* Single-dose, subcutaneous recombinant phenylalanine ammonia lyase conjugated with polyethylene glycol in adult patients with phenylketonuria: an open-label, multicentre, phase 1 dose-escalation trial. *Lancet* **384**, 37–44 (2014).
17. Ishida, T. *et al.* Accelerated blood clearance of PEGylated liposomes following preceding liposome injection: effects of lipid dose and PEG surface-density and chain length of the first-dose liposomes. *J. Control. Release* **105**, 305–317 (2005).