Isoagglutinin reduction in intravenous immunoglobulin (IgPro10, Privigen) by specific immunoaffinity chromatography reduces its reporting rates of hemolytic reactions: an analysis of spontaneous adverse event reports

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BACKGROUND: Hemolysis is an infrequent but recognized and potentially serious adverse effect of intravenous immunoglobulin (IVIG). Relatively elevated hemolysis reporting rates were seen with some IVIG products with high anti-A/B isoagglutinin content, among which IgPro10 (Privigen, CSL Behring). For IgPro10, two isoagglutinin reduction measures were successively implemented: 1) anti-A donor screening and 2) immunoaffinity chromatography (IAC; Ig IsoLo)-based isoagglutinin reduction step included in the production process. The aim of this analysis was to investigate the effects of these isoagglutinin reduction measures on the reporting rates of IgPro10 hemolysis worldwide.

STUDY DESIGN AND METHODS: Between February 2008 and December 2018, hemolysis reports from the CSL Behring Global Safety Database were analyzed in relationship to changes in IVIG IgPro10 production methods. Further analysis classified hemolysis reports by indication and blood group.

RESULTS: Median (minimum-maximum) anti-A/anti-B titers were 32 (8-64)/16 (8-32) at baseline, 32 (8-64)/16 (8-32) after donor screening, and 8 (8-32)/4 (2-8) after implementation of IAC. The reporting rate of hemolytic reactions per 1000 kg IgPro10 sold was 4.05 cases at baseline, 2.00 after donor screening, and 0.50 after implementation of IAC. In 2018, there were seven reports of hemolytic reactions; representing 0.18 cases per 1000 kg IgPro10 sold, with a reduction of 95.6% versus baseline.

CONCLUSION: Following implementation of the IAC isoagglutinin reduction step, spontaneous reports of hemolytic events with IgPro10 were significantly and consistently reduced versus IgPro10 without isoagglutinin reduction, offering patients a more favorable benefit-risk profile.

INTRODUCTION: Intravenous immunoglobulin (IVIG) is used as immunomodulatory therapy in autoimmune diseases such as primary immune thrombocytopenia (ITP), Kawasaki disease (KD), Guillain-Barré syndrome (GBS), and chronic inflammatory demyelinating polyneuropathy (CIDP), and as replacement therapy in patients with primary and secondary immunodeficiency.1,2 Although generally showing a favorable safety profile,3,4 clinically significant hemolysis is an infrequent but known adverse event associated with IVIG products and can result in severe complications.5–8 Severe hemolytic events can result in anemia and reticulocytosis9,10; in very rare cases, hemoglobinuria leading to renal failure, disseminated intravascular coagulation and death have occurred. Risk factors for hemolytic events with IVIG include non-O blood group, high IVIG...
dose, underlying inflammatory state, and high isoagglutinin content in the IVIG product.\(^5\)

IVIG consists of immunoglobulin G (IgG) purified from donor plasma, potentially containing antibodies of the IgG class to the blood group A and B antigens (isoagglutinins). Originally, IgG was purified by a cold ethanol fractionation protocol,\(^11\) which removes isoagglutinins in a precipitation step (separation of Cohn fraction III). Unfortunately, this step is quite wasteful and eliminates other useful antibodies as well.\(^12,13\) Some newer IgG purification processes have partially or totally replaced cold ethanol fractionation with other techniques, such as octanoic acid precipitation and/or chromatography. While these processes result in IVIG products with an overall high purity and a high yield, they may have reduced capacity to reduce isoagglutinins. An increase in hemolytic events was reported when several IVIG manufacturers with widely used IVIG products changed from cold ethanol fractionation to chromatographically purified products.\(^5,14\) This coincided with a change in presentation of the products from lyophilized powders to stabilized liquid solutions, but the lyophilization itself was not responsible for reducing the isoagglutinins. Some IVIG manufacturers have continued to use cold ethanol fractionation, use other purification techniques such as polyethylene glycol precipitation, and/or have switched to a chromatographic process including a specific isoagglutinin reduction step.\(^15\) These products did not contribute to the observed increase in hemolytic cases with IVIG.\(^3\)

Current guidelines require IVIG products to have isoagglutinin titers below a reference standard (anti-A titer of 1:32-1:64; anti-B titer of 1:16-1:32), assessed by the agglutination assay according to Ph Eur (Ph Eur direct method).\(^14,16,17\) This limit has been criticized for not adequately addressing the haemolytic risk with high-dose, immunomodulatory IVIG therapy. Indeed, a study driven by data in the EudraVigilance database reported 373 IVIG-related severe hemolytic reactions between 2008 and 2013, with a relationship between isoagglutinin titers above 1:16 in IVIG products and increased rates of hemolytic events.\(^3\)

The manufacturing process for the IVIG product IgPro10 (Privigen, CSL Behring), based on octanoic acid fractionation and chromatography, initially did not include any isoagglutinin reduction step. The manufacturer consecutively implemented two isoagglutinin reduction measures. First, CSL Behring’s plasma collection organization, CSL Plasma, introduced a screening program in 2014 to exclude plasma with high anti-A titers. The plasma of about 5% of donors (those with high anti-A titers) was excluded from plasma pools used for IgPro10 production.\(^17\) Some IgPro10 was still produced from plasma obtained from other suppliers and was not screened for anti-A. Starting at the end of 2015, donor screening was progressively replaced with an immunoaffinity chromatography (IAC; Ig IsoLo) step included in the IgPro10 production process. The IAC column contains trisaccharides that mimic A/B antigens; as the immunoglobulin solution passes through the column, anti-A/Bs bind to the resin and are removed from the product. Loss of antibodies other than isoagglutinins is minimal, and other product characteristics are not modified.\(^17\)

Upon approval of the manufacturing change by the relevant health authorities, IAC was implemented into each market; global implementation was completed during 2017.

Donor screening had a limited impact on isoagglutinin titers in the final product, with no change in median (minimum-maximum) anti-A and anti-B titers (anti-A: 32 [8-64], anti-B: 16 [8-32]), but titers above the median became less frequent. IAC produced a two-titer step (75%) reduction in isoagglutinins (anti-A: 8 [4-16], anti-B: 4 [2-8]). A flow cytometry assay showed anti-A and anti-B reductions of 39% and 27%, respectively, with donor screening; and of 89% and 88% with IAC (Table 1).\(^17,18\)

We analyzed spontaneous reports of hemolytic reactions in patients receiving IgPro10 as well as the impact of anti-A donor screening and of the IAC manufacturing step on hemolytic reactions reporting rates worldwide.

### TABLE 1. IgPro10 production time periods: isoagglutinins testing results

| IgPro10 production years | Chromatography, no isoagglutinin reduction Baseline | Chromatography, anti-A donor screening After donor screening | Chromatography, IAC step After IAC step |
|--------------------------|-----------------------------------------------------|-------------------------------------------------------------|----------------------------------------|
| Ph Eur direct method,\(^{14,16,17}\) titers, median (min-max) | Anti-A | 2007-2013 | 2014-2015 | Since 2016 |
| Ph Eur direct method,\(^{14,16,17}\) titers, | Anti-B | 32 (8-64) | 32 (8-64) | 32 (8-64) |
| | Anti-B | 16 (8-32) | 16 (8-32) | 16 (8-32) |
| | Flow cytometry,\(^{18}\) % reference, mean (SD) | Anti-A | 109.5 (33.7) | 66.6 (9.2) | 13.3 (4.4) |
| | Anti-B | 82.3 (22.2) | 60.3 (5.4) | 10.3 (4.5) |

IAC = immunoaffinity chromatography; IgPro10 = 10% intravenous immunoglobulin preparation; n = number of IgPro10 lots tested; SD = standard deviation.
METHODS

IgPro10 production methods
Between 2008 and 2013, IgPro10 was produced by octanoic acid precipitation and chromatographic purification without isoagglutinin reduction (referred to as baseline). Between 2014 and 2015, IgPro10 was produced following donor screening, resulting in exclusion of plasma from donors with high anti-A titers (approx. 5% of donors; referred to as donor screening). From 2016 onward, the IgPro10 production method replaced the donor screening with an IAC step (Ig IsoLo), with complete implementation by mid-2017 (referred to as after IAC step).

Hemolysis cases
The reporting rates of spontaneous cases of hemolysis were compared across the three time periods until the end of 2018. Hemolysis reporting rates were calculated as the number of cases per 1000 kg of IgPro10 sold.

Spontaneously reported cases of hemolytic reactions in patients receiving IgPro10 were identified from the CSL Behring Global Safety Database from February 2008 to December 2018. Cases of hemolytic reactions (referred to as hemolysis in this analysis) were identified using the Standard Medical Dictionary for Regulatory Activities Query hemolytic disorders “broad.” Hemolysis cases from clinical trials or those from unbranded products (i.e., no brand name included but only the international nonproprietary name reported) were excluded.

Outcomes
The main outcome of this analysis is the comparison of reporting rates of spontaneous hemolysis cases in patients receiving IgPro10 over the defined time periods corresponding to the different IgPro10 production methods (as outlined in Table 1).

Furthermore, we analyzed hemolysis cases by indication and by ABO blood group, distinguishing between immunomodulatory (generally high-dose) and replacement (generally low-dose) therapy. Due to insufficient dosing data, the impact of dose could not be assessed directly.

IgPro10 lots have a 3-year shelf life. Consequently, the lots available in the market in the after IAC step period (2016-2018) originated from either the baseline, donor screening, or IAC period. IAC lots became more prominent in the market as time elapsed. As a result, the hemolysis cases occurring in the after IAC step period were associated with non-IAC lots, IAC lots, or both.

Statistical analysis
Data were analyzed descriptively. A post hoc analysis for comparing the relative rates of reported cases of hemolysis per quantity of IgPro10 sold after the different steps compared to baseline was performed using a Poisson regression model (SAS version 9.4, SAS Institute) to obtain ratios, 95% confidence intervals, and p values. Results were not adjusted for multiplicity. A p value of less than 0.025 was considered statistically significant.

Role of the funding source
The funder of this analysis was responsible for the data collection and analyses included in this manuscript. All authors had access to the data included in this manuscript, and all authors have agreed to submit the manuscript.

RESULTS

Hemolysis reporting rates
A total of 438 spontaneous hemolysis cases were identified from the CSL Behring Safety Database between February 2008 and December 2018.

Before introduction of donor screening, the overall hemolysis reporting rate was 4.05 cases/1000 kg IgPro10 sold (262 reported cases). After incorporation of donor screening, the rate dropped to 2.00 cases/1000 kg (122 reported cases, unadjusted p value <0.0001), a reduction of 50.6% compared to baseline.

| Year | Total cases | No. of cases associated with only non-IAC lot(s) | No. of cases associated with only IAC lot(s) | No. of cases associated with both IAC and non-IAC lot(s) | ≤14 days | >14 days | Missing dates | No. of cases with onset of hemolysis within 14 days after an IAC lot |
|------|-------------|-----------------------------------------------|---------------------------------------------|-------------------------------------------------|----------|----------|---------------|---------------------------------------------------------------|
| 2016 | 30          | 18                                             | 3                                           | 7                                               | ≤14 days | >14 days | Missing dates | No. cases with onset of hemolysis within 14 days after an IAC lot |
| 2017 | 7           | 2                                               | 4†                                          | 1                                               | 0        | 0        | 0             | 10 (3 cases with only IAC lots)                                |
| 2018 | 4           | 1                                               | 3                                           | 0                                               | 0        | 0        | 0             | 4 (3 cases with only IAC lots)                                 |
| Total | 41          | 21                                              | 10                                          | 10                                              | 0        | 0        | 0             | 17                                                             |

Only 41/54 hemolysis cases with lot number data were available; for the remaining 13 cases, lot number data were missing. Cases with only non-IAC lots were not analyzed for time to onset of hemolysis.

* Independent of cases associated with IAC and non-IAC lots.
† One case (not included) was a transfusion reaction without any signs of hemolysis and missing dates.
‡ A subset number of cases with IAC and non-IAC lot(s) and number of cases with only IAC lots.

IAC = immunoaffinity chromatography.
with baseline. After implementation of the IAC step, the hemolysis reporting rate decreased to 0.50 cases/1000 kg IgPro10 sold (54 reported cases, unadjusted p value <0.0001), signifying an 87.7% reduction in the hemolysis rate compared with baseline.

Among the 54 cases reported after IAC implementation, between 2016 and 2018, IgPro10 lot numbers were reported in 41 cases (75.9%). Of these cases, 21 were associated with non-IAC lots, 10 were associated with both IAC and non-IAC lots, and 10 were associated with only IAC lots. Cases associated with non-IAC lots occurred mainly in 2016 (Table 2). Among the seven cases reported in 2018, lot number information was available in four cases. Only three were associated with IAC lots, all had risk/confounding factors (e.g., concomitant medications known to cause hemolysis and/or decreased hemoglobin such as azathioprine, rapid onset proximal myopathy and history of systemic lupus erythematosus) (Table 3).

Regarding the data gathered after IAC implementation, the hemolysis reporting rate per 1000 kg IgPro10 sold decreased from 1.11 in 2016 (72.6% reduction compared with baseline) to 0.32 in 2017 (92.1% reduction compared with baseline) and to 0.18 in 2018 (95.6% reduction compared with baseline) (Fig. 1), likely reflecting the progressive replacement of non-IAC IgPro10 lots with IAC lots in the market and decreased hemolysis with the latter.

**Impact of indication**

The CSL Behring Global Safety Database includes adverse event reports with IgPro10 in all indications for immunomodulatory and replacement therapy, including “off-label” use. Indication was reported for 83.2% of cases before and 82.7% after incorporation of donor screening, and 92.6% after the introduction of IAC. The majority of reported hemolysis cases were from patients receiving IgPro10 for immunomodulatory (i.e., high-dose) therapy (Fig. 2). For immunomodulatory therapy, the reporting rate of hemolysis was 3.09 at baseline, 1.41 (54% reduction) after the introduction of donor screening and 0.39 (87% reduction) after IAC. Patients receiving IVIG as replacement therapy showed much lower hemolysis rates at baseline (0.28), which also decreased following introduction of donor screening (0.25) and IAC (0.08) (Fig. 2). This indicates that IVIG-related

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**TABLE 3. IgPro10 hemolysis cases in 2018 (after IAC implementation)**

| IgPro10 hemolysis cases (2018) | Indication | Blood group | Dose | Non-IAC/IAC lots (anti-A [A] and anti-B [B] titer by Ph Eur direct method) | Outcome | Confounding factors |
|-------------------------------|------------|-------------|------|-------------------------------------------------|---------|-------------------|
| 1                             | Kawasaki disease | A | 2 g/kg bw | Non-IAC (A-1/32 B-1/16) | Recovered | Underlying Kawasaki disease, endocarditis |
| 2                             | Guillain-Barré syndrome | Not reported | Not reported | Not reported | Recovered | Temporarily-associated with another IVIG product (co-suspect drug) |
| 3                             | Immune thrombocytopenia | A | 0.9 g/kg bw | IAC (A-1/16 B-1/8) (A-1/8 B-1/8) (A-1/8 B-1/4) | Recovering | Concurrent pulmonary embolism (and its treatments), lymphoma, rapidly onset proximal myopathy and history of systemic lupus erythematosus |
| 4                             | Chronic inflammatory demyelinating polyradiculoneuropathy | Not reported | Not reported | Not reported | Not reported | Consumer report – underlying inflammatory state including bacterial meningitis |
| 5                             | Demyelinating polyneuropathy | Not reported | 1.9 g/kg bw | Not reported | Not reported | Onset latency of 22 days, omeprazole (hemolytic anemia a known side effect) |
| 6                             | Myasthenic syndrome | AB | Not reported | IAC (A-1/16 B-1/4) | Recovered | Temporarily-associated with another IVIG product (co-suspect drug) |
| 7                             | Chronic inflammatory demyelinating polyradiculoneuropathy | Not reported | Induction: 1.2 g/kg bw, Maintenance: 0.5 g/kg/day every 2 weeks | IAC (A-1/4 B-1/2) (A-1/8 B-1/4) | Recovered | Azathioprine (co-suspect drug, symptoms started with its start and subsided with its discontinuation) |

bw: body weight; IAC, immunoaffinity chromatography; IgPro10, 10% intravenous immunoglobulin preparation; IVIG, intravenous immunoglobulin.
hemolysis is primarily a high-dose adverse reaction as shown in previous scientific literature.8,10,19

The five indications with the highest frequency of reported hemolysis cases were all indications where IgPro10 was used as an immunomodulatory therapy: GBS, ITP, KD, myasthenia gravis (MG), and CIDP (Fig. 3). The rate of hemolysis was reduced by 43% to 72% and 82% to 92% (values represent range across indications) following donor screening and IAC, respectively. Patients receiving IVIG for MG and KD showed the greatest reduction in hemolysis reporting rates.

IgPro10 hemolysis cases with KD indication after IAC

In this analysis, the effect of IAC on reporting rates of hemolysis in patients with KD was also evaluated. There were 19 cases of hemolysis reported in pediatric patients with KD in the baseline period (i.e., before donor screening and the implementation of IAC), representing 0.29 cases/1000 kg of IgPro10. After the IAC implementation, there were three cases reported, which represent 0.03 cases/1000 kg of IgPro10. In patients with KD, after incorporation of the IAC step, the reporting rate of hemolysis cases was reduced by 89.7% (p = 0.0002) (Fig. 3).

The three KD pediatric cases (aged 16 months to 2 years) reported after the IAC implementation had multiple confounding factors. Blood group was reported for two cases; both were blood group A (non-O). Use of high doses of IVIG was reported for two cases. One case reported two co-suspect drugs (IVIGs from different manufacturers) with insufficient information on the temporal relationship to IgPro10 and the other suspected IVIG products. Lot numbers were reported for two cases: One was a non-IAC lot and the other an IAC lot (Table 4).
Impact of ABO blood group

ABO blood group data were available for 223 hemolysis cases presented in this analysis. Blood groups were reported for 54.2% of hemolysis cases at baseline, 40.2% after donor screening and 59.3% after implementation of IAC. The majority of hemolysis cases (70%) were reported in patients with blood group A, followed by AB; hemolysis was rare in blood groups B and O (Fig. 4; Table S1, available as supporting information in the online version of this paper). For each blood group, the majority of hemolysis cases occurred during the baseline period.

DISCUSSION

The relative rate of reported cases of hemolysis per quantity of IgPro10 sold decreased following introduction of donor screening; a greater decrease was identified following IAC incorporation compared with baseline. These findings coincided with observed decreases in product isoagglutinin titers following introduction of anti-A donor screening and, subsequently, a more pronounced reduction in titers following the introduction of IAC in the IgPro10 production process.14,17 Very few hemolysis cases were associated with IAC-treated IgPro10 lots. Our analysis further supports the causal role of isoagglutinins in the final product in hemolytic events associated with IVIG, which has been previously reported.3,6,13,14,20

Isoagglutinins can be efficiently reduced during IVIG manufacturing by an IAC step, as used for IgPro10 and some other products.14,15,21 A donor screening and exclusion program is a less efficient measure to reduce isoagglutinins in chromatographically purified IVIG. CSL Behring implemented a donor screening and exclusion program for IgPro10 from 2014 to 2016. Exclusion of approximately 5% of donors with high anti-A titers resulted in a modest reduction of isoagglutinin titers in the final IVIG product, which was followed by a reduction of hemolytic events as seen here and in an observation study.19 Both isoagglutinin content and hemolysis data shown here suggest that donor screening is less effective than IAC.17 Another IVIG manufacturer evaluated a different donor screening program and concluded it would have “minimal” impact on isoagglutinin titers in the final IVIG product.22 Moreover, donor exclusion reduces availability of plasma for IVIG production, which is frequently in short supply.

| IgPro10 hemolysis cases (2018) | Gender/Age | Blood group | Dose | non-IAC/IAC lots (anti-A [A] and anti-B [B] titers by Ph Eur direct method) | Outcome | Confounding factors |
|-------------------------------|------------|-------------|------|---------------------------------------------------------------------------|---------|--------------------|
| 1                             | Male/17 mo | A           | 2 g/kg bw | Non-IAC (A-1/32 B-1/16) IAC (A-1/16 B-1/4) | Recovered | Endocarditis        |
| 2                             | Female/2 y | A           | 2 g/kg bw over 2 hr 10 min | IAC                              | Recovered | IVIG product from a different manufacturer given after IgPro10 and before the onset of hemolysis |
| 3                             | Male/16 mo | Not reported | Not reported | Not reported                  | Recovered | Two IVIG products from different manufacturers but insufficient information regarding the temporal relationship |

* Age at the time of event. bw = body weight; IAC = immunoaffinity chromatography; IgPro10 = 10% intravenous immunoglobulin preparation; IVIG = intravenous immunoglobulin.
Analyzing spontaneous reports of hemolysis with IVIG to the EudraVigilance database from 2008 to 2013, Bellac et al.\textsuperscript{13} found that IVIG products with anti-A titers of 16 or less had low hemolysis reporting rates (0.08–0.28 cases/1000 kg), while IVIG with higher anti-A titers (≥16) had higher hemolysis reporting rates (0.60–3.08 cases/1000 kg). Acknowledging that comparisons between the two investigations are limited due to methodological differences, hemolysis rates with IgPro10 without isoagglutinin reduction and with anti-A donor screening appear consistent with the latter category. With the IAC step, hemolysis reporting rates with IgPro10 (0.18 cases/1000 kg in 2018) are as low as products in the former category, including products with isoagglutinin reduction by Cohn-like ethanol fractionation.

We compared hemolysis reporting rates in patients receiving IgPro10 by indication. The majority of hemolysis cases were in patients receiving IgPro10 as an immunomodulatory therapy. Cases in patients receiving replacement therapy were rare. This likely reflects dose dependence of IVIG-induced hemolysis, which we could not assess directly, as dose was rarely reported. Replacement therapy is typically low dose (0.2–0.4 g/kg body weight), while immunomodulatory therapy is usually in the range of 1 to 2 g/kg body weight.

The highest hemolysis reporting rates were seen in GBS, KD, and ITP, where IVIG is typically given at a high dose (2 g/kg) for the treatment of acute disease. Somewhat lower hemolysis rates were seen in CIDP and MG, where IVIG is typically given as maintenance therapy at a dose of 1 g/kg per month (in addition to occasional treatments at high dose for induction and treatment of acute exacerbations).

KD is associated with a systemic inflammatory response and an increase in immune system activation, complement activation, and antibody-dependent cell-mediated cytotoxicity, all of which could theoretically lead to increased hemolysis. A higher IVIG dose is often needed, especially in complete or refractory KD, and may magnify the intensity of hemolysis.\textsuperscript{10}

An inflammatory milieu is thought to constitute the first “hit” in a two-hit model of IVIG-mediated hemolysis, where the second hit represents intrinsic factors (presumably isoagglutinins) of the IVIG product.\textsuperscript{5,23,24} In an 8-year retrospective analysis of 123 KD-evaluable patients, hemolysis occurred in 15% of KD patients evaluated for anemia and was strongly associated with high-dose (≥4 g/kg) IVIG.\textsuperscript{10} That study, which investigated cases occurring between 2008 and 2015, did not report the IVIG brand or isoagglutinin titers in the IVIG lots used.

As expected, the data presented here suggest the greatest benefit of isoagglutinin reduction on the hemolysis reporting rates in immunomodulatory indications, including high-dose indications such as KD. In the present analysis, the incorporation of the IAC step successfully reduced the incidence of hemolysis reporting rates in patients with KD from 0.29 cases/1000 kg IgPro10 to 0.03 cases/1000 kg IgPro10. This significant reduction of hemolysis in high-risk indications such as KD ($p = 0.0002$) indicates that IVIG products with low isoagglutinins have a benefit-risk advantage in the treatment of KD patients, who often require higher doses of IVIG, to minimize the hemolysis risk and its complications in this vulnerable patient population.

In our analysis, blood group information was not available for all cases of hemolysis, and the blood group composition of the population exposed to IgPro10 during this investigation is unknown. However, assuming that the analysis population is similar to the US and Western European population, our analysis confirms that the risk of hemolysis is highest in blood group AB, followed by A, B, and O. The majority of hemolysis cases occurred during the baseline period for all blood groups. The higher density of blood group A antigens versus B antigens on erythrocytes, and the average higher anti-A versus anti-B titers in plasma donors, can explain the different patterns between A, B, and AB patients.\textsuperscript{11,25} From our analysis, blood group A and AB recipients would benefit most from isoagglutinin reduction in IVIG. In blood group B, the risk was small at baseline, and only minor changes were seen following donor screening or IAC. Blood group O patients do not express blood group A or B antigens, as such in this blood group hemolysis is not likely to be isoagglutinin-mediated and should not change following anti-A donor screening or IAC. The mechanism of hemolysis after IVIG in blood group O patients, if causally related, remains unknown. The database used in this analysis contains too few cases for an in-depth analysis, and in most, confounding factors were noted, raising doubt about the causal role of IVIG. A role for other blood group antibodies potentially present in IVIG (such as D) appears unlikely.\textsuperscript{25}

Our analysis has some limitations. First, the analysis relied upon spontaneous reporting of hemolytic events. These data were gathered from the central CSL Behring Global Safety Database. This is subject to variability in reporting behaviors and, as such, could be subject to missing information. Mild to moderate hemolytic events can be easily missed without careful clinical and laboratory follow-up.\textsuperscript{5} Second, due to changes in IVIG prescribing practices, the analysis is possibly subject to confounder effects. The changes in approved indications, and potential off-label use, of IgPro10 could underestimate the impact of IAC. Moreover, since 2016, IgPro10 lots produced with donor screening and IAC were in circulation concomitantly, and information on the lot number used was not always available. The result of which could underestimate the effect IAC had on the incidence of hemolysis. Finally, the Weber effect, which describes the effect where spontaneous reporting of adverse events peaks at the end of the second year after regulatory approval and declines steadily thereafter, is a potential confounder for reporting database studies.\textsuperscript{26} However, considering that IVIG has been around for decades, the clear directional shift in hemolysis reporting rates following implementation of IAC in 2016 is unlikely to be attributable to the Weber effect.
In summary, IAC was more efficacious than anti-A donor screening and exclusion. Implementation of the IAC step substantially reduced the anti-A and anti-B antibodies in the IVIG IgPro10 and significantly and consistently reduced spontaneous reporting rates of hemolytic reactions for patients receiving IgPro10 globally.

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CONFLICT OF INTEREST

All authors are employees of CSL Behring; ASH, KVD, AH, JPL, and SW own stock in CSL Behring.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

Table S1. Frequency of cases of hemolysis by ABO blood group and comparison with US population.