Foundational Analysis of the Impact of Mechanical Stress on Molecular Size Distribution in Immune Globulin Intravenous, Human-stwk 10%

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BACKGROUND

- Intravenous immunoglobulin (IVIG) therapy is the standard of care for patients with primary immunodeficiencies and is increasingly used in other immune-mediated conditions[1,2]
- It is imperative to minimize protein aggregates in commercial IVIG therapy, as they have been implicated in adverse events and can enhance immunogenicity in patients receiving IVIG therapy[2]
- Increased demand for IVIG has necessitated robust and optimized manufacturing processes to ensure product quality and minimize impurities, including aggregates[1]
- Shear stress, agitation, mixing speed, and filtration can impact aggregate formation during the manufacturing process, while shipping conditions and temperature excursions may contribute after manufacturing[3]

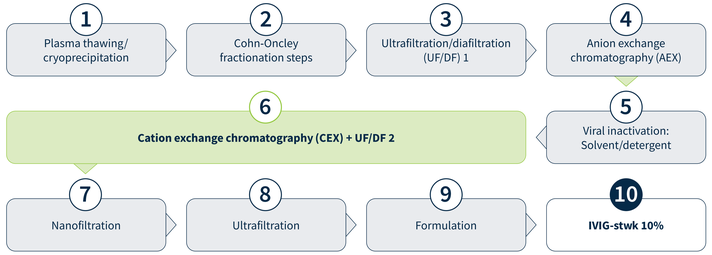
PURPOSE

The purpose of this study was to assess the impact of agitation on the molecular size distribution of IVIG-stwk 10%.

METHODS

- ALYGLO® immune globulin intravenous, human-stwk, 10% (IVIG-stwk 10%) was developed using a multistep manufacturing process that includes cold ethanol fractionation, ultrafiltration and diafiltration, anion exchange chromatography, solvent/detergent virus inactivation, cation exchange chromatography + UF/DF 2, nanofiltration, an additional ultrafiltration step, and formulation **(Figure 1)**

Figure 1. ALYGLO® (IVIG-stwk 10%) Manufacturing Process



- 10 mL of IVIG-stwk 10% was dispensed into sterile washed vials which were stoppered and sealed
- Vials were then placed on a Green SSeriker shaker (Vision Science), and agitation started at 50 rpm in an upright position at a temperature of between 20–25°C to induce mechanical stress
- On days 0, 1, 3, and 11, samples were withdrawn and diluted 1:16 with saline for analysis
- The molecular size distribution of monomers, dimers, high-molecular-weight aggregates, and fragments were quantified using size-exclusion high-performance liquid chromatography (SE-HPLC)
- For SE-HPLC, chromatographic separations were performed using a size exclusion column (TSKgel G3000SW) using a sample size of 50 µl and a flow rate of 0.5 mL/min at a detection wavelength of 280 nm

ABBREVIATIONS

IVIG: intravenous immunoglobulin

SE-HPLC: size-exclusion high-performance liquid chromatography

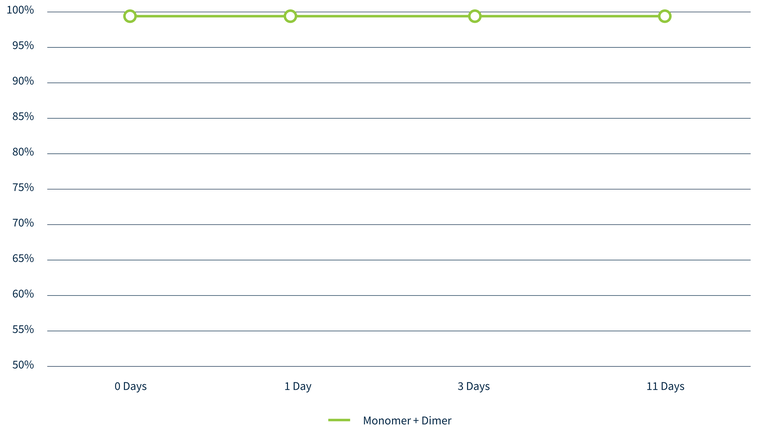
METHODS, CONT.

1. IVIG-stwk 10% was dispensed into sterile vials
2. Vials were agitated on a Green SSeriker shaker (Vision Science) at 50 rpm in an upright position between the temperatures of 20–25°C to induce mechanical stress
3. On days 0, 1, 3, and 11, samples were withdrawn and diluted 1:16 with saline for analysis
4. The molecular size distribution was determined using SE-HPLC (TSKgel G3000SW)

RESULTS

The monomer + dimer percentage for IVIG-stwk 10% on Day 0 was 99.576%; Day 1, 99.582%; Day 3, 99.591%; Day 11, 99.526%. The percentage of polymers and fragments remained below their respective validated limits of quantitation (0.3% and 0.5%) for each time point.

Figure 2. Monomer + Dimer (%) Upon Agitation Over Time



RESULTS, CONT.

Table 1. Molecular Size Distribution of Immune Globulin Intravenous, Human-stwk, 10% Upon Agitation

MOLECULAR SIZE AND U.S. FDA ACCEPTANCE CRITERIA	0 Days	1 Day	3 Days	11 Days
MONOMER + DIMER ≥ 95%	99.576%	99.582%	99.591%	99.526%
POLYMERS ≤ 2%	< 0.3%[a]	< 0.3%[a]	< 0.3%[a]	< 0.3%[a]
FRAGMENTS ≤ 3%	< 0.5%[b]	< 0.5%[b]	< 0.5%[b]	< 0.5%[b]

[a]Polymer limit of quantitation is 0.3%. [b]Fragment limit of quantitation is 0.5%.

POLYMERS AND FRAGMENTS
remained below validated limits of quantitation at all time points.

CONCLUSION

- Mechanical agitation for up to 11 days shows minimal impact on IVIG-stwk 10% molecular size distribution
- A very high percentage of monomers and dimers (>99.5%) remained throughout the duration of the study, compared to polymers and fragments below quantifiable limits.
- Further assessments more analogous to real world conditions, along with additional measurements of opacity and particle size across multiple lots to assess variability are warranted

REFERENCES

1. Kang GB, et al. *J Immunotoxicol.* 2023;10:1253177. **2.** Cook D. *BioSupply Trends Quarterly.* 2024. **3.** Ratanji KD, et al. *J Immunotoxicol.* 2014; 11(2):99-109.

DISCLOSURES AND ACKNOWLEDGEMENTS

SN, AH, SS, and LRM are employees of GC Biopharma USA. NK is an employee of GC Biopharma.