GC Biopharma USA

Alyglo™ (immune globulin intravenous, human-stwk), 10%:

Advancing the IVIG Manufacturing Process with Cation Exchange (CEX) Chromatography

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Ryan Dorfman, PhD | Prolytix, Chief Operating Officer
Stacey Ness, PharmD, IgCP, CSP, MSCS, AAHIVP | GC Biopharma USA, Medical Science Liaison
Suzanne Strasters MSN, FNP-C, IgCN | GC Biopharma USA, Head of Clinical Education

Speaker Introductions and Disclosures

Alan Huber, BSc Pharm, PharmD, MBA
Director of Medical Affairs
GC Biopharma USA

- Clinical pharmacist and plasma industry executive with over 20 years of expertise, including management roles in nationally accredited home infusion companies and leadership of medical affairs teams
- Served as Adjunct Assistant Professor of Pharmacy Practice at the University of Southern California School Of Pharmacy and as an educator and preceptor for pharmacy schools across the country
- Nationally recognized immune globulin (Ig) therapy expert called upon for publications, presentations, and service in an advisory capacity
- **Dr. Huber is an employee of GC Biopharma USA**

Ryan Dorfman, PhD
Chief Operating Officer
Prolytix

- Seasoned expert with over 25 years experience pharmaceutical product and analytical method development, and the manufacturing of reagents and custom collection devices.
- Technical expertise in protein biochemistry, enzymology, blood coagulation, and analytical method development
- Since completing his doctorate in 2000, he has held various key roles at Prolytix, including Senior Scientist, Scientific Director, and Vice President of R&D Operations and Manufacturing.
- **Dr. Dorfman is an employee of Prolytix and a paid consultant for GC Biopharma USA**

Stacey Ness, PharmD, IgCP, CSP, MSCS, AAHIVP
Medical Science Liaison
GC Biopharma USA

- Clinical pharmacist with 20 years of Ig experience
- Immediate past president of Immunoglobulin National Society (IgNS) after serving 2 terms
- Tenured on the IgNS Executive Leadership Team, IgNS Standards of Practice Committee, Education Committee, Pharmacist Steering Committee, and IgCP development committee resulting in presentation of the IgNS Leadership Award in 2019
- **Dr. Ness is an employee of GC Biopharma USA**

Suzanne Strasters MSN, IgCP, FNP-C
Head of Clinical Education
GC Biopharma USA

- Nurse Practitioner with 20 years of experience in nursing
- Over 13 years of experience in Ig and professional clinical education
- Served as clinical educator for several immunology products including a wide portfolio of plasma derived therapies
- Educated on plasma therapies and medical devices to deliver Ig therapies
- Immediate past Medical Science Liaison aiding in the launch of a new infusion device
- **Ms. Strasters is an employee of GC Biopharma USA**

02 ALYGLO™

Manufacturing with CEX chromatography to reduce FXIa to undetectable levels

IVIG, thromboembolic events (TEE) and FXIa – a brief history

TEE in patients receiving IVIG

- Estimated incidence of 0.5%–17%
- Arterial events (e.g., stroke, myocardial infarction, pulmonary embolism) most common

Patient-related risk factors:

- Underlying disease (renal failure, diabetes)
- Thrombotic risk factors (use of oral contraceptives, history of thrombosis, sedentary lifestyle, smoking, advanced age)

IVIG-related risk factors:

- High doses, high infusion rates
- Presence of activated coagulation factors

2003

- FDA requires manufacturers to include TEE warning for all IVIG products

2010

- An increased rate of TEE was observed associated with an IVIG product
- **Contaminating FXIa was identified as root cause**

2013

- FDA requires Boxed Warning for risk of TEE for all IVIG products

An effective means of removing FXIa from IVIG preparations has been a longstanding goal

References: **1**. Ammann EM, Haskins CB, Filman KM, et al. *Am J Hematol.* 2016;91:594-605. doi:10.1002/ajh.24358. **2.** Guo Y, Tian X, Wang X, Xiao Z. *Front Immunol.* 2018;9:1299. doi: 10.3389/fimmu.2018.01299. **3**. Roemisch JR. Webmed Central *IMMUNOTHERAPY* 2011;2(6):WMC002002. doi:10.9754/journal.wmc.2011.002002

Importance of Factor XI/XIa in Hemostasis and Thrombosis

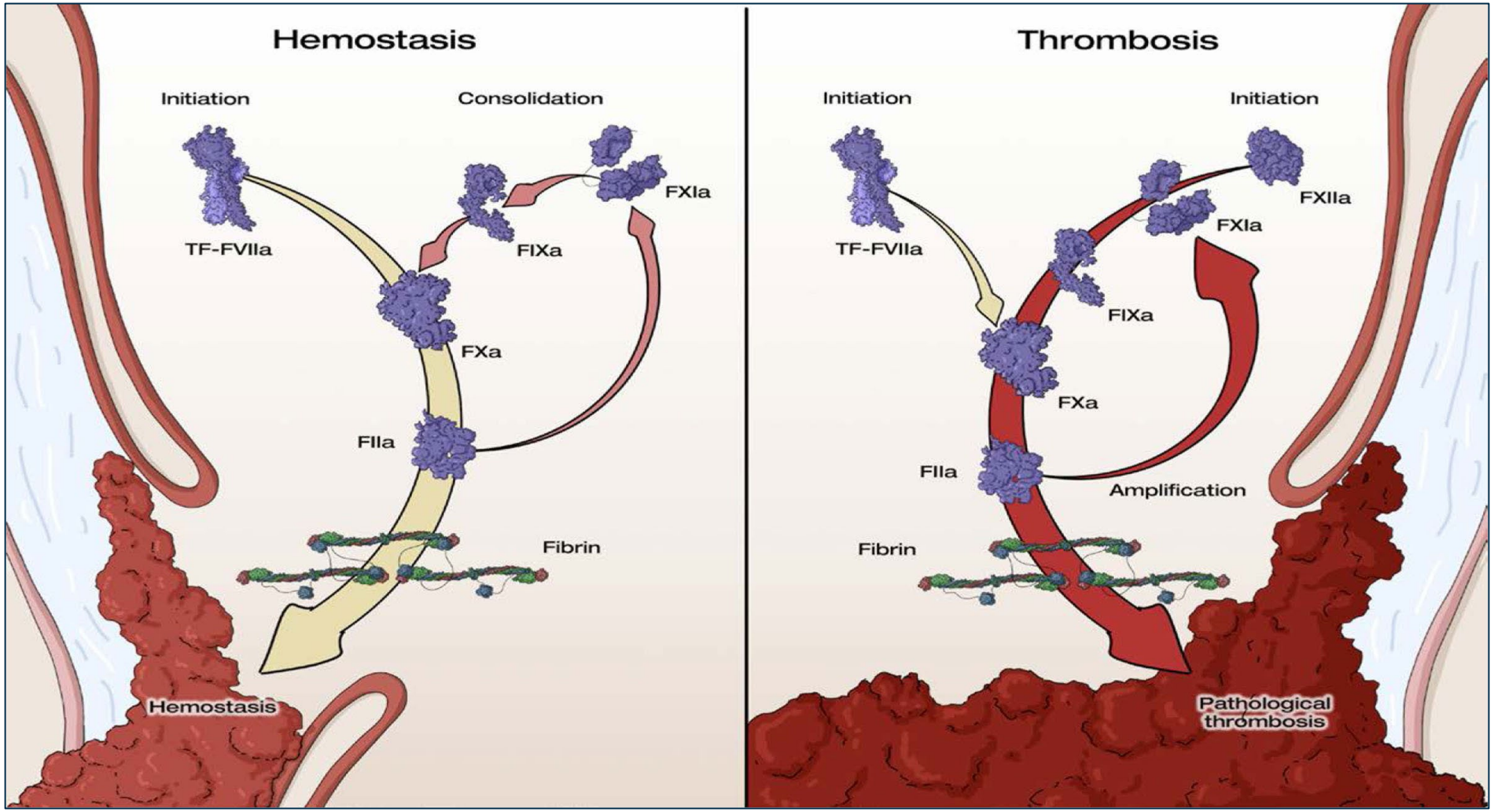
- **Role in Hemostasis**
  - Primarily activated by thrombin in a feedback loop as a result of Extrinsic Pathway activation
  - Augments final hemostatic clot by activation of FIX to FIXa
  - Plays a relatively minor role

- **Role in Thrombosis**
  - Primarily activated by as a result of Intrinsic Pathway (Contact Pathway) activation
  - Plays a major role in pathological thrombus formation
  - FXI drives thrombus growth and propagation in a process called amplification

Differential contributions in Hemostasis versus Thrombosis have made FXI a highly anticipated target for therapeutic anticoagulation

References: 1. Ali AE, Becker RC. Factor XI: structure, function and therapeutic inhibition. J Thromb Thrombolysis. 2024 Apr 16. doi: 10.1007/s11239-024-02972-5. Epub ahead of print. PMID: 38622277.

Positive and Negative Consequences of Factor XI/XIa



References: **1.** Greco A, Laudani C, Spagnolo M, Agnello F, Faro DC, Finocchiaro S, Legnazzi M, Mauro MS, Mazzone PM, Occhipinti G, Rochira C, Scalia L, Capodanno D. Pharmacology and Clinical Development of Factor XI Inhibitors. Circulation. 2023 Mar 14;147(11):897-913. doi: 10.1161/CIRCULATIONAHA.122.062353. Epub 2023 Mar 13. PMID: 36913497.

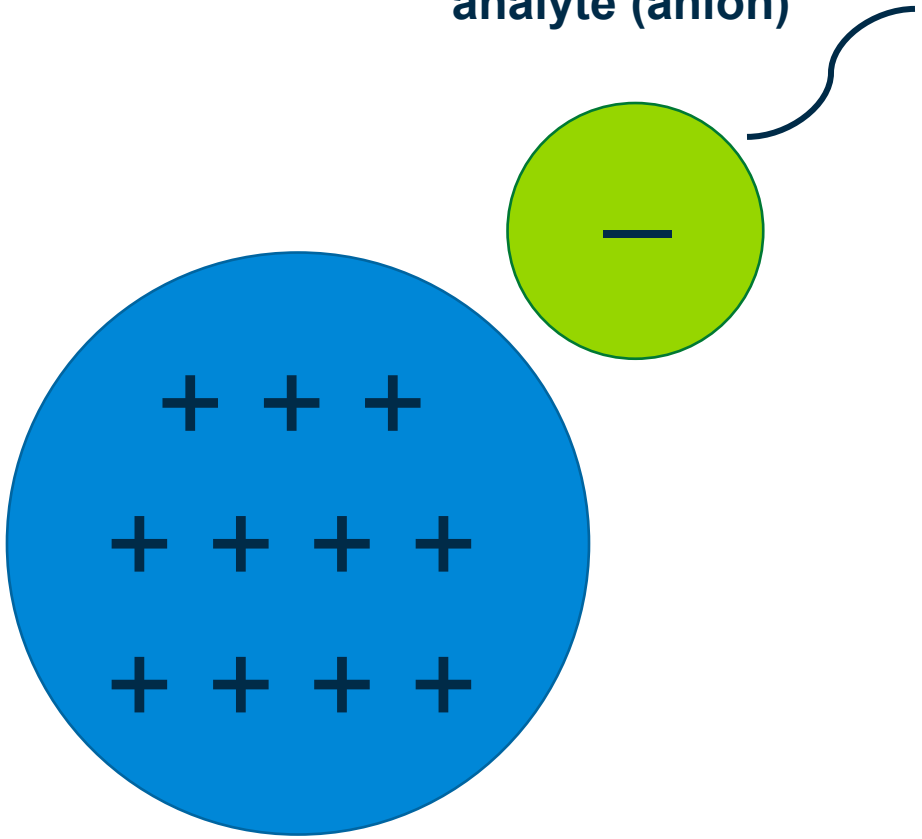
ALYGLO™ manufacturing process

1. Plasma thawing/ cryoprecipitation
2. Cohn/Oncley Fractionation steps
3. Ultrafiltration/ diafiltration (UF/DF) I
4. Anion exchange chromatography (AEX)
5. Viral inactivation: Solvent/detergent
6. **Cation exchange chromatography (CEX)** + UF/DF II
7. Nanofiltration
8. Ultrafiltration
9. Formulation

Alyglo™
immune globulin intravenous, human-stwk 10% liquid

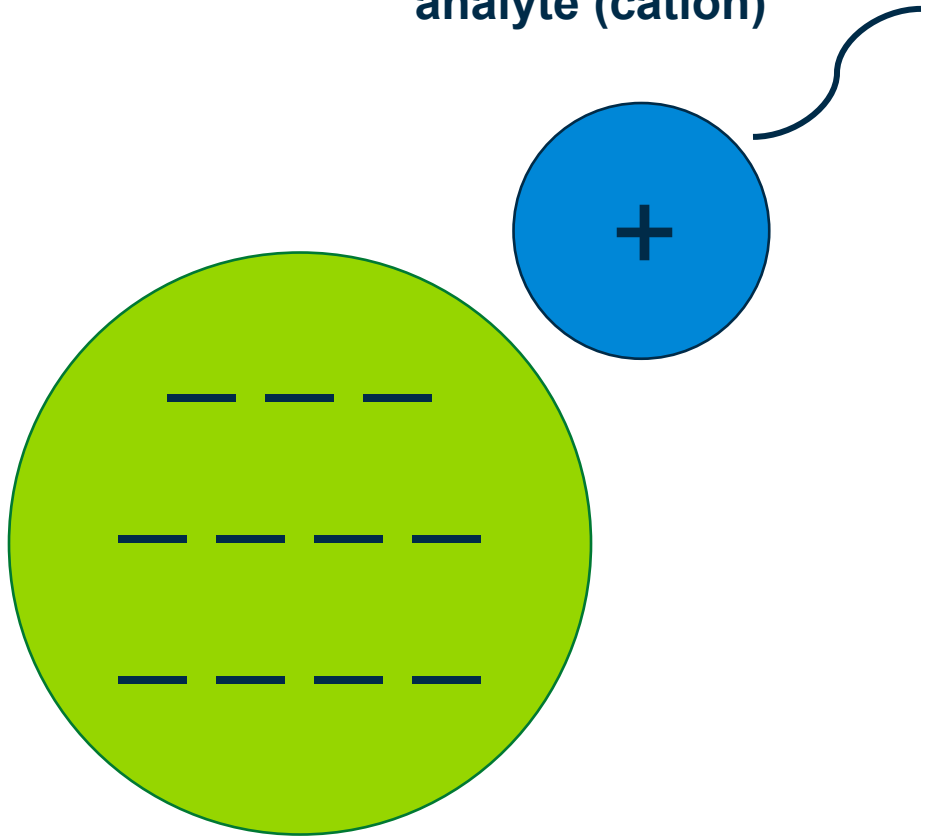
Ion-Exchange Chromatography

Negatively charged analyte (anion)



Anion exchanger stationary phase particle

Positively charged analyte (cation)

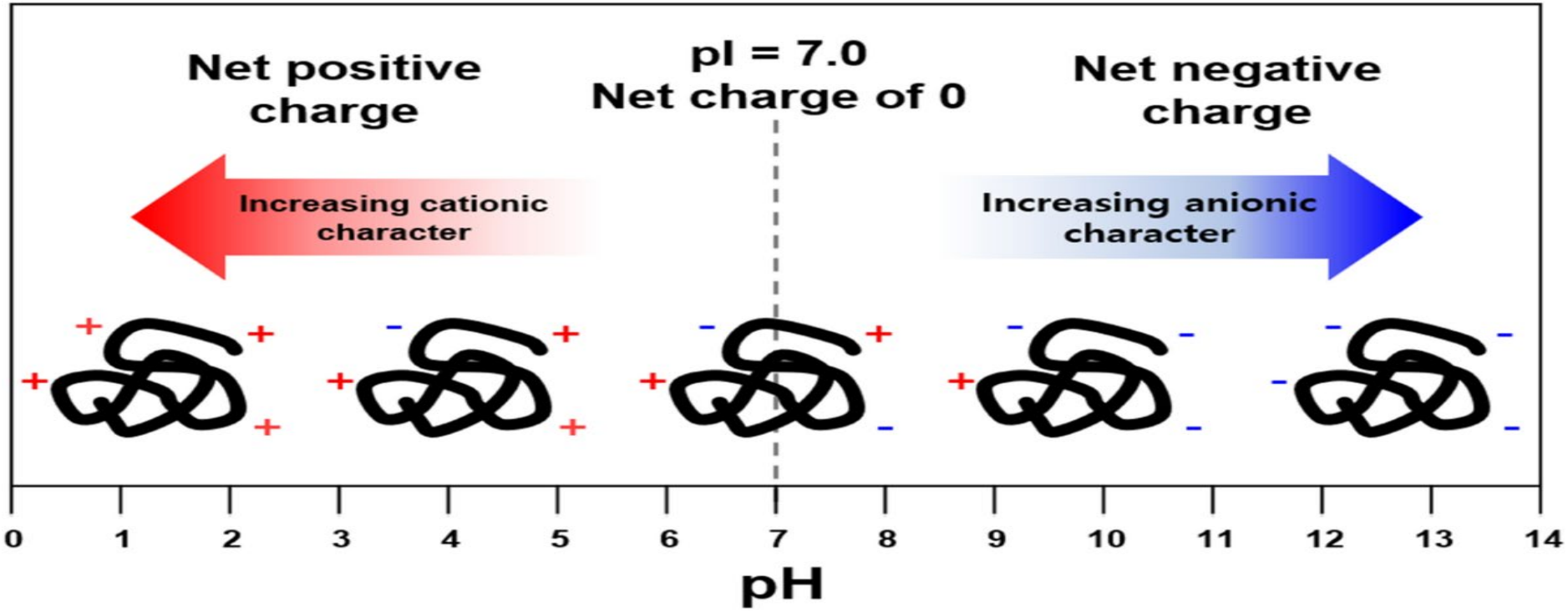


Cation exchanger stationary phase particle

Reference: 1. Bahadir, O. (2013). Ion-Exchange Chromatography and Its Applications. InTech. doi: 10.5772/55744

Isoelectric Point: pH and the Impact on Global Net Charge

- The isoelectric point (pI) of IgG (immunoglobulin G) is 6.1–8.5
- The isoelectric point (pI) of FXI is 8.9-9.1

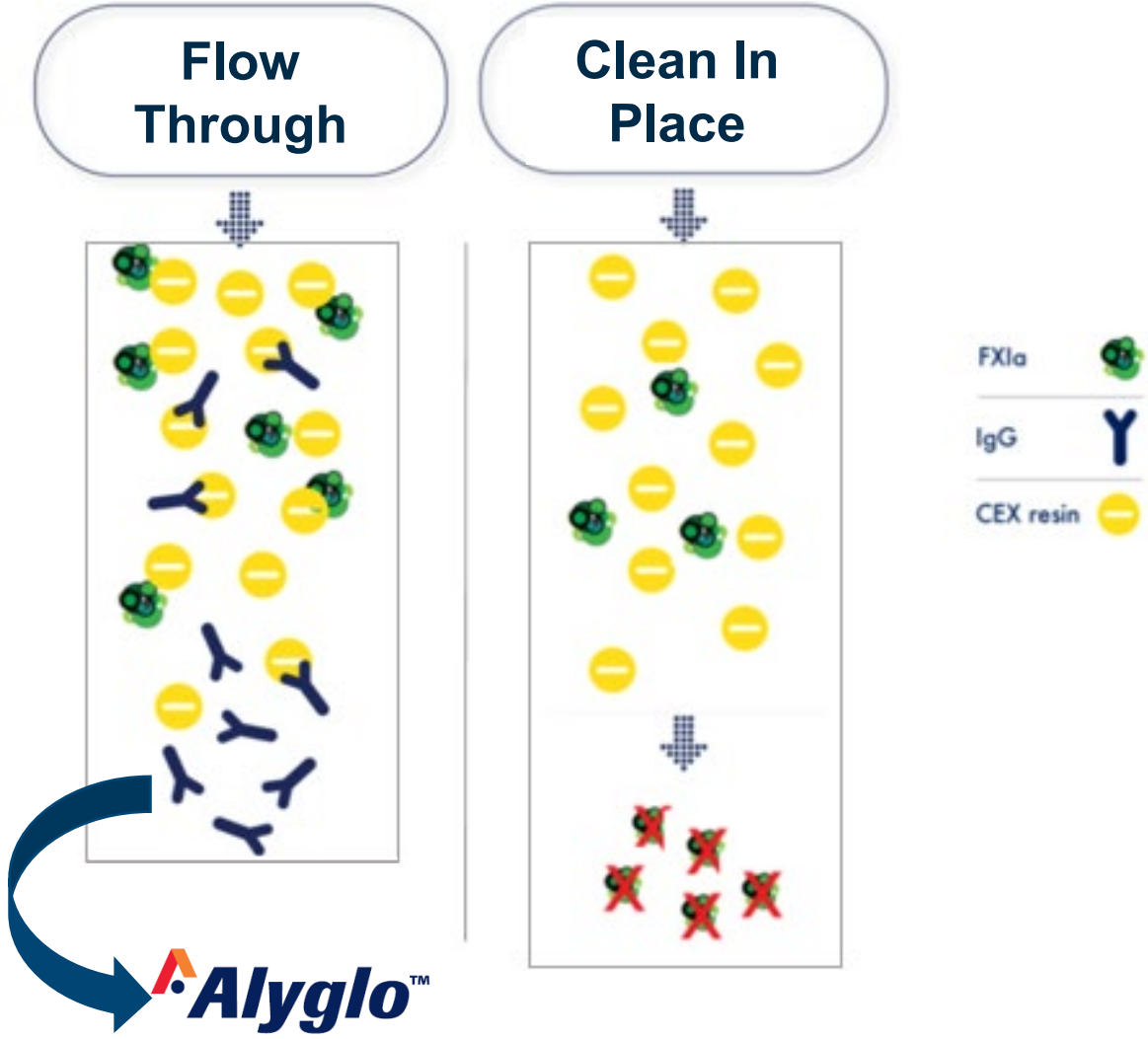


Reference: 1. Noh, G., Keum, T., Bashyal, S. *et al*. Recent progress in hydrophobic ion-pairing and lipid-based drug delivery systems for enhanced oral delivery of biopharmaceuticals. *J. Pharm. Investig.* **52**, 75–93 (2022). https://doi.org/10.1007/s40005-021-00549-5

CEX chromatography: Designed to separate FXIa from IgG

The difference in isoelectric points between FXI and Ig allows the molecules to be separated under specific pH conditions

- CEX resin binds IgG with 1.5 times higher capacity than standard resin
- While FXIa binds tightly to resin, purified IgG flows through more easily under these pH conditions
- FXIa is then removed from the column in the Clean in Place step only after the purified IgG has been collected



Reference: **1.** Burnouf-Radosevich M, Burnouf T. *Transfusion* (1992) 32:861-7. **2.** Park DH, Kang GB, Kang DE, et al. *Biologicals.* 2017:45;1–8. doi: 10.1016/j.biologicals.2016.11.002

FXI/FXIa levels assessed throughout the ALYGLO™ manufacturing process

1 Antigen assays

- Western blot analysis
- Enzyme-linked immunosorbent assay (ELISA)

2 Activity assays

- Thrombin generation assay (TGA)
- Chromogenic FXIa assay
- Non-activated partial thromboplastin time (NaPTT)

3 Spiking study

1. Large quantities of FXIa added to the pre-CEX intermediate
2. FXIa levels assessed after CEX chromatography

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Cation exchange chromatography removes FXIa from a 10% intravenous immunoglobulin preparation

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[1]R&D Center, GC Biopharma Corp., Yongin-si, Republic of Korea, [2]Department of Medical Affairs, GC Biopharma USA, Inc., Fort Lee, NJ, United States

The presence of residual activated coagulation factor XI (FXIa) in some commercial intravenous immunoglobulin (IVIG) products has been identified as the root cause of a small number of thromboembolic events in patients who had received such therapy. Our objectives here were to design and evaluate the manufacturing process of GC5107, a 10% glycine-stabilized IVIG product, for its capacity to remove FXIa. The manufacturing process included a cation exchange chromatography (CEX) step, which employs a resin that binds immunoglobulin G (IgG) with high capacity. Procoagulant activity was assessed using Western blot analysis, enzyme-linked immunosorbent assay, thrombin generation assay, chromogenic FXIa assay, and non-activated partial thromboplastin time (NaPTT) assay. A spiking study in which large quantities of FXIa were added to samples before CEX chromatography was used to examine the robustness of the process to remove FXIa. Western blot and ELISA analyses demonstrated that residual FXIa remained in the intermediate manufacturing products until after CEX chromatography, when it was reduced to undetectable levels. The spiking study demonstrated that CEX chromatography removed >99% of FXI protein and reduced FXI activity to below detection limits, even in samples containing 158-fold greater FXIa levels than that of normal samples. Procoagulant activity in 9 consecutive lots of GC5107 was reduced to below the detection limits of the thrombin generation and chromogenic FXIa assays (<1.56 IU/ml and <0.16 IU/ml, respectively). The NaPTT of >250 s in all 9 lots indicated very low levels of procoagulant activity. We demonstrate that a novel 10% IVIG manufacturing process including CEX chromatography is a robust means of removing FXIa from the final preparation.

KEYWORDS
intravenous immune globulin, thromboembolic event, coagulation FXIa, procoagulant activity, cation exchange chromatography

Introduction

Immunoglobulin (IG) therapy is a mainstay of treatment for patients with primary immunodeficiencies (1). There are numerous licensed IG products available, formulated for either intravenous (IV) or subcutaneous administration. While all human IG is manufactured from pooled plasma using variations on the Cohn–Oncley cold ethanol fractionation process, differences in starting material, downstream purification steps and added stabilizers result in final products that differ in composition in ways that may be important for individual patients (2–4).

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Reference: **1.** Kang GB, Huber AH, Lee J, et al. *Front. Cardiovascular Med.* 2023; 10:1253177. doi:10.3389/fcvm.2023.1253177

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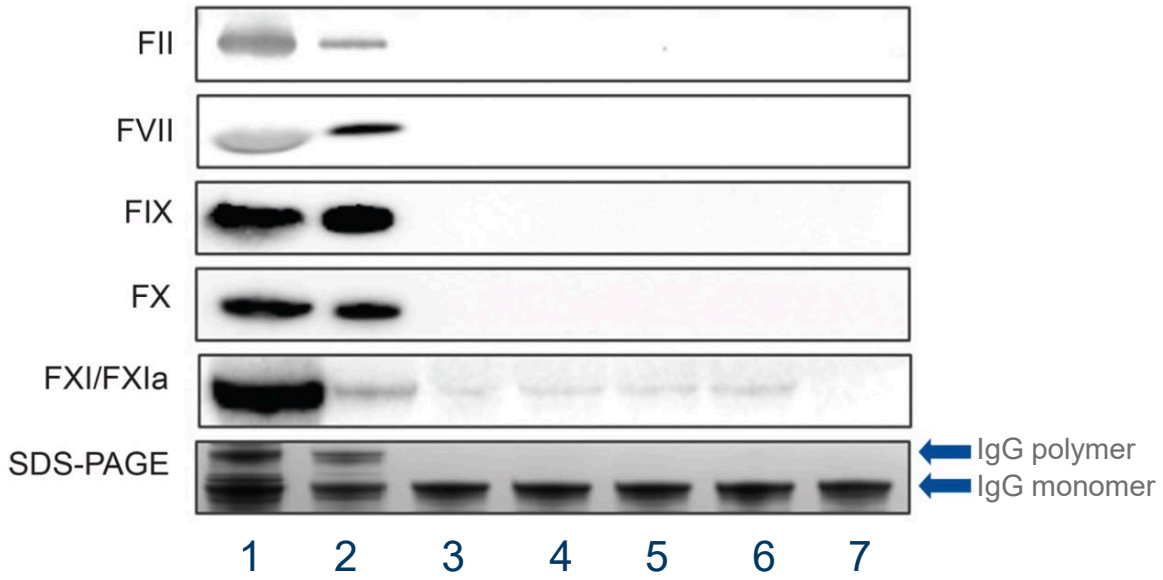
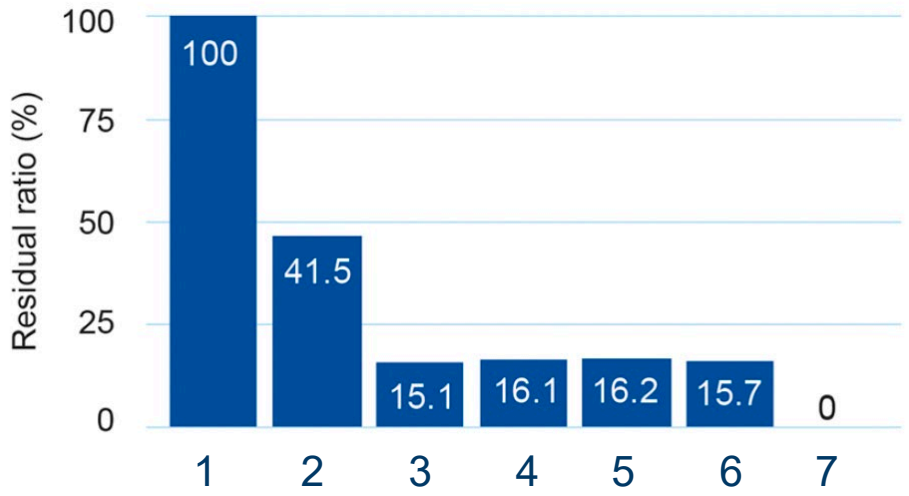
CEX reduces FXI/FXIa antigen to below detection limits

WESTERN BLOT



BAND DENSITY ANALYSIS



(**1**) Cryo-poor plasma; (**2**) Fraction I + II + III paste; (**3**) Fraction I + III filtrate; (**4**) Fraction II paste resuspension; (**5**) UF/DF1 solution; (**6**) AEX flow through; **(7) CEX +UF/DF 2 solution**

FXI/FXIa is undetectable only after CEX chromatography

***Abbreviations:** UF/DF = Ultrafiltration/diafiltration; AEX = Anion exchange chromatography; CEX = Cation exchange chromatography

Reference: **1.** Kang GB, Huber AH, Lee J, et al. *Front. Cardiovascular Med*. 2023; 10:1253177. doi:10.3389/fcvm.2023.1253177

Capacity for FXIa removal verified in spiking study

	No spike		FXIa spike 1 (2.8 µg/mL)		FXIa spike 2 (14.0 µg/mL)	
	Pre-CEX	Post-CEX	Pre-CEX	Post-CEX	Pre-CEX	Post-CEX
FXI/XIa Protein (ELISA*), ng/mL	83.79	<0.31	2679.48	0.96	13264.79	5.56
FXIa Activity (TGA†), mIU/mL	22.26	<1.56	73159.54	<1.56	367549.59	<1.56
IgG Recovery (%)	103.3		100.8		102.4	
FXI Residual Ratio (%)	0.9		0.1		0.1	

32X and 158.3X higher than normal samples

*ELISA = enzyme linked immunosorbent assay

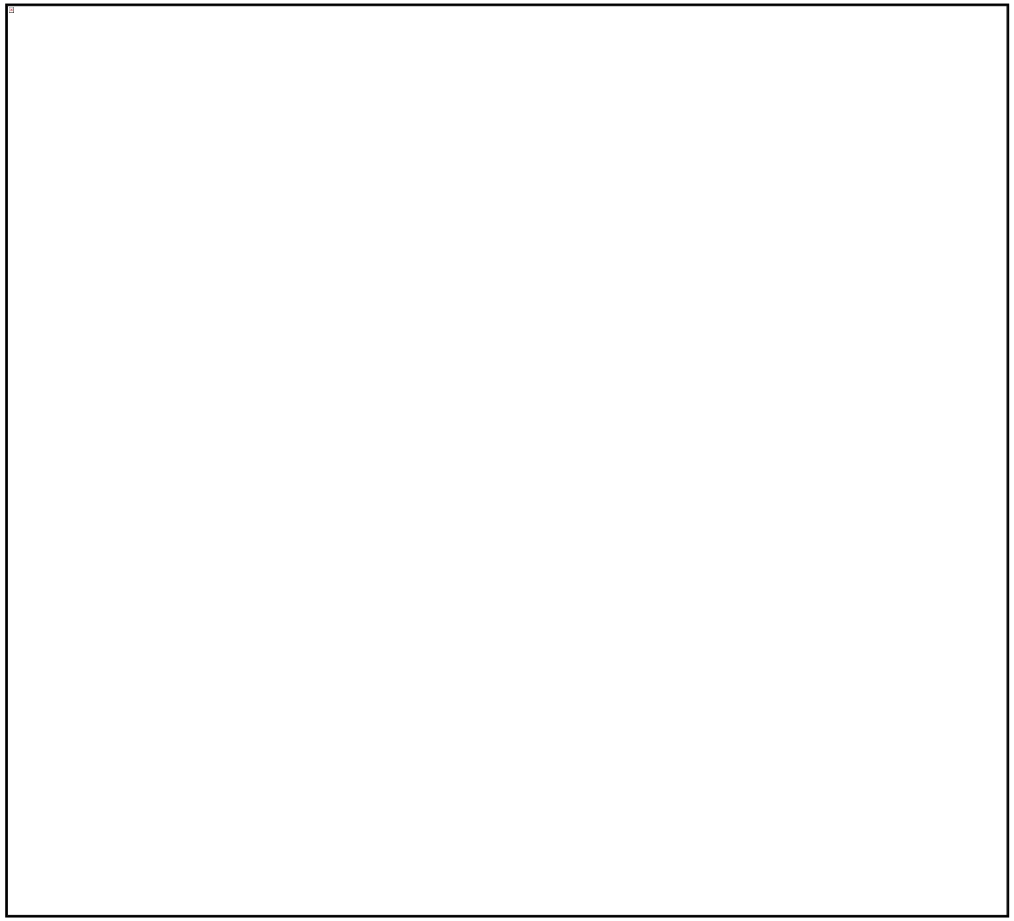
†TGA = thrombin generation assay

FXIa effectively reduced while retaining IgG recovery

Reference: **1.** Kang GB, Huber AH, Lee J, et al. *Front. Cardiovascular Med*. 2023; 10:1253177. doi:10.3389/fcvm.2023.1253177

Thrombin Generation Assays (TGA)

- TGA assays measure thrombin generation as a function of thrombin's cleavage of a synthetic fluorogenic substrate.
- There are three phases of thrombin generation
  - Initiation (clot time/Lag phase)
  - Propagation (burst of thrombin after the clot)
  - Termination (the thrombin machinery is shut down)
- Global assays like PT/aPTT/NAPTT terminate upon formation of a fibrin clot
- At clot time (AKA lag phase), about 10% of total thrombin has been produced
- Engineered for FXIa Specificity – FXIa in the IVIG test article rescues Factor XI deficient plasma



Global assays are simply a measure of Tlag

Reference: **1.** Thrombin Generation Assay Aids Development, May 2012. Genetic Engineering & Biotechnology News, 32(9):52-52.

Procoagulant assessment in 9 batches of final product

Batch no.	TGA* (mIU/mL)	Chromogenic FXIa (mIU/mL)	NaPTT*(s)			
			1:5	Ratio[b]	1:10	Ratio[c]
1	<1.56	<0.16	267.0	1.0	266.2	1.0
2	<1.56	<0.16	282.6	1.1	268.8	1.1
3	<1.56	<0.16	268.1	1.0	275.6	1.1
4	<1.56	<0.16	299.4	1.0	291.9	1.0
5	<1.56	<0.16	302.4	1.0	304.8	1.0
6	<1.56	<0.16	300.8	1.0	296.6	1.0
7	<1.56	<0.16	265.9	1.1	265.9	1.1
8	<1.56	<0.16	256.8	1.1	259.6	1.0
9	<1.56	<0.16	271.3	1.1	266.7	1.1
Mean ± SD	<1.56	<0.16	279.4 ± 17.5	1.0 ± 0.1	277.3 ± 16.2	1.0 ± 0.1

Below limits of detection

> 250s = no coagulation activity

***Abbreviations:** TGA = Thrombin generation assay; NaPTT = Non-activated partial thromboplastin time

Reference: **1.** Kang GB, Huber AH, Lee J, et al. *Front. Cardiovascular Med.* 2023; 10:1253177. doi:10.3389/fcvm.2023.1253177

ALYGLO™ manufacturing process summary

- ✓ The potential presence of residual FXIa in IVIG preparations puts patients at risk of thromboembolic events
- ✓ FXI/FXIa is difficult to remove from IVIG preparations using ethanol precipitation alone
- ✓ CEX chromatography is a robust means of reducing FXIa activity and antigen in ALYGLO™ to below detection limits

The future of IVIG manufacturing

- ✓ Plasma is a limited resource with an ever-increasing demand.
- ✓ IVIG is the most substantial driver of this demand
- ✓ Technological advances are needed to keep up with the demand
  - Eliminate the use of ethanol and pH extremes
  - Streamline the process (process cycles in just 48 hours)
  - Increase IgG yields (>80% is expected at industrial scale)
  - Significantly lower cost per manufacturing cycle
  - Increased safety profiles

Reference: **1.** Hartmann J, Klein HG. Supply and demand for plasma-derived medicinal products - A critical reassessment amid the COVID-19 pandemic. Transfusion. 2020 Nov;60(11):2748-2752. doi: 10.1111/trf.16078. Epub 2020 Sep 9. PMID: 32856742; PMCID: PMC7460929.

- Different IVIG products can be tolerated differently because they vary in their purification methods, excipients, and other factors.

- Explanation
- **Purification methods**
- Different companies may use different methods to purify the immunoglobulins from human plasma. These methods can affect the levels of impurities, which can impact safety and tolerability.
- **Excipients**
- Different IVIG products may contain different excipients, such as sugars, amino acids, or other immunoglobulins.
- **Other factors**

- IVIG products can differ in their sodium levels, pH levels, osmolality, and IgA content.