Minimizing Procoagulant Impurities in IGIV Products Baxter´s Approach

Risk Mitigation Strategies to Address Procoagulant Activity
in Immune Globulin Products
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Agenda

- Background
- Tests established at Baxter to detect procoagulant impurities in IgG products
  - Product comparison
- In vivo Thrombogenicity of FXIa
- Removal of FXI(a) during commercial Cohn fractionation
  - Pharmacovigilance (PV)/ Safety Assessment
  - Conclusions

Background

- "On August 20, 2010, in the interest of patient safety, Octapharma USA Inc. initiated a voluntary market withdrawal of selected lots of Octagam® [Immune Globulin Intravenous (human)] 5% Liquid Preparation]. This was performed as a result of an increased number of reported thromboembolic events, some of which were serious"[1].
- Globally, Octapharma received 30 thromboembolic event reports (spontaneous reporting) during Q3, 2010 compared to 18 throughout 2009[2].
- "This increase is thought to be related to problems with the medicine's manufacturing process"[3].
- Other products followed
  - The Omrix IgG product Omr-IgG-am was voluntarily recalled on Nov 7, 2011[4]
  - Warning letter issued for Vivaglobin, CSL Behring, an s.c. immune globulin on March 11, 2011[5]
- Coagulation factors of the contact activation pathway were considered to play a a major role as procoagulant impurities of IgG products.
  - Focus on Factor XIa
- **Although Baxter has seen no similar signal, Baxter proactively initiated a preliminary assessment to evaluate the thromboembolic potential of IGIV products.**

Accessed May 6, 2011

1. http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/Recalls/ucm227133.htm
2. http://www.afssaps.fr/Infos-de-securite/Autres-mesures-de-securite/Octagam-50-mg-ml-solution-pour-perfusion-Octapharma-France-Mise-en-quarantaine-de-tous-les-lots
3. http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/public_health_alerts/2010/09/human_pha_detail_000021.jsp&murl=menus/medicines/medicines.jsp&mid&jsenabled=true
4. http://www.omrix.com/IVIG%20HepB%20Combined%20Voluntary%20Recall%20Dr%20Doctor%20FINAL.pdf
5. http://www.cslbehring-us.com/docs/499/498/Dear%20Dr%20Letter%20Vivaglobin%2011Mar11.pdf

Test Methods established at Baxter

Tests Established at Baxter to Detect Procoagulant Impurities in IgG Products

- NAPTT - Non-activated Partial Thromboplastin Time
  - In normal and FXI deficient plasma
- Prekallikrein activator assay (PKA)
- Protein and protease profiling
  - ELISA method with specific antibodies (incl. FXI ELISA)
  - Proteolytical activity using different chromogenic and fluorogenic substrates
- Thrombin Generation Assay - TGA
  - Variation of the Calibrated Automated Thrombograph (CAT)
- FXIa activity assay
  - Details see next slide

FXIa Assay

	FIX based assay
FXIa	HFXIa 1111a (Enzyme Research Laboratories)
Method	**Indirect** FXIa incubated with FIX, FVIII, FX, PL, Ca →the generated FXa, which depends on the FXIa concentration is measured by a FXa-specific chromogenic substrate
Substrate	**FIX** amount of FXa cleaved by the activated FIX is measured by FXa-1 + αNAPAP-chromogenic substrate
Measurement	Endpoint (405 nm)
Reader	Benchmark, BioRad
Control	* Control 1: for FIXa (and/or FXa)-like activity ** Control 2: for FXa-like activity

* Highest sample concentration tested without addition of FIX to exclude FIXa (and/or FXa)-like activities

** Highest sample concentration tested with buffer only to verify the absence of any FXa like-activity in the samples

Most Relevant *in vitro* Methods for Assessment of the Thromboembolic Potential of IGIV Products

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- Global assays
  - A non-activated partial thromboplastin time (NAPTT) assay with factor XI-deficient plasma in the past had been used routinely in Baxter QC to assess thrombogenic potential of IGIV products*****
  - Thrombin generation assays are established and routinely used in Baxter R&D for research, preclinical/clinical studies and for product support (incl. regulatory submissions; e.g. FEIBA, ADVATE)
- Specific assay
  - *"…we suggest that factor XIa present in some IGIV preparations could contribute to the in vivo risk of thrombosis after IGIV therapy."* (Wolberg et al., Am J Hematol 2000; 65(1):30-34) FXIa can be determined with a highly sensitive activity assay by Baxter

✓ **Distributed IGIV products have been tested with the above three methods and the results have been compared**

***Limit for evaluation depends on the clotting time of the reference plasma**

Comparison of Immunoglobulin Products for FXIa Content, NAPTT and TGA

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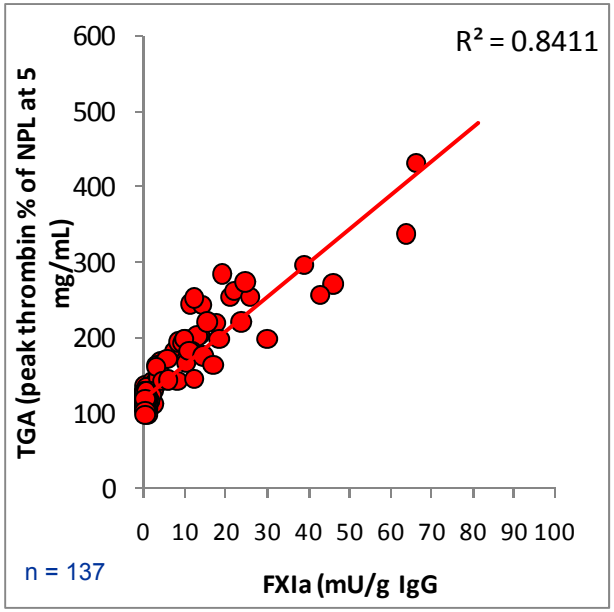
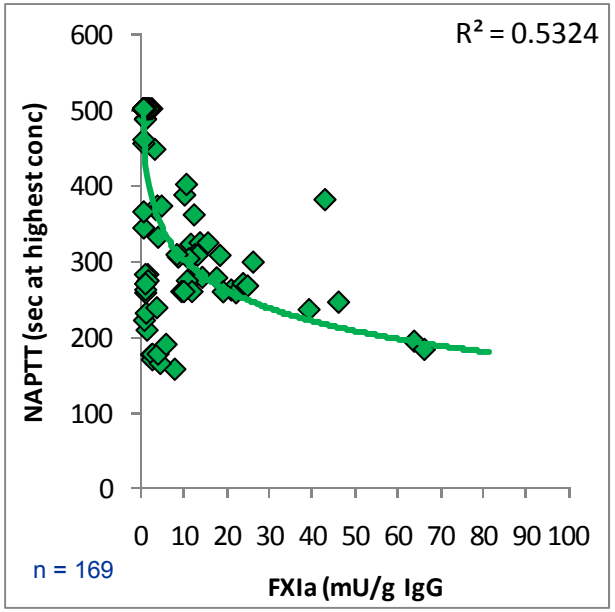
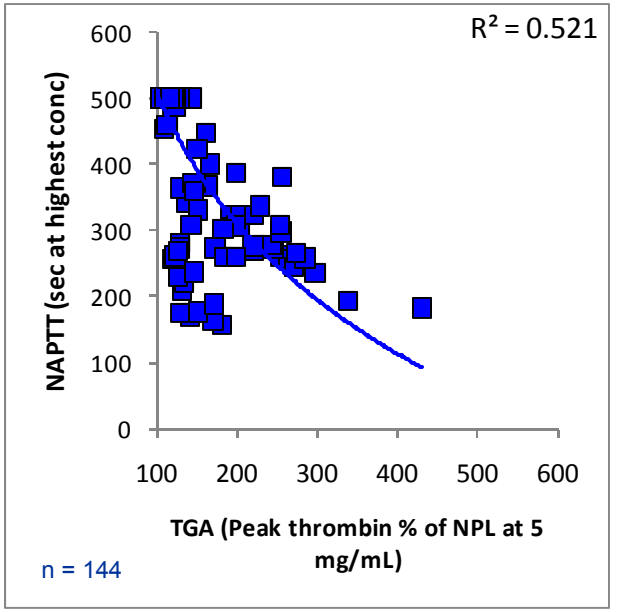
Product	Protein (%)	TGA (% of normal plasma control at 5% protein)	FXIa specific (mU/mL)	NAPTT (mg at 180 sec)
Product A	10	106.79	<0.04	>10
Product B	12	97.23	<0.04	>10
Product C	10	108.64	<0.04	>10
Product D	5	124.72	<0.04	>5
Product E	5	124.82	<0.04	>5
Product F	5	120.67	<0.04	>5
Product G	5	124.65	<0.04	>5
Product H	5	268.95	<0.04	>5
Product I	10	194.37	1.95	>10
Product J	5	451.05	4.06	1.44
Product K	5	115.64	<0.04	>5
Product L	5	110.13	<0.04	>5
Gammagard Liquid Baxter Lot # 1	10	106.28	<0.04	>10
Gammagard Liquid Baxter Lot # 2	10	113.29	<0.04	>10
Gammagard Liquid Baxter Lot # 3	10	112.34	<0.04	>10
Gammagard Liquid Baxter Lot # 4	10	110.35	<0.04	>10

FXIa levels determined with a specific FXIa assay correlate well with results of TGA and NAPTT

Correlation Between FXIa, TGA and NAPTT Levels in Various Immunoglobulin Products







A good correlation has been observed between FXIa and TGA
Both NAPTT and TGA-CAT are sensitive to FXIa levels.

FXIa

In vivo Thrombogenicity

In vivo Thrombogenicity of IGIV Spiked with FXIa Rabbit Wessler Stasis Model

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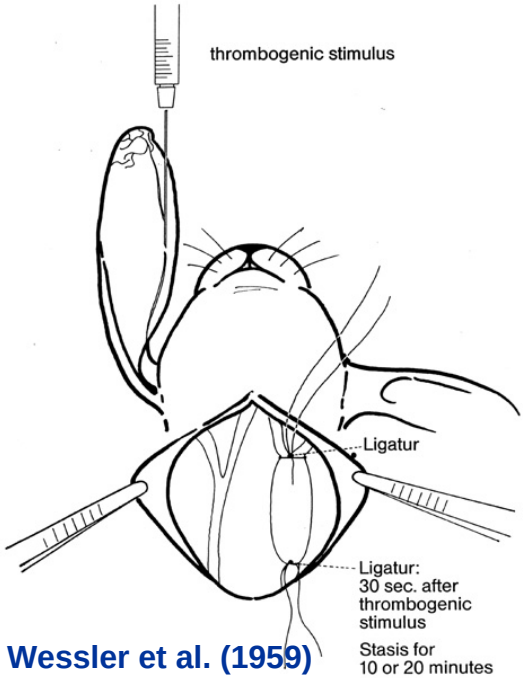
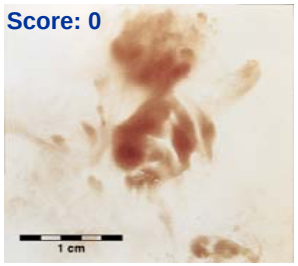
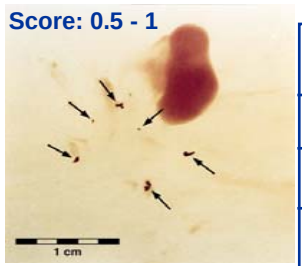
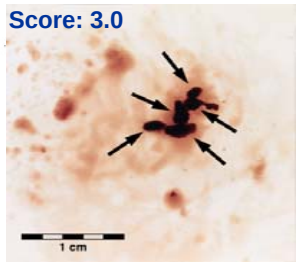
- Study design:
  - Two lots of a Baxter IGIV product were spiked with purified FXIa
  - 5% IgG solution spiked with FXIa to reach a similar concentration as found in samples A and G of EMA-FDA Blood Cluster CS 469 on Thrombogenicity Tests for IGIV, April/Mai 2011: ~ 30 mU FXIa/mL IgG solution ~600 mU/g IgG
  - Treatment dose: Volume: 10 mL/kg; Protein: 500mg/kg

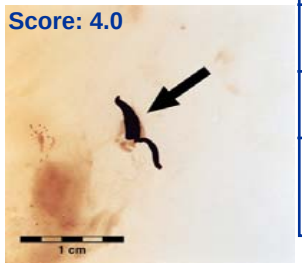


Wessler et al. (1959)
Fareed et al. (1985)
Thomas et al. (1989)









QUANTITATIVE EVALUATION OF THROMBOGENICITY	SCORE
no clot	0
few macroscopic strands of fibrin	0.5 - 1
several small thrombi	2
two or more large thrombi	3
several large thrombi	3.5
single thrombus forming a complete clot in vein segment	4

In vivo Thrombogenicity of IGIV Spiked with FXIa Rabbit Wessler Stasis Model

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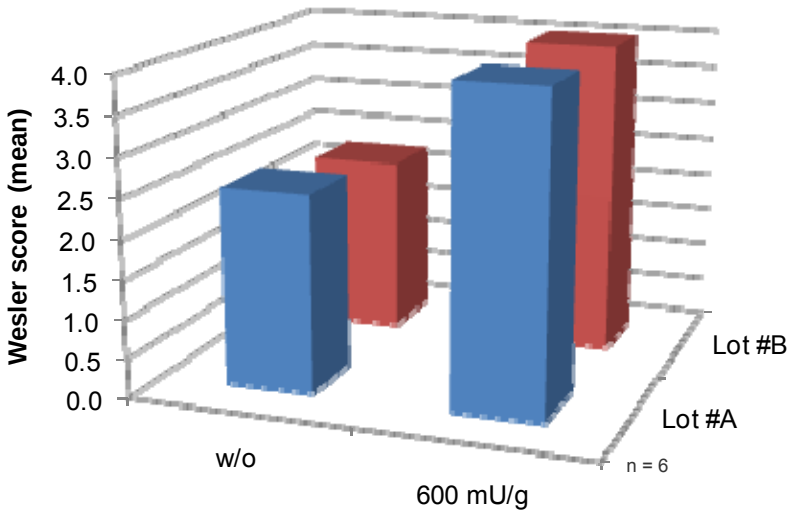
- Results:
  - Mean thrombogenicity score of IGIV w/o FXIa spiking as expected from rabbits treated with high dose of a human IgG_{pr}oduct with low numbers of spontaneous TE reports
    - Results show variability of model
  - An amount of 600 mU FXIa/g protein results in complete clots in the rabbit thrombogenicity model



	Wessler scores IGIV lot #A (after addition of FXIa)		Wesler scores IGIV lot #B (after addition of FXIa)	
Animal no#	w/o	600 mU XIa/g	w/o	600 mU XIa/g
1	4.0	4.0	3.0	4.0
2	2.0	4.0	2.0	4.0
3	2.0	4.0	3.5	4.0
4	3.0	4.0	1.0	4.0
5	2.0	4.0	2.0	4.0
6	2.0	4.0	2.0	4.0
Mean	**2.50**	**4.00**	**2.25**	**4.00**
SD	0.84	0.00	0.88	0.00

Removal of FXI(a) by Gammagard Liquid/Kiovig Cold Alcohol Fractionation

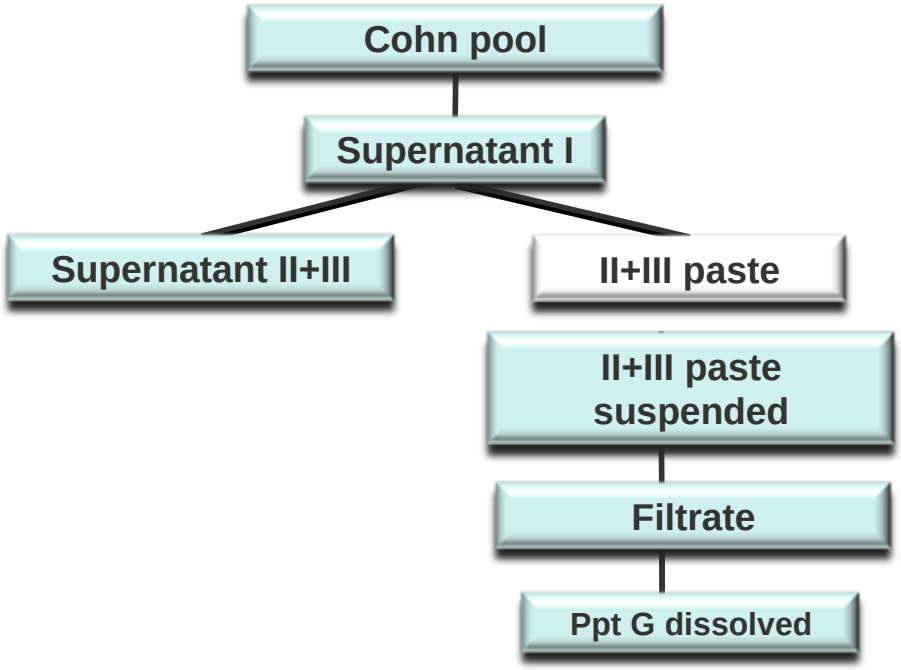
Gammagard Liquid Upstream Investigation

Scope

- **Determine partitioning of FXI zymogen in the Gammagard Liquid (Kiovig) upstream process from Cohn pool to Precipitate G**

Study outline

- **Samples were drawn in alcohol fractionation and analyzed**
  - **0.45µm filtration was applied before analysis if samples were turbid (e.g. for II+III paste suspended)**



Partitioning of FXI zymogen in the Gammagard Liquid (Kiovig) Upstream Process

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Mean values of 3 large-scale manufacturing batches are shown

Sample	FXI zymogen	
	(U/mL)	(% of Cohn pool)
Cohn pool	1.25	100.0
Supernatant I	1.01	87.2
Supernatant II+II	0.21	19.1
II+III paste resuspended*	1.31	71.6
Filtrate	0.04	2.8
Ppt G dissolved	0.31	1.8

*II+III paste re-suspension sample was analyzed after 0.45 µm filtration

- **Precipitation I does not remove substantial amounts of Factor XI zymogen from the IgG**
- **Most of the FXI zymogen precipitates at the fractionation II+III**
- **The main removal step for Factor XI zymogen in the Gammagard Liquid process is the II+III paste resuspension and filtration step**

➢ **The Gammagard Liquid upstream process removes more than 98% of Factor XI zymogen present in the Cohn pool**

  - **Further removal of pro-coagulant impurities occurs during down-stream purification of the Gammagard Liquid process**

Kiovig / Gammagard Liquid Pharmacovigilance Experience 2005 - 2010

Postmarketing Reporting Frequencies of Thromboembolic Events

Methodology and Limitations

Methodology

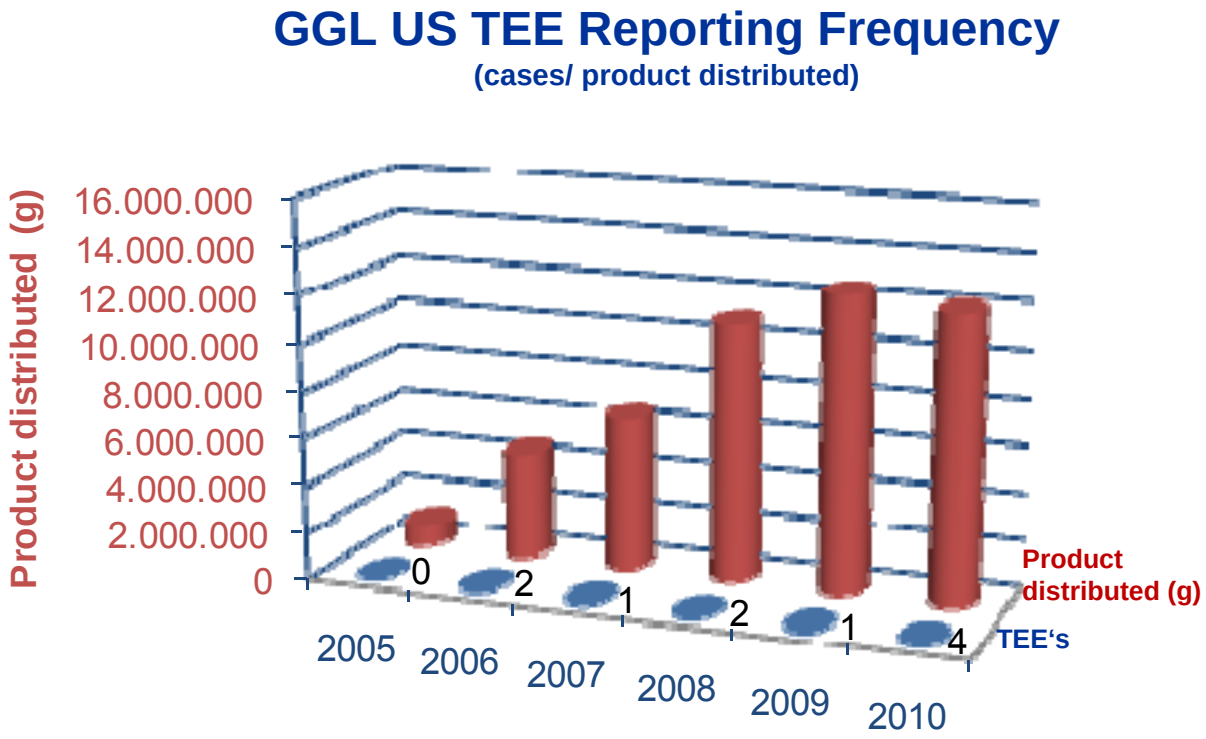
- All spontaneous reports of Thromboembolic events (TEEs) included (i.e., reports received from healthcare professionals and from regulatory agencies)
- Solicited, clinical and literature reports are excluded
- Data presented separately for US and EU

Limitations

- Underreporting in spontaneous reporting schemes occurs to an unknown extent
- Reporting frequencies do not represent incidence rates
- Due to missing denominator data no incidence rates can be calculated from spontaneous pharmacovigilance data
- Regionally different reporting cultures result in different reporting frequencies

US Experience

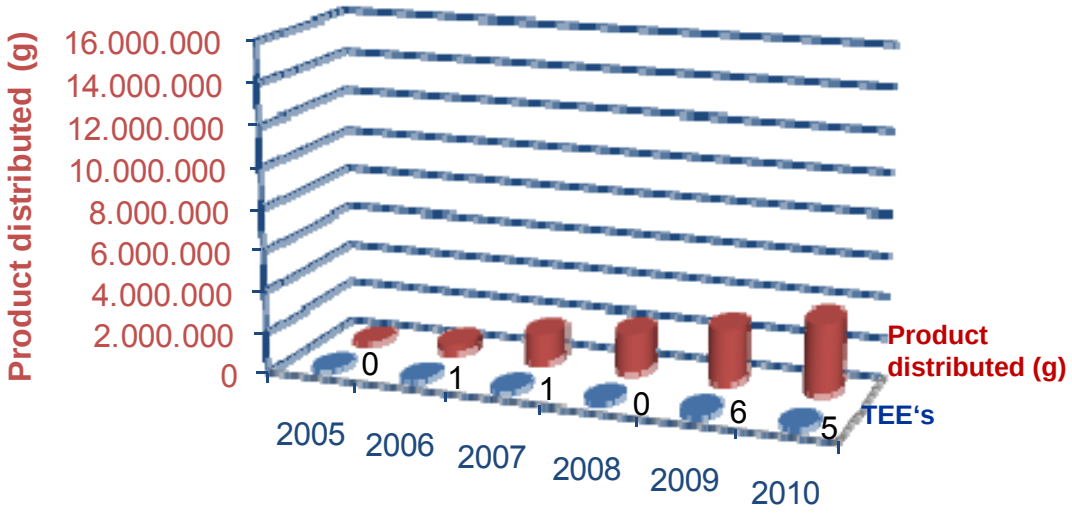
- Licensure of Gammagard Liquid: 27 April 2005



EU Experience

- Kiovig was registered on 19 January 2006 through Centralized Procedure

Kiovig EU TEE Reporting Frequency
(cases/ product distributed)



PV Field Safety Analysis and Conclusions

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Summary:

- **The absolute numbers of spontaneous TE reports are low for GGL during the reported data analyzed from 2005-2010**
- **The reporting frequencies of TEs for GGL have remained low and consistent during the reported data analyzed from 2005-2010**
- **Differences in reporting frequency of TEEs observed for US and Europe**
- **Non-product risk factors (i.e., patient risk profiles), as listed in the product insert, are also likely to play a major independent risk factor for the development of TE‘s**

Thromboembolic events are known and appropriately labeled AEs. Risk-benefit in labeled indications is positive and remains unchanged based on Baxter‘s field experience

Summary & Conclusions

- **Baxter has proactively evaluated IGIV products by *in vitro* methods indicative of thromboembolic potential**
- **FXIa was measured in different IgG products and a possible procoagulant effect of the product was assessed by two global tests (TGA in normal plasma pool and NAPTT in FXI deficient plasma)**
- **The three assays showed very good correlation, both global tests were dependent on the FXIa levels of the product**
  - **The specific FXIa test based on FIX as its natural substrate correlates well with the TGA**
  - **Historically, NAPTT* has been used by Baxter for IGIV product characterization (i.e.: support of conformance lots, process changes, exceptions)**
  - **Baxter's preferred method is the NAPTT assay because of its simplicity**
  - **Spiking of an IVIG product with FXIa increases thrombogenicity in the Wessler stasis model**
  - **A commercial Cohn fractionation process (Gammagard Liquid / Kiovig) removes more than 98% of Factor XI zymogen present in the Cohn pool**
    - **Further removal occurs during down-stream purification**
  - **The number of spontaneous TE reports are low for Gammagard Liquid / Kiovig a widely used IGIV product**
  - **Thromboembolic events are known and appropriately labeled AEs. Risk-benefit in labeled indications is positive and remains unchanged based on Baxter's field experience**

* FXI deficient plasma