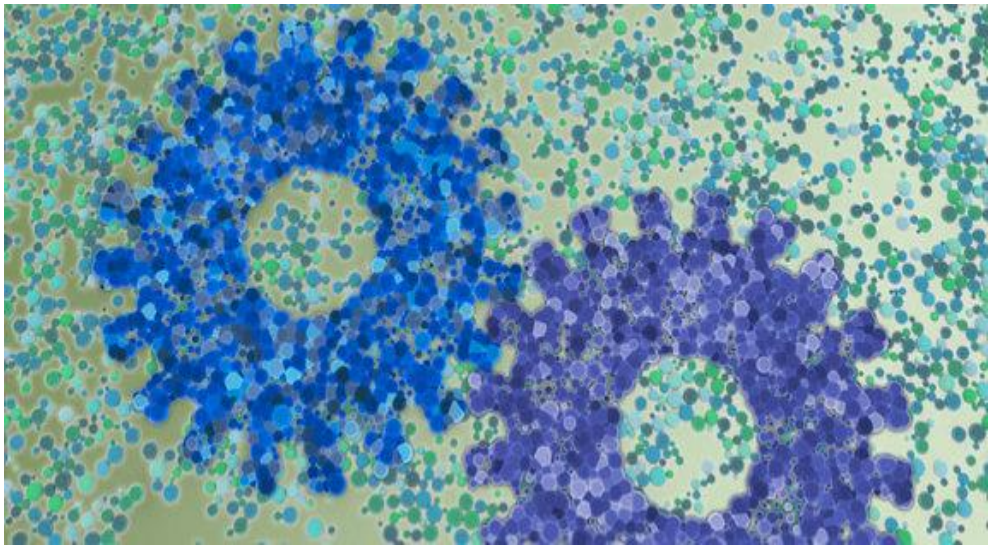


I nuovi concentrati di FVIII e di FIX: evidenze

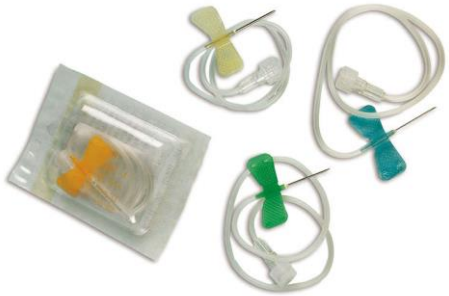
Elena Santagostino

A. Bianchi Bonomi Hemophilia & Thrombosis Center
Ca' Granda Foundation, Maggiore Hospital Policlinico, Milano, Italy

Napoli, 23 marzo 2016

Issues with current treatment

- Prophylaxis should be started at very young ages
- Repeated intravenous injections can be problematic even in some adults
- Compliance and adherence to treatment (adolescents)
- No universal regimen = treatment individualization
- Inhibitor development



Long-acting products

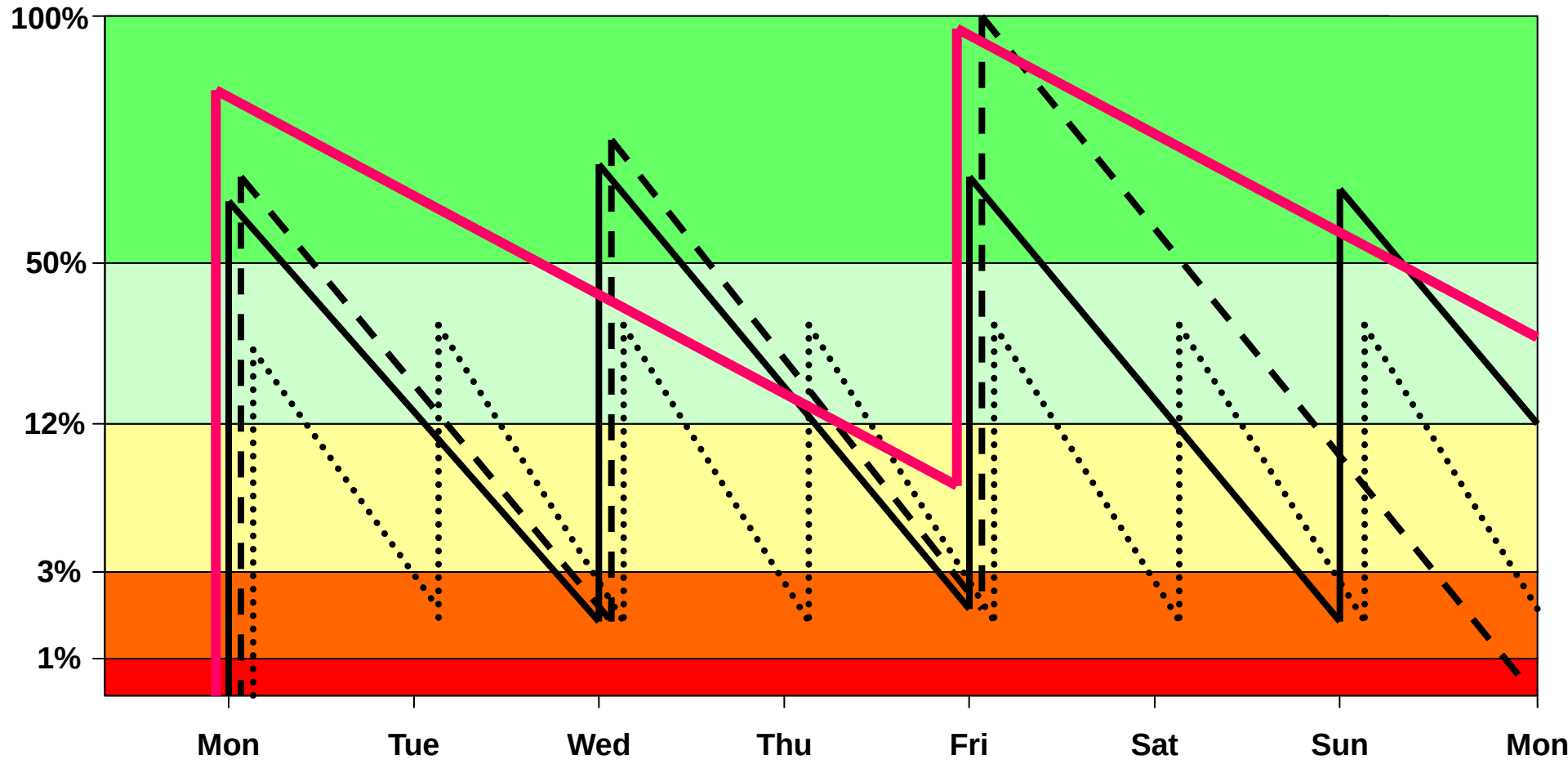
Expected changes in prophylaxis patterns

	Current products (# yearly i.v. injections)	Long-acting products (# yearly i.v. injections)
Hemophilia A	150-180 →	80-100
Hemophilia B	**100-120** →	**30-40**

How will these longer acting concentrates impact on prophylaxis?

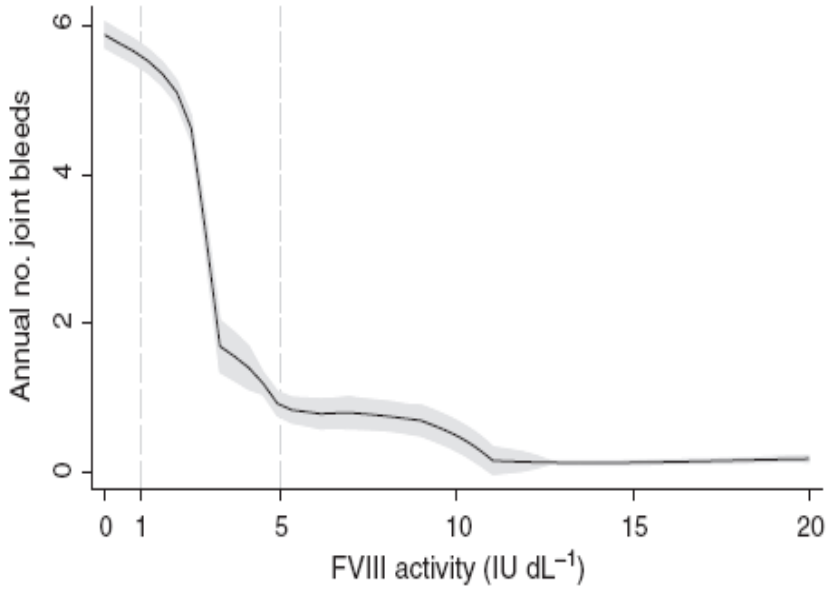
Fewer infusions

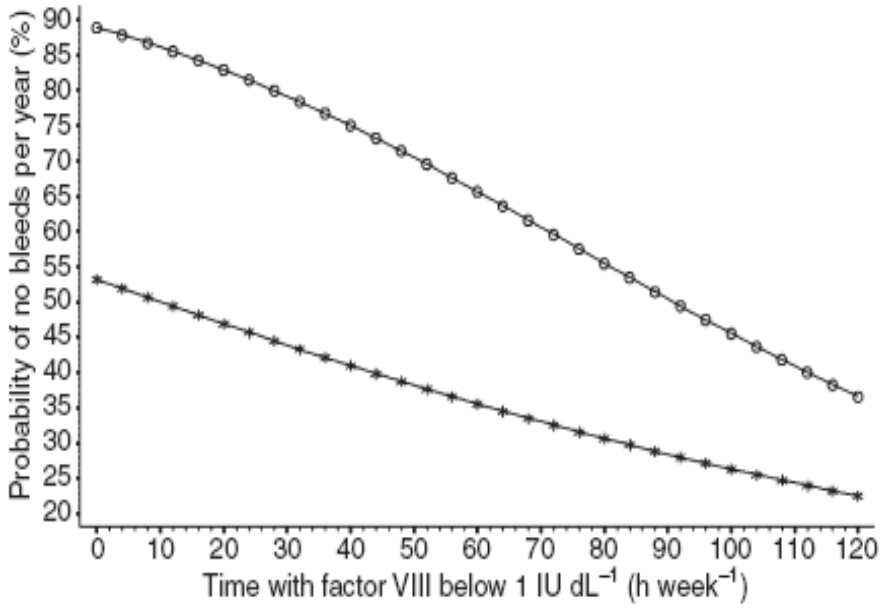
Higher troughs



The importance of higher troughs

- In the past trough levels between 1 and 3% were considered “enough”[1]
- To protect from joint bleeds higher troughs are needed[2]
- The time spent below 1% resulted proportional to the incidence of break-through bleeds[3]





[1] *Ahlberg A. Acta Orthop Scand 1965; 77 (Suppl): 3-132*

[2] *Den Uijl IE et al. Haemophilia 2011; 17: 849-53*

[3] *Collins P et al. JTH 2009; 7: 413-20*

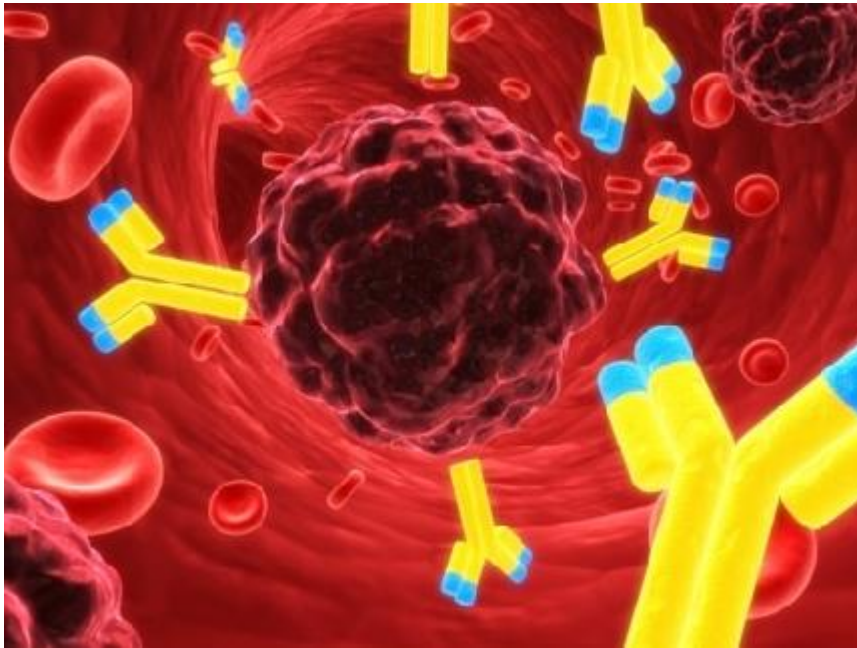
Immunogenicity

Will they result in:

- ✓ ***More (>25-30%) – won't be accepted***
- ✓ ***SAME – will be tolerated***
- ✓ ***Less (<25%) - hopefully***

So far so good in PTPs

Awaiting for PUPs studies



Long-acting FVIII and FIX products

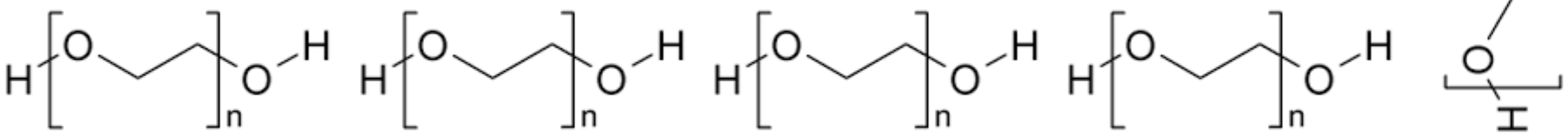
Molecule name	Structure	Availability	Brand/Company
rFIXFc	**rFIX Fc fusion**	**- Marketed in USA/Canada** **- PUPs trial ongoing**	**Alprolix®/Biogen Idec**
Nonacog beta pegol (N9-GP)	GlycoPEGylated FIX	- Extension study	NA/NovoNordisk
CSL-654 (rIX-FP)	rFIX albumin fusion	- Extension study - PUPs trial ongoing	NA/CSL Behring
rFVIIIFc	**rBDD-FVIII Fc fusion**	- **Marketed in USA/Canada** - **PUPs trial ongoing**	**Eloctate®/Biogen Idec**
BAY 94-9027	PEGylated BDD-FVIII (60 KDa)	- Extension study	NA/Bayer Healthcare
N8-GP	GlycoPEGylated BDT-FVIII (40 KDa)	- Extension study - PUPs trial ongoing	NA/NovoNordisk
BAX-855	PEGylated FVIII (20 KDa)	- Extension study	NA/Baxter

BDD: B-Domain deleted; BDT: B-Domain truncated; NA: not applicable

Different technologies, different outcomes?

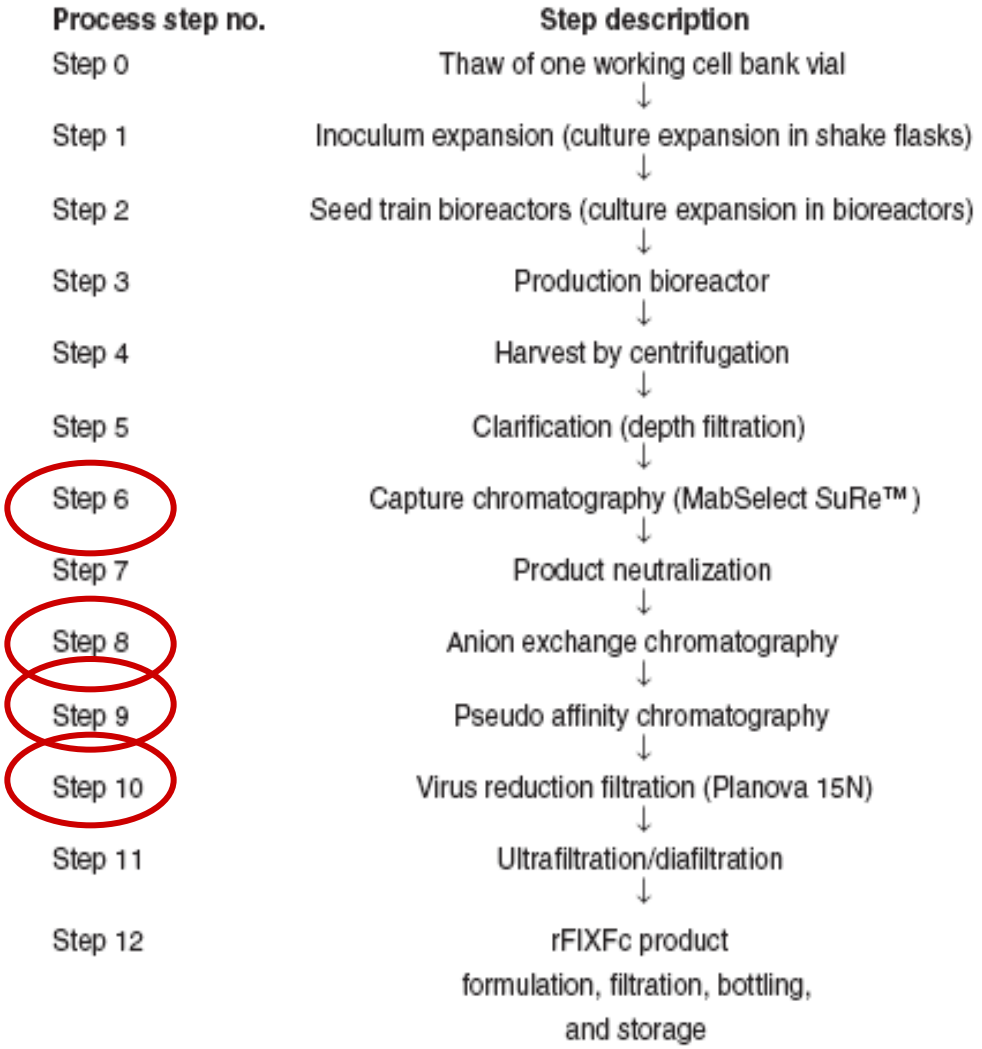
- ✓ Fusion technologies with physiological proteins as Fc fragment or albumin seems safer
- ✓ Fusion of FVIII to albumin failed to preserve effective coagulation activity
- ✓ Concerns about long-term exposure to PEG moiety

  - antibody production
  - accumulation???
  - long-term toxicity?
  - 20, 40, 60 kDa



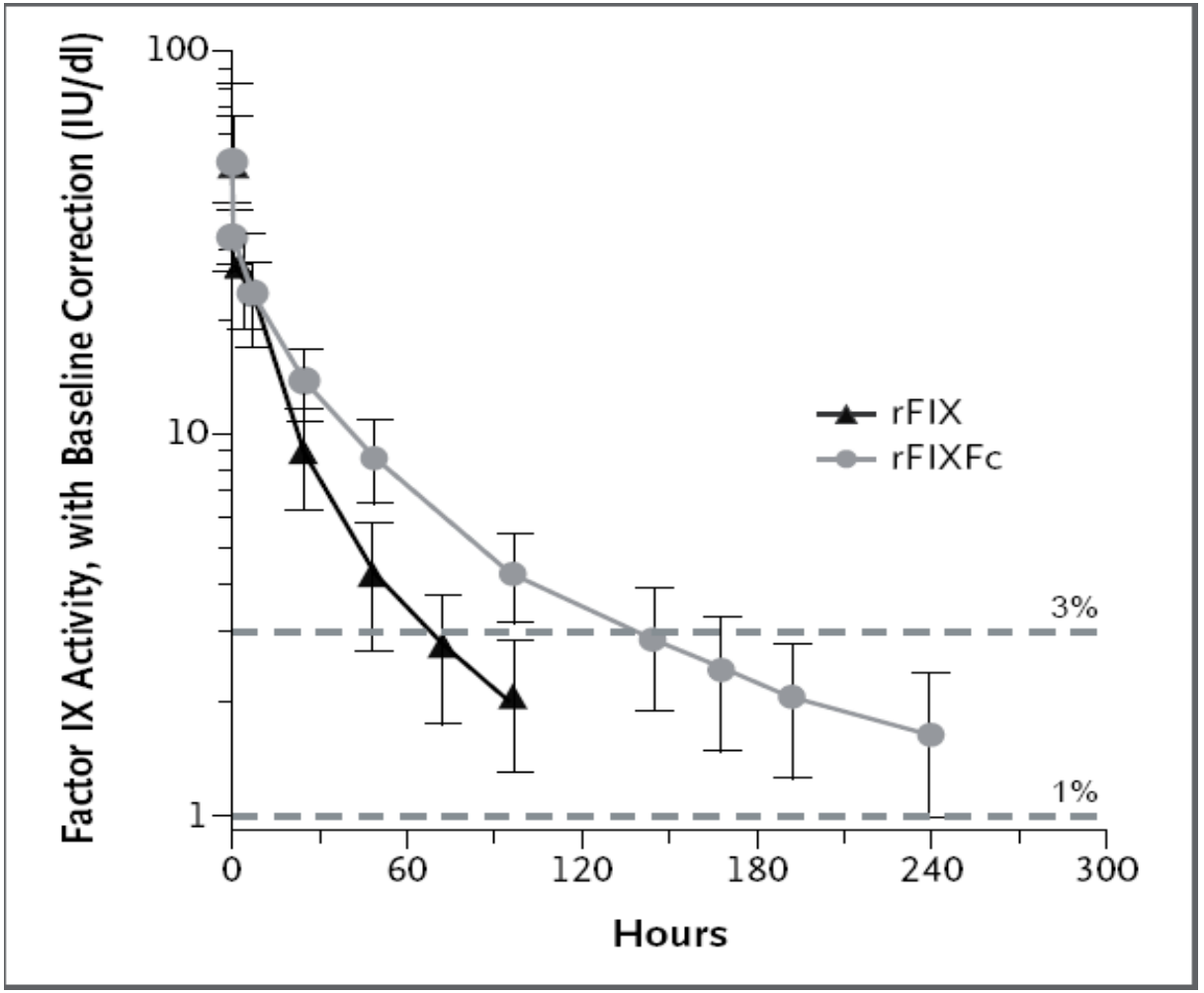
Manufacturing process of rFIXFc

- Human embryonic kidney (HEK) 293H cells
- A single molecule of rFIX covalently fused to the Fc domain of human IgG1
- Transfected HEK 293H cells are grown in serum-free medium
- Specific analytical tests were used to assess identity, purity, activity and safety



McCue et al Haemophilia 2014; 20: e327-35

B-LONG: Phase 3 Study of rFIXFc in PTPs



- **The median weekly dose was 45 IU/kg in group 1**
- **The median dosing interval was 12.5 days in group 2**

Powell et al NEJM 2013; 369: 2313-23

B-LONG: Phase 3 Study of rFIXFc in PTPs

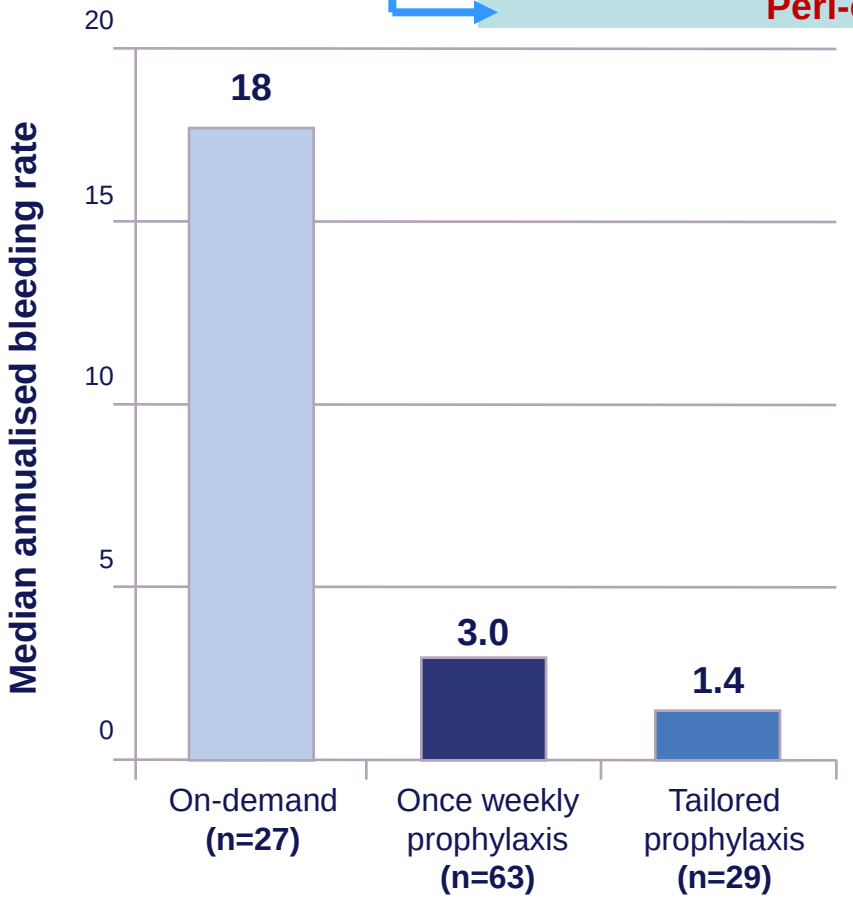
SCREENING

Current regimen

On demand → **On demand (20–100 IU/kg as required)**

Prophylaxis or on demand

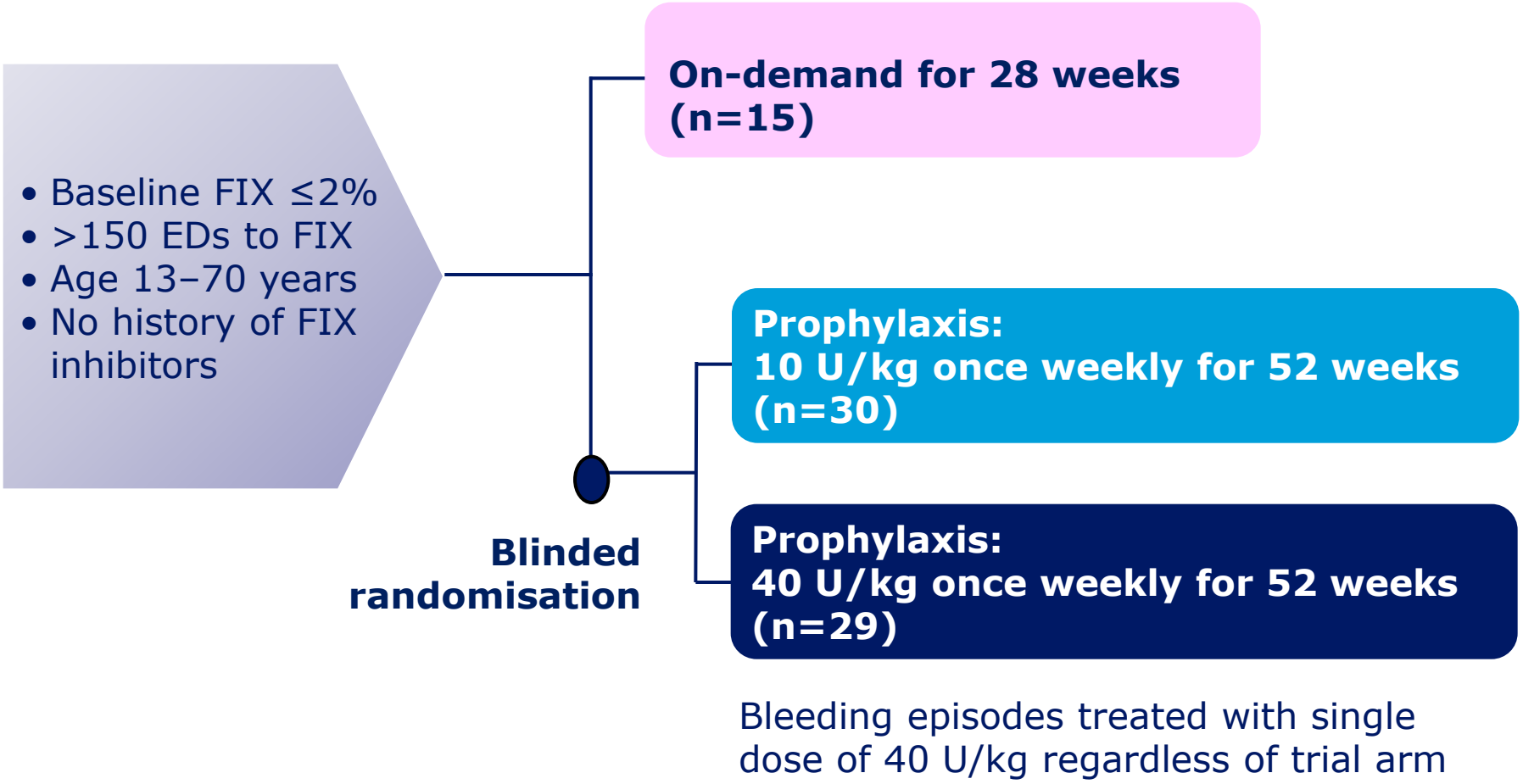
- → **1 - Weekly prophylaxis (50 IU/kg to start, PK driven dosing)**
- → **2 - Tailored prophylaxis (100 IU/kg q10d to start, PK driven interval)**
- → **Peri-operative treatment (Surgical study)**



- ***123 patients (≥ 12 yrs)***
- **$t^{1/2}$: ~ 82 hours; IVR: 0.92**
- **Target trough: 1-3% or higher**
- **In the tailored prophylaxis arm 54% of patients were treated every 14 days**
- **No inhibitors were detected (55 pts with ≥ 50 EDs)**
- **No cases of anaphylactic reactions or thromboembolic events were reported**

Powell et al NEJM 2013; 369: 2313-23

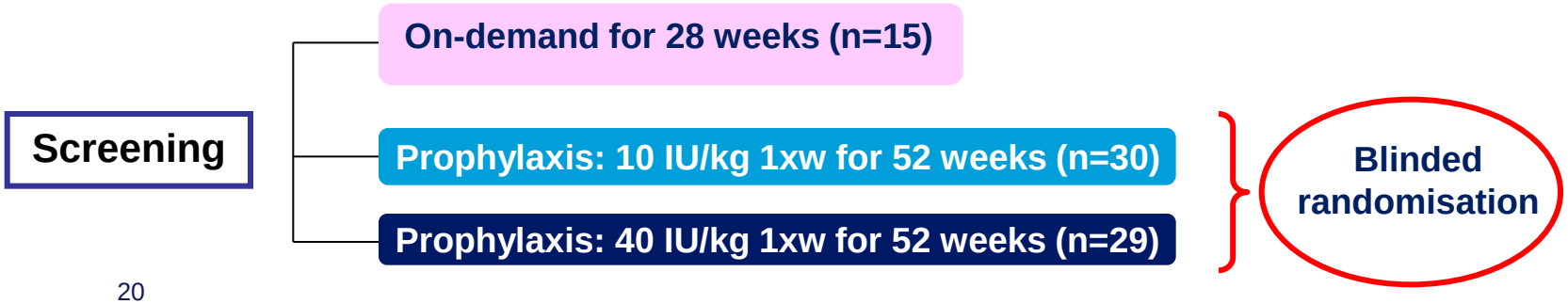
Paradigm 2: Phase 3 study of N9-GP

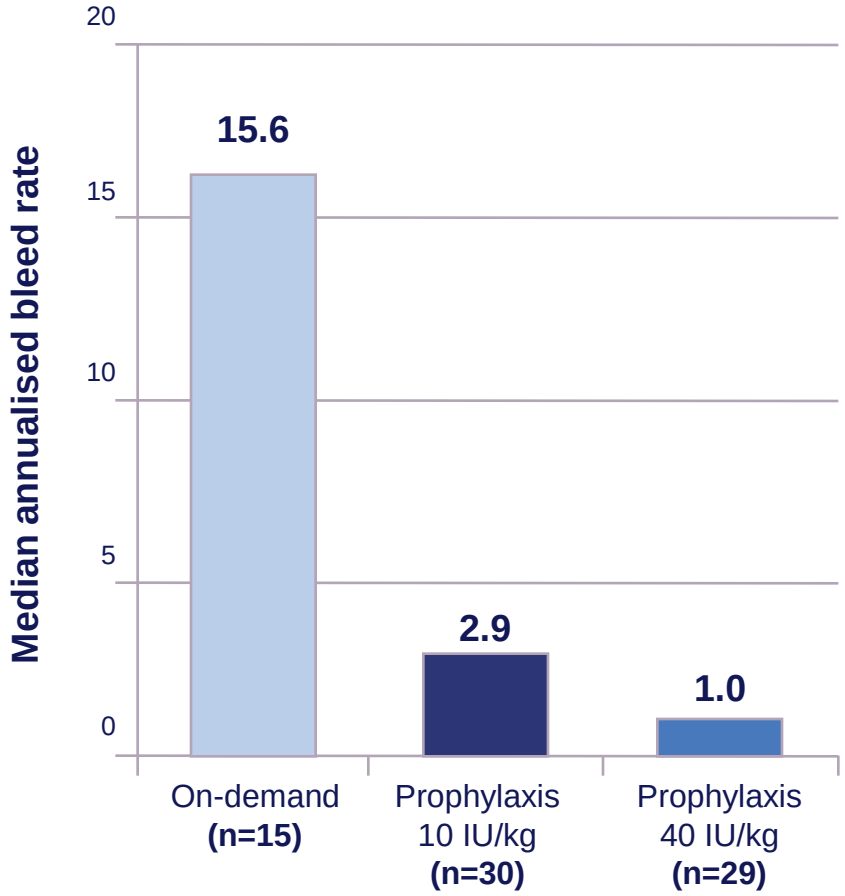


Bleeding episodes treated with single dose of 40 U/kg regardless of trial arm

Collins P et al Blood 2014; 124: 3880-6

Paradigm 2: Phase 3 study of N9-GP

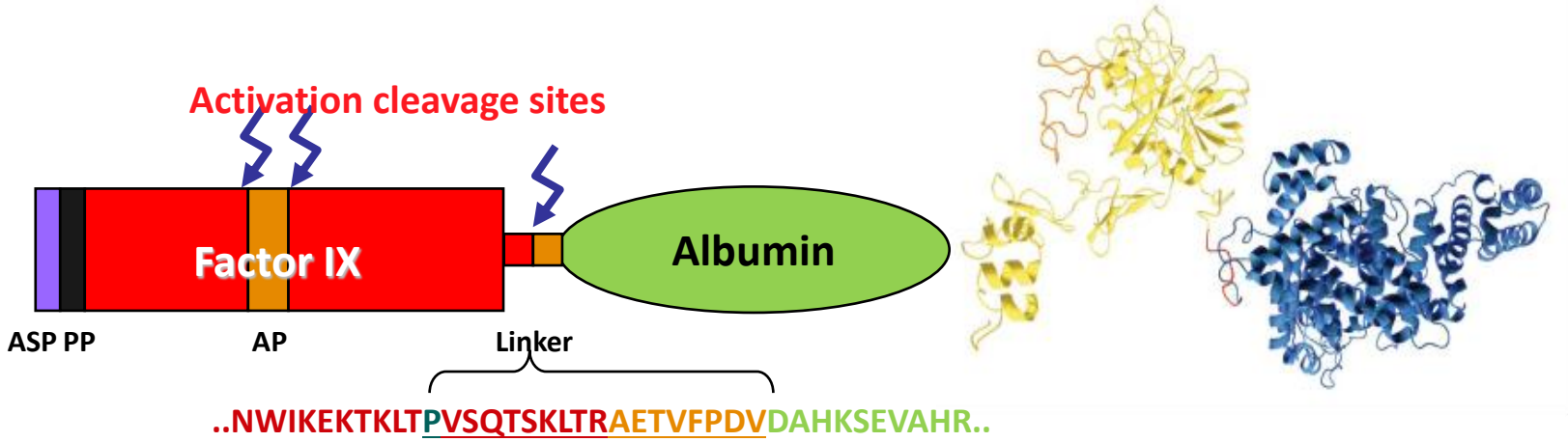




- $t^{1/2}$: 93 hours
- Overall success rate for treatment of bleeds: 92.4% (excellent or good)
- Bleeds were treated with a single dose of 40 IU/kg
- 91.7% of bleeds successfully treated with a single dose
- No inhibitory antibodies to N9-GP
- No safety issues

Collins P et al Blood 2014; 124: 3880-6

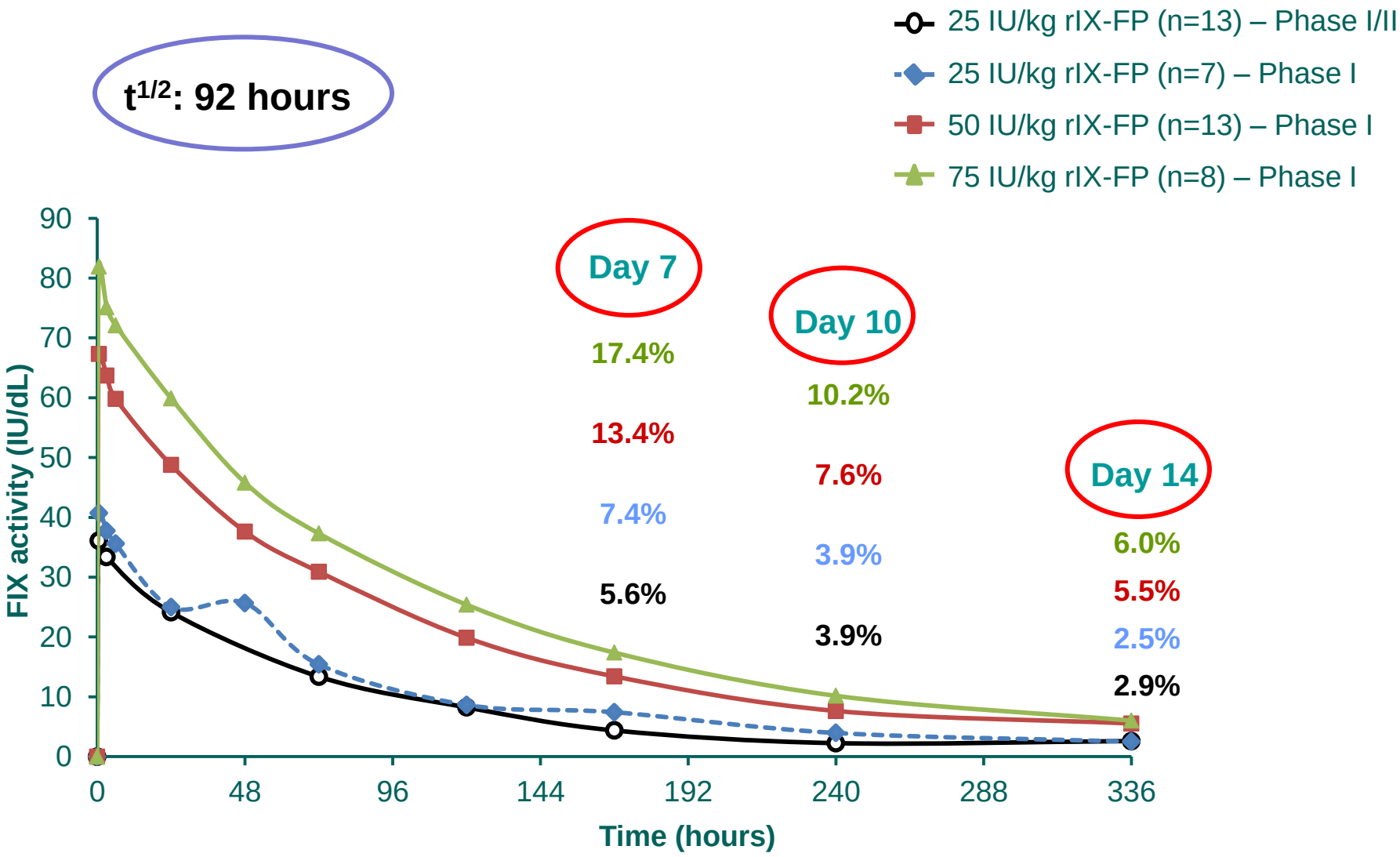
rFIX Albumin Fusion Protein



- rIX-FP is a recombinant protein purified from CHO cells
- rIX-FP is generated by the genetic fusion of human recombinant albumin to the c-terminus of rFIX
- Cleavable linker between rFIX and albumin derived from rFIX activation region

rIX-FP yielding a longer duration of action could address the existing unmet medical needs by requiring less frequent dosing

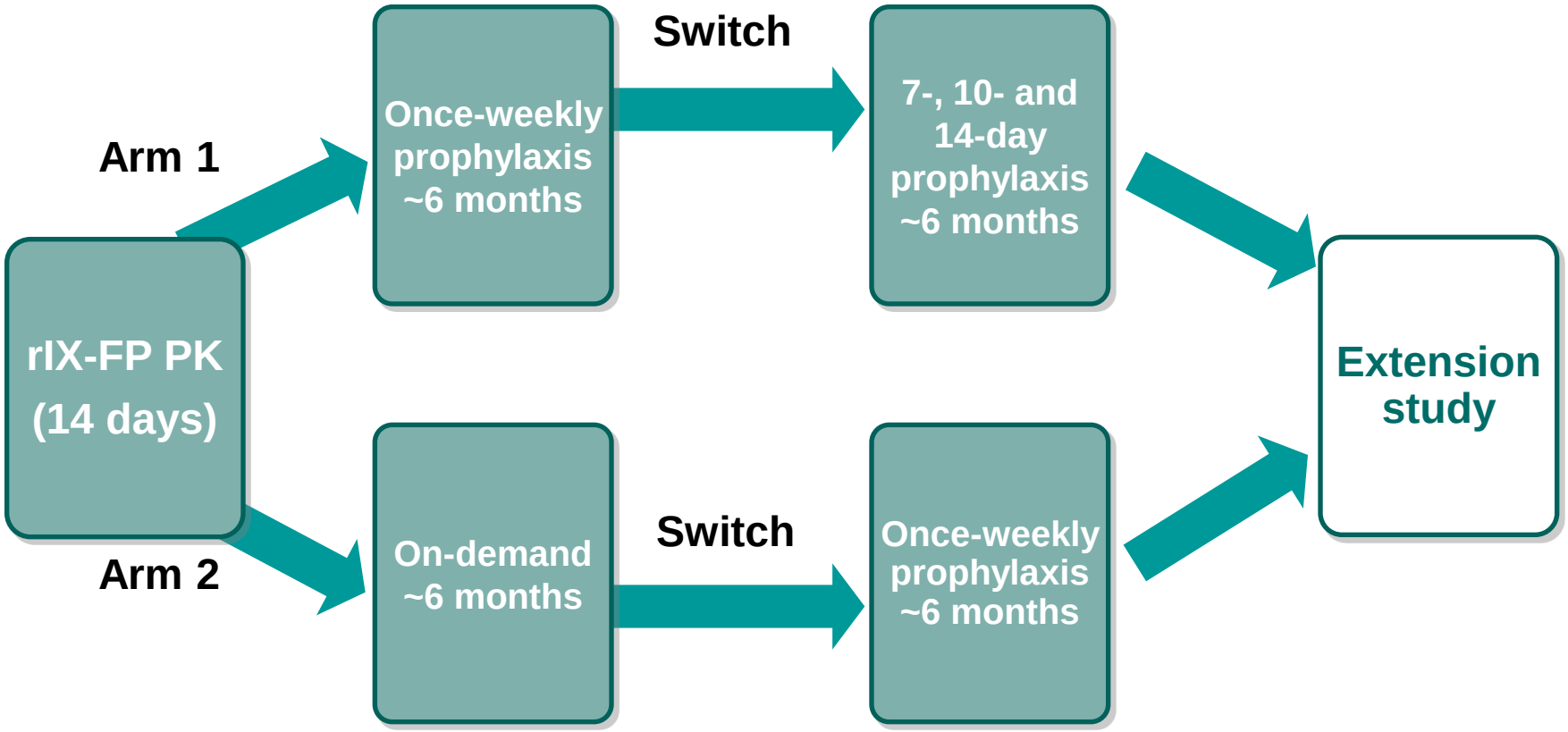
Prolong9-FP: Phase I and I/II PK study



Santagostino E et al Blood 2012;1 20: 2405-11

Martinowitz U et al Thromb Res 2013; 131 (Suppl 2): S11-14

Prolong9-FP: Phase 3 study of rIX-FP

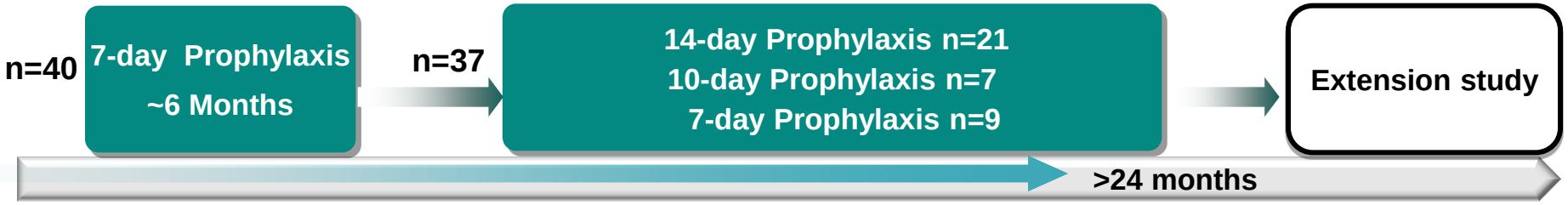


www.clinicaltrials.gov.

On-Demand vs. Prophylaxis with rIX-FP

	Within-subject comparison (n=19) rIX-FP		AsBR reduction
	On-demand period ~6 months	Prophylaxis period ~12 months	
AsBR, median (IQR)	**15.43** (7.98–17.96)	**0.0** (0.00–0.96)	**100% (p<0.0001)**
Target joint(s), n (%)	**10** (53)	**0**	
Estimated AsBR (95% CI)†	**13.62** (11.00–16.87)	**0.55** (0.23–1.32)	
Estimated total ABR (95% CI)†	**18.22** (15.38-21.58)	**1.81** (0.97–3.37)	
Median dose (IU/kg)		**40 IU/kg**	

7-, 10- and 14-Day Prophylaxis Regimens



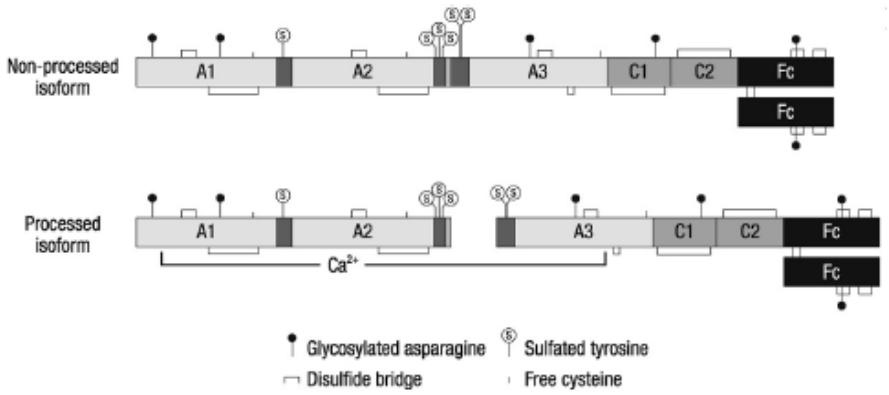
	7-Day Regimen (n=40)	10-Day Regimen (n=7)	14-Day Regimen (n=21)
AsBR			
Median (IQR)	**0** (0, 0)	**0** (0, 0)	**0** (0, 1.0)
Estimated mean AsBR (95% CI)†	**0.65** (0.37–1.13)	**0.56** (0.27–1.17)	**0.83** (0.38–1.77)
Total ABR			
Median (IQR)	**0** (0, 1.87)	**0** (0, 1.78)	**1.08** (0, 2.7)
Estimated mean ABR (95% CI)†	**1.58** (1.02–2.44)	**1.69** (0.87–3.28)	**1.61** (0.93–2.80)

Manufacturing process of rFVIIIFc

- Human embryonic kidney (HEK) 293H cells
- A single molecule of rFVIII covalently fused to the Fc domain of human IgG1
- Transfected HEK 293H cells are grown in serum-free medium
- Specific analytical tests were used to assess identity, purity, activity and safety

Levels of A) galactose-α-1,3-galactose (α-Gal) and B) N-glycolylneuraminic acid (NGNA) in rFVIIIFc and three commercially available rFVIII products.

Sample	α-Gal		NGNA	
	Average % mol/mol (n = 3)	Standard deviation (n = 3; %)	Average % mol/mol (inter-day; n = 9)[a]	RSD[b] (inter-day; n = 9; %)[a]
rFVIIIFc	<LOD[c]	NA	<LOD[d]	NA
Xyntha	10.2	1.6	20.31 (0.73)	3.6
Advate	3.3	0.6	1.33 (0.14)	10.8
Kogenate	1.3[e]	0.8	5.99 (0.32)	5.3
Positive control	41.7	0.4	–	–



Process Step No.	Step Description
Step 0	Thaw of one working cell bank vial
Step 1	Inoculum expansion (culture expansion in shake flasks)
Step 2	Seed train bioreactors (culture expansion in bioreactors)
Step 3	Production bioreactor
Step 4	Harvest by centrifugation
Step 5	Clarification (depth filtration)
Step 6	Virus inactivation (Triton X-100 addition)
Step 7[a]	Affinity chromatography (VIIISelect column)
Step 8[a]	Anion exchange chromatography (TMAE HiCap column)
Step 9	Anion exchange membrane (Mustang Q membrane)
Step 10[a]	Virus reduction filtration (Planova 15N)
Step 11	Hydrophobic interaction chromatography (Octyl Sepharose fast flow)
Step 12	Ultrafiltration/diafiltration
Step 13	rFVIIIFc product formulation, filtration, bottling, and storage

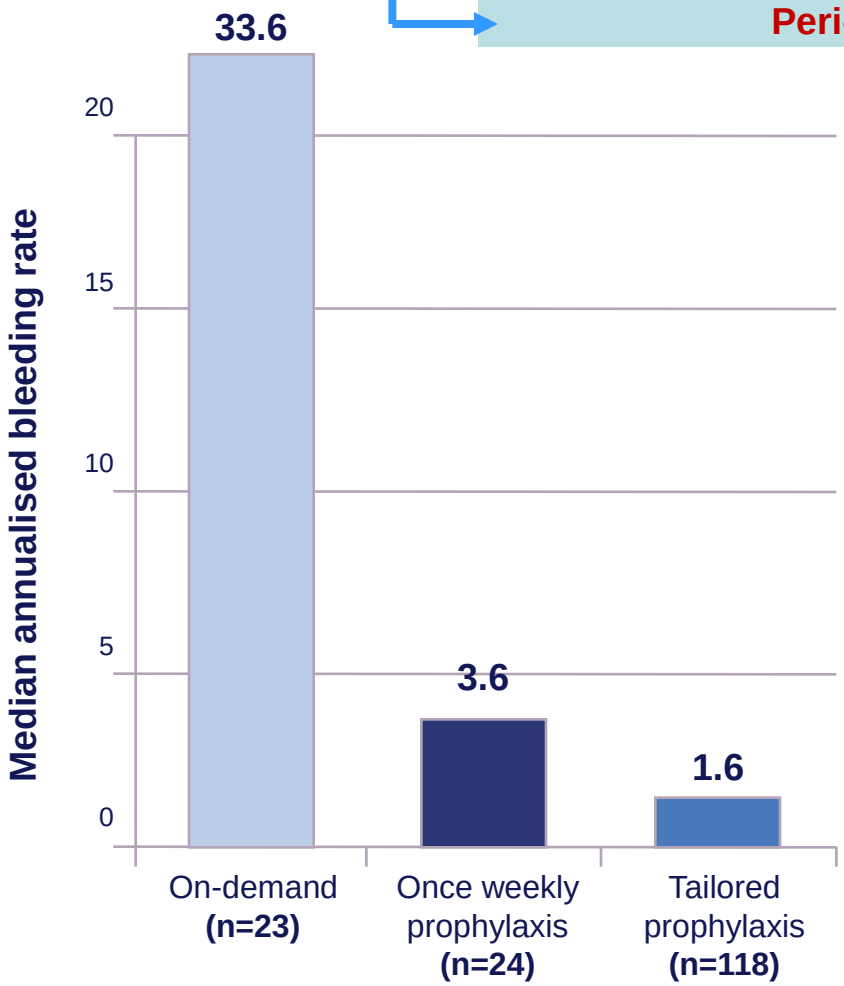
McCue et al Biologicals 2015; 43: 213-9

A-LONG: Phase 3 Study of rFVIIIFc in PTPs

Current regimen

SCREENING

- **On demand** → On demand (10–50 IU/kg as required)
- **Prophylaxis or on demand** → Weekly prophylaxis (65 IU/kg)
- **Prophylaxis or on demand** → Tailored prophylaxis (25-65 IU/kg every 3-5 days)
- **Prophylaxis or on demand** → Peri-operative treatment (Surgical study)



- ***165 patients (≥ 12 yrs)***
- **$t^{1/2}$: 19.0 hours; IVR: 2.2**
- **Target trough: 1-3% or higher**
- **In the tailored prophylaxis arm the median weekly dose was 78 IU/kg**
- **30% of subjects achieved a 5-day dosing interval**
- **No inhibitors were detected (110 pts with ≥ 50 EDs)**
- **9 major procedures in 9 pts**

Mahlangu J et al Blood 2014; 123: 317-25

ASPIRE: Extension Study with rFVIIIFc in PTPs

A-LONG
165 enrolled
153 completed

150 ≥ 12 yrs in the extension study

61 < 12 yrs in the extension study

Kids A-LONG
71 enrolled
67 completed

42 changed prophylaxis regimen

211 enrolled in the ASPIRE

All but 2 maintained twice weekly prophylaxis

A-LONG infusion frequency (end of study)	Every 3 days *N* = 28 (18.7%)	Twice weekly *N* = 43 (28.7%)	Every 4 days *N* = 9 (6.0%)	Every 5 days *N* = 26 (17.3%)	Every 6 days *N* = 2 (1.3%)	Once Weekly *N* = 33 (22%)
Every 3 days *N* = 35 (23.3%)	26	6	3	0	0	0
Twice weekly *N* = 33 (22.0%)	0	26	2	3	0	2
Every 4 days *N* = 4 (2.7%)	1	1	2	0	0	0
Every 5 days *N* = 37 (24.7%)	0	1	2	22	2	10
	--	--	–	--	–	--
Once weekly* *N* = 19 (12.7%)	0	3	0	0	0	16

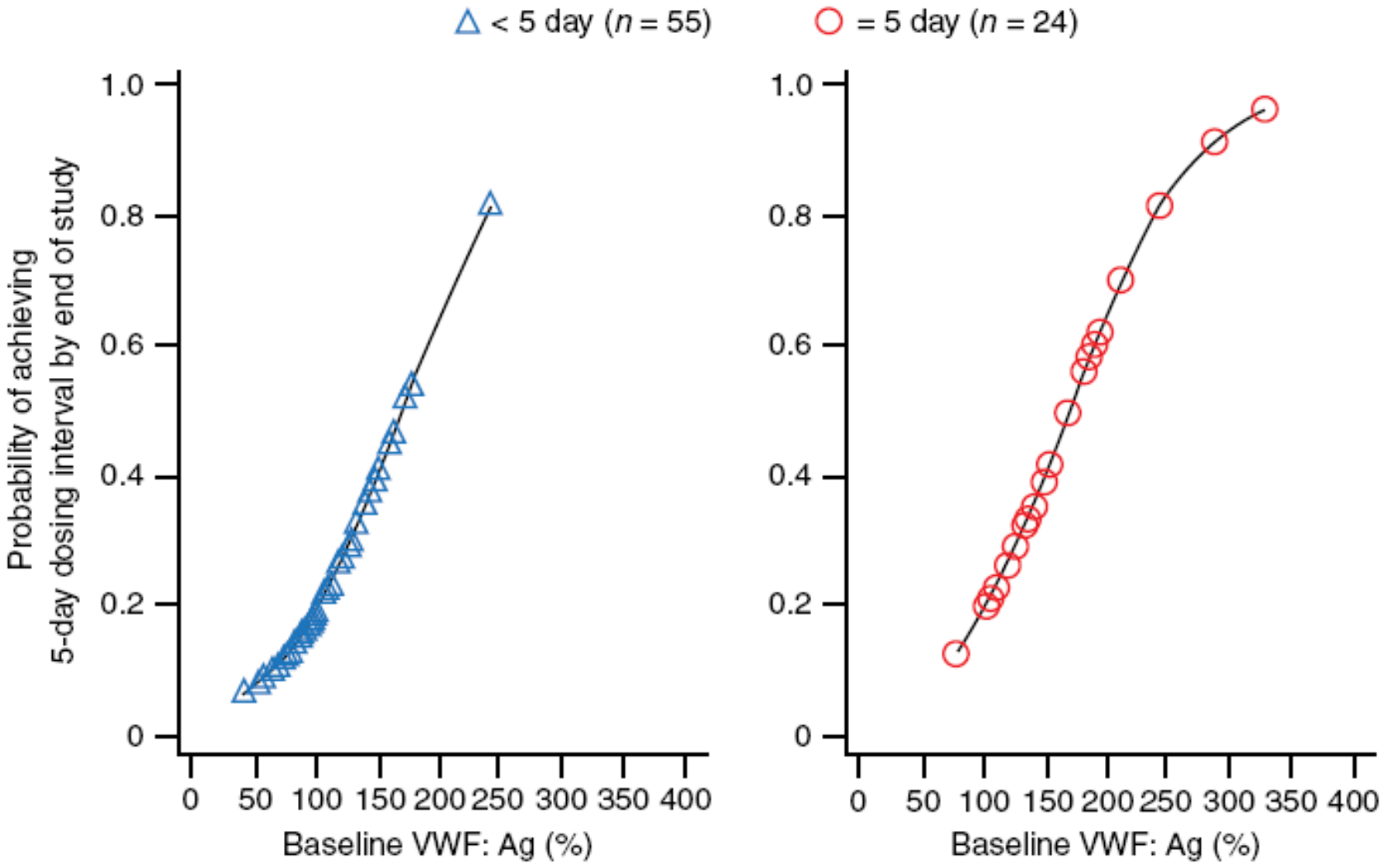
Change in infusion interval
- Lengthened (*n* = 28, 21.9%)
- No change (*n* = 92, 71.9%)
- Shortened (*n* = 8, 6.3%)

	Every 3 days	Twice weekly	Every 4 days	Every 5 days	Every 6 days	Once Weekly	Other *N* = 2 (1.3%)	Episodic *N* = 7 (4.7%)
Episodic treatment *N* = 22 (14.7%)	1	6	0	1	0	5	2†	7

- ***No inhibitor development***

Nolan B et al Haemophilia 2015; epub ahead of print

Post-hoc analysis on bleeding rates



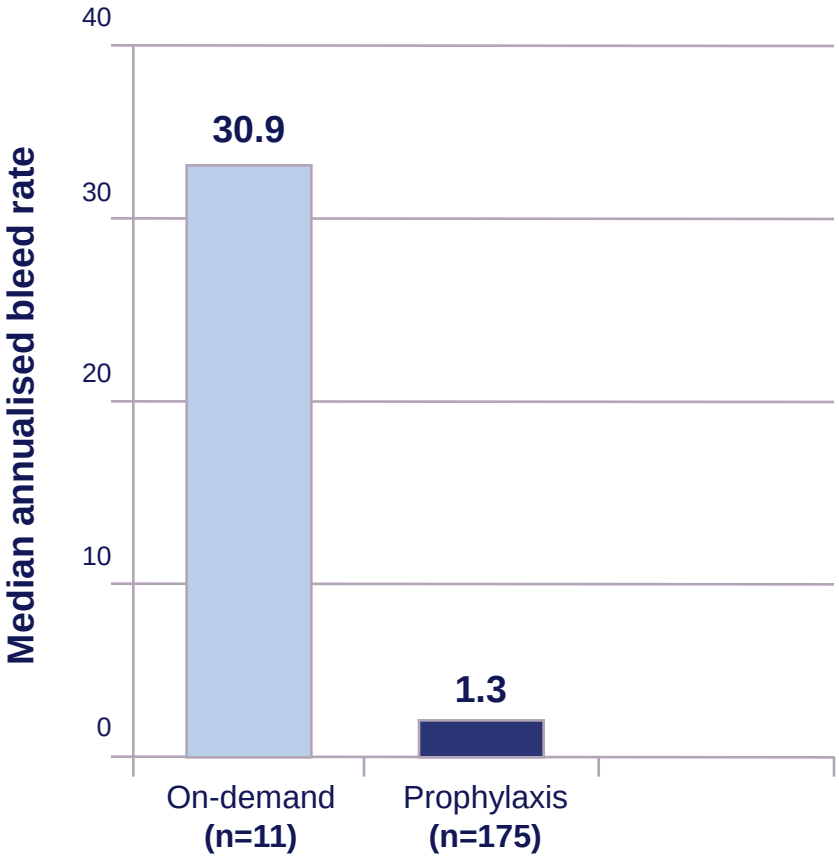
Shapiro AD et al JTH 2014; 12: 1788-1800

Pathfinder 2: Phase 3 Study of N8-GP

SCREENING

On demand (n=11)

Prophylaxis (50 IU/kg every 4 days) (n=175)



- **$t^{1/2}$: 18.4 hours**
- **One patient developed anti-FVIII inhibitors**
- **Inhibitor development in line with expectations in PTPs (1 inhibitor)**
- **Mean trough level: 8%**

ClinicalTrials.gov identifier: NCT01480180
Company announcement 19 march 2014

PROTECT-VIII: Phase 2/3 Study of BAY 94-9027 in PTPs

Screening

On-demand therapy individual dosage (n=20)

2x/wk 25 IU/kg (n≈120)

2 or more breakthrough bleeds:

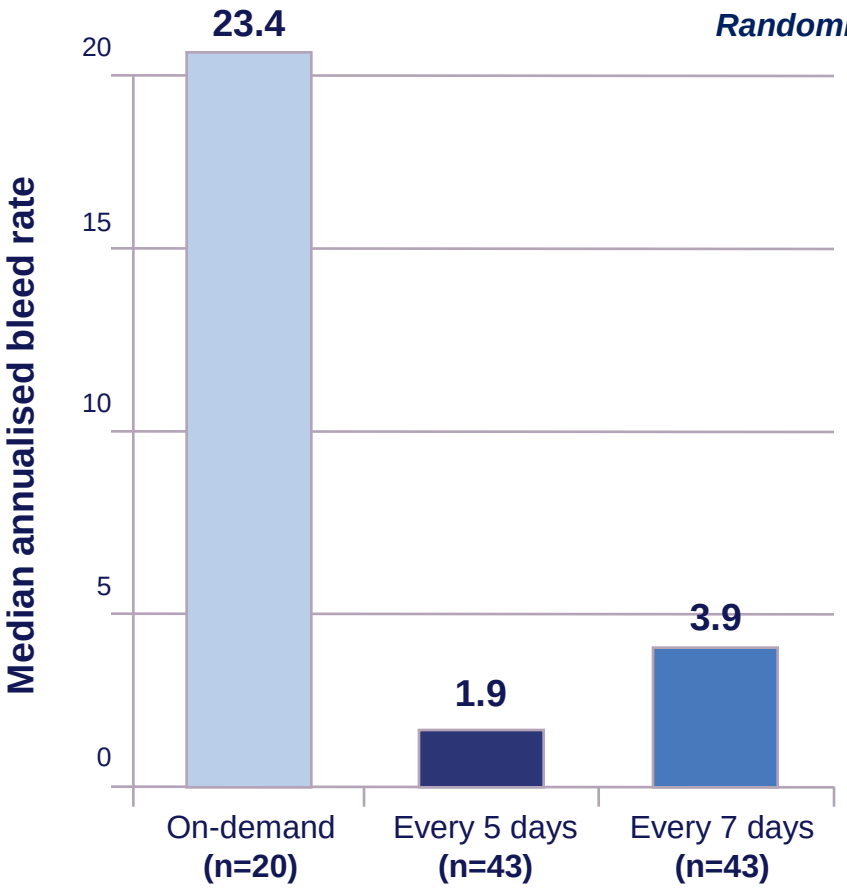
2x/wk 30–40 IU/kg (n≥20)

No or 1 breakthrough bleeds:

Randomization

Every 5 days 45–60 IU/kg (n≤40)

Every 7 days 60 IU/kg (n≤40)



- **$t^{1/2}$: 18.7 hours**
- **Treatment tailored to individual bleeding tendency**
- **Mean dose every 5 days: 45 IU/kg**
- **Mean dose every 7 days: 57 IU/kg**

ClinicalTrials.gov identifier: NCT01580293

PROLONG-ATE: Phase 2/3 Study of BAX 855

SCREENING

Current regimen

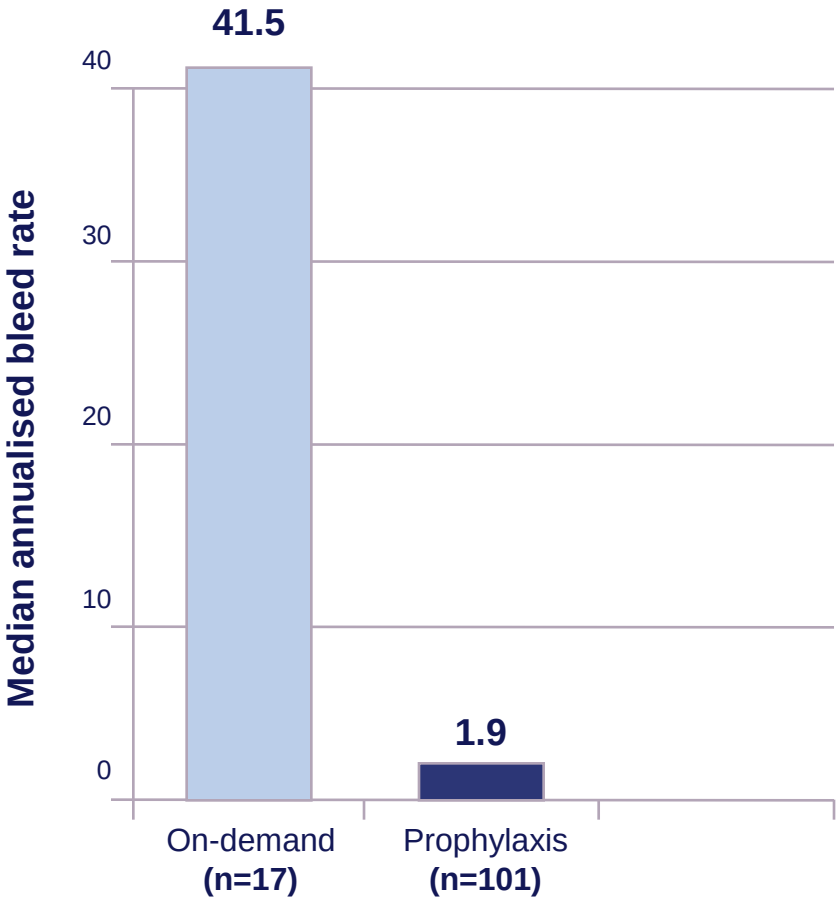
Prophylaxis or on demand → **PK assessment**

Assigned per patient's previous treatment regimen

→ On demand (n=17)

→ **Prophylaxis (45 ± 5 IU/kg 2x/wk) (n=109*)**

**21 were previously on demand*



- **Full-lenght rFVIII covalently bound to a branched 20 kDa PEG moiety (random PEGylation)**
- **60% of PEG localized on the B-domain**
- **Target trough: ≥ 1%**
- **$t^{1/2}$: 15 hours**
- **No inhibitor development**

Konkle B et al Blood 2015; 126: 1078-85

Longer-acting products: a new era for hemophilia prophylaxis?

Challenges and Perspectives

- All novel investigative therapies are promising, however still associated with potential risks and real benefits are to be proven

- Treatment individualization is the best strategy

- Open issues:
  - long-term safety
  - laboratory monitoring
  - availability
  - costs