Il bambino con emofilia

Padova, 13-14 Maggio 2016

# L'INIBITORE

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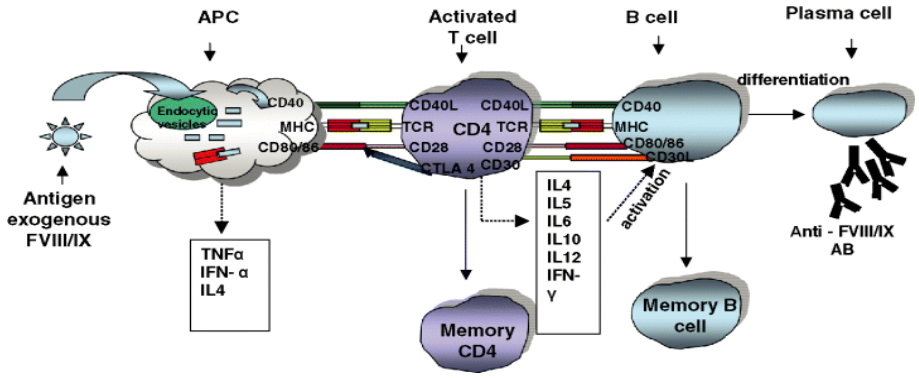
# Summary

- Definition and Epidemiology
- Mechanisms and diagnosis
- Risk factors
- Prevention

# Definition and Epidemiology

# INHIBITORS

- Antibodies that neutralize infused FVIII or FIX
- Frequency: ~25% in hemophilia A / ~ 3% in hemophilia B
- Typically developing at beginning of treatment




**Hemophilia A**

- Onset after a median of 15 exposures to exogenous FVIII
- After 50-75 exposures: plateau
- Incidence in PTP (≥ 150 ED): 2/1000 pat/year

blood

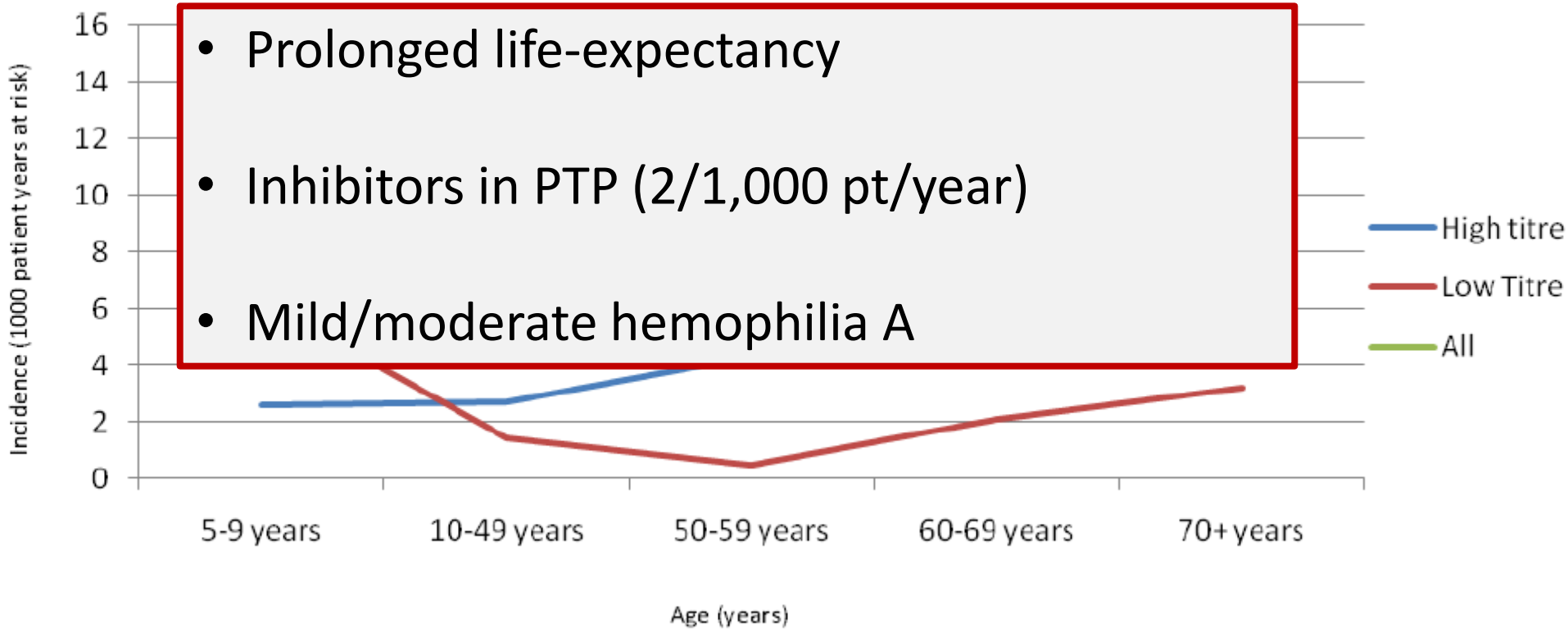
Prepublished online April 6, 2011;
doi:10.1182/blood-2010-09-308668

# The incidence of factor VIII inhibitors throughout life in severe hemophilia A in the United Kingdom

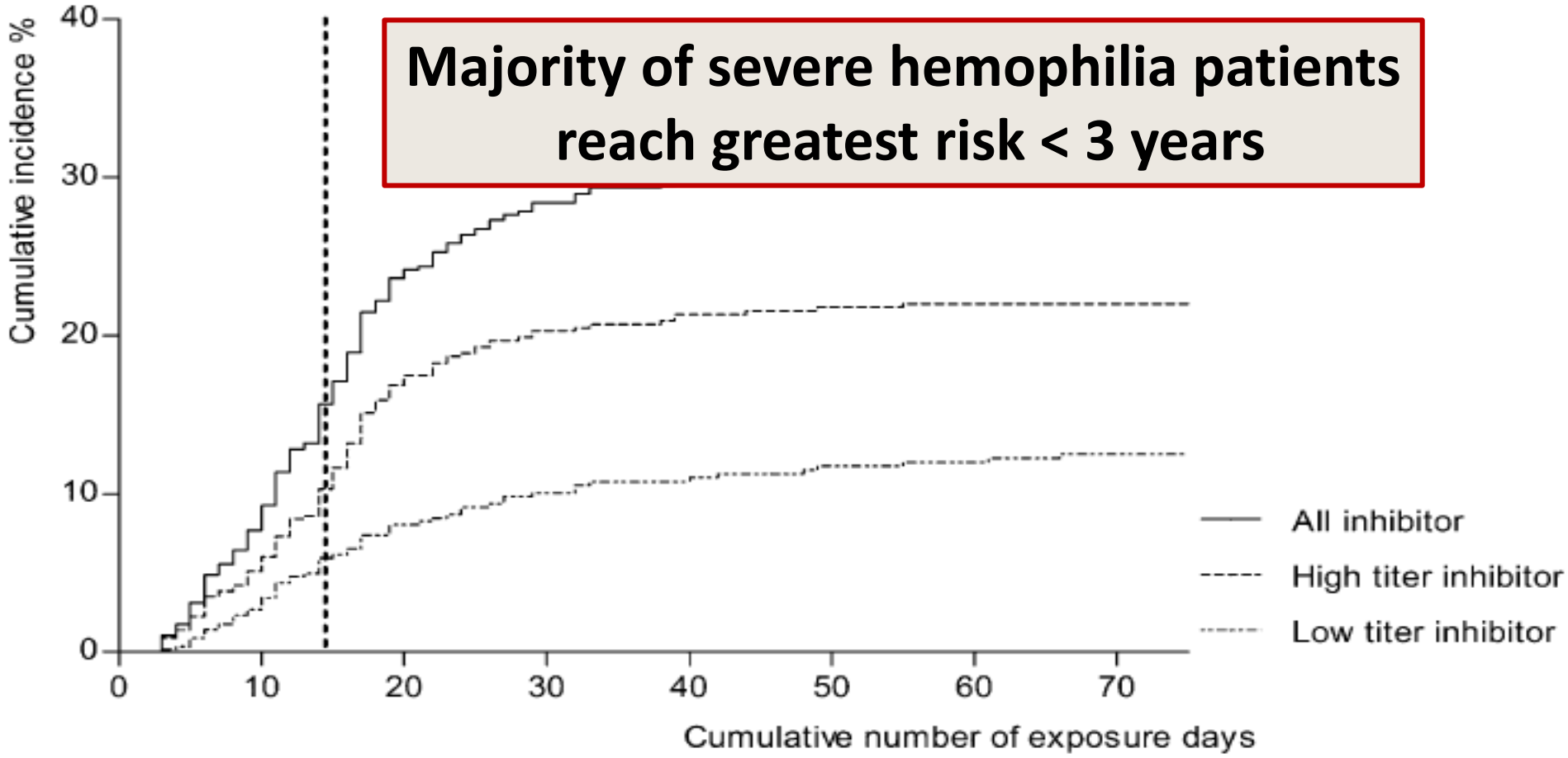
Charles RM Hay, Ben Palmer, Elizabeth Chalmers, Ri Liesner, Rhona Maclean, Savita Rangarajan, Michael Williams and Peter W Collins

**Blood 2011; 117: 6367**

- Prolonged life-expectancy
- Inhibitors in PTP (2/1,000 pt/year)
- Mild/moderate hemophilia A



# Inhibitor incidence in severe patients




Gouw SC *et al*. Blood 2013;121:4046–55

# *F8* genotype and adjusted inhibitor risk

| ***F8* mutation** | **Patients** *n* | **Inhibitor** *n (%)* | **Inhibitor risk at 20 ED** *% (95%CI)* | **Inhibitor risk at 50 ED** *% (95%CI)* |
|---|---|---|---|---|
| ***Mutations in >10 patients*** | | | | |
| R531C | 35 | 1 (2.9) | .0 | .0 |
| N618S | 58 | 1 (1.7) | 3 (0-9) | 3 (0-9) |
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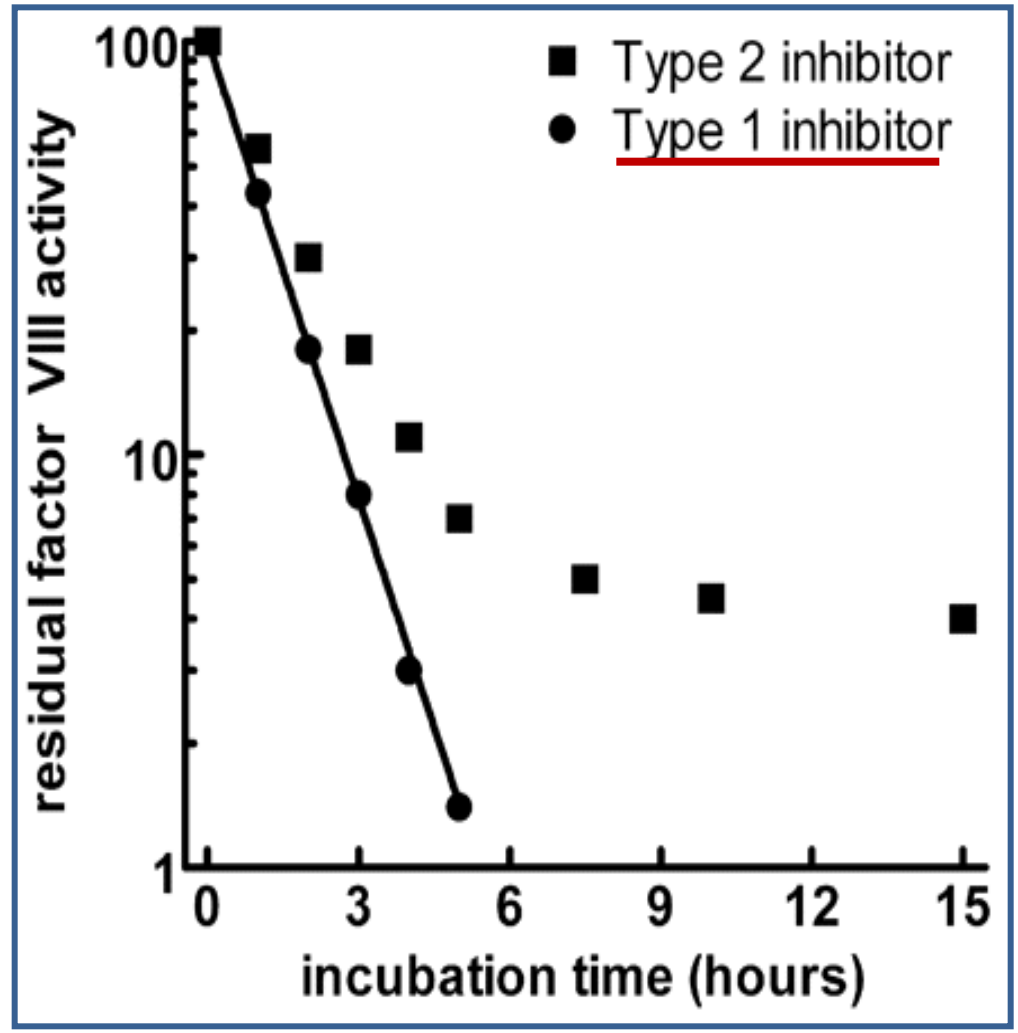
| R2159C | 21 | 3 (14.3) | 9 (0-26) | 39 (3-75) |
| W2229C | 10 | 5 (50.0) | 42 (5-78) | 42 (5-78) |

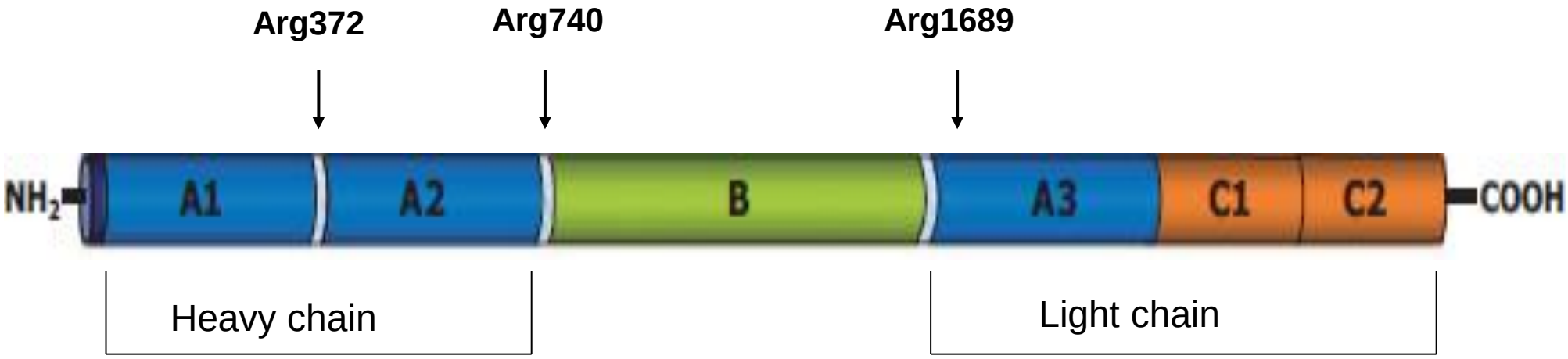
# Mechanisms and diagnosis

# Characteristics of anti-FVIII antibodies

- Largely polyclonal IgG4
- They do not fix complement
- Time-dependent
- Type 1 inactivation kinetic



# Structural domains of Human FVIII




- **A2, A3 and C2 doamins, and light chain most immunogenic**
- Often Abs anti C2 domain inhibiting phospholipids and VWF binding VWF and cleavage by Fxa (present in ~ 70 % of cases)
- Abs anti A2 domain (aa 454-509) inhibit binding with FX
- Abs anti B domain increase clearance of F VIII

# When should an Inhibitor be suspected ?

- **Clinically:**

 - Worsening of clinical phenotype

- Bleeding hardly responsive to replacement therapy

- **Laboratory:**

- PTT not corrected by normal plasma
- FVIII not appropriately recovered after infusion
- Inhibitor testing

# Mixing studies

- *No inhibitor*:

$$\frac{Patient+Normal}{Normal} < 1.2$$

- *Inhibitor* $(e.g. LAC, heparin)$:

$$\frac{Patient+Normal}{Normal} > 1.2$$

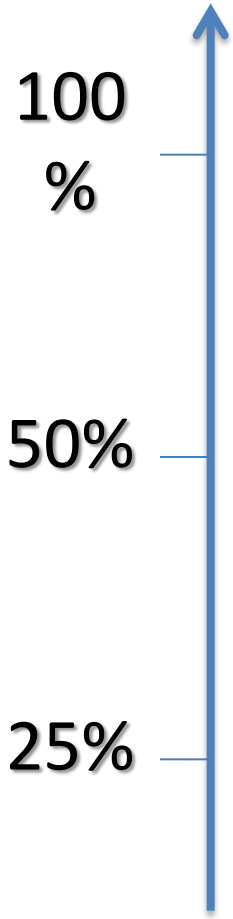
- *Time-dependent* $(FVIII)$:

$$\frac{Patient120min+Normal120min}{Normal120} > \frac{Patient+Normal}{Normal}$$

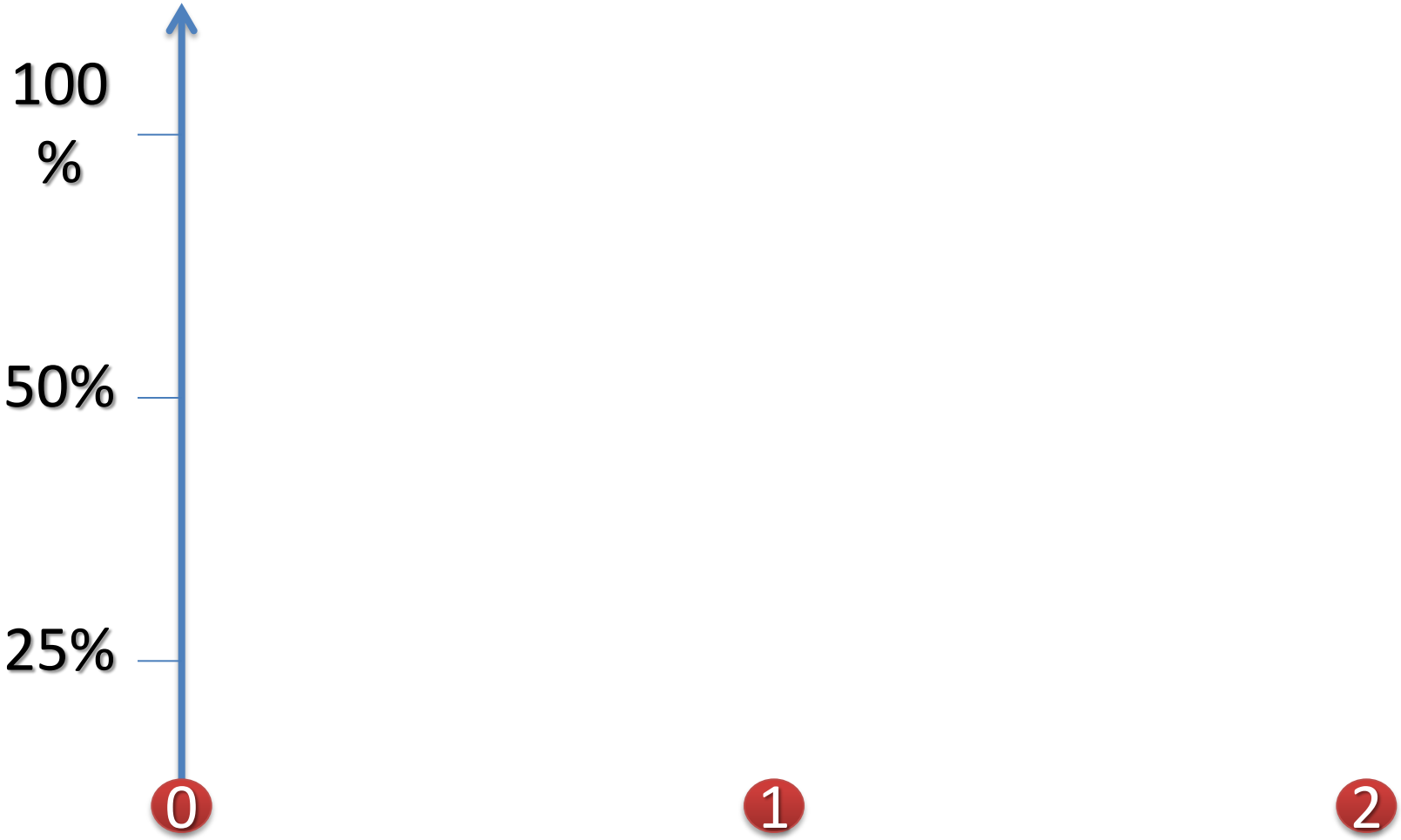
# Bethesda assay

- Patient plasma is serally diluted (1:2 – 1:1024) in buffer, hence diluting the inhibitor. Each dilution is then mixed 1:1 in normal plasma
- A normal plasma is serially diluted (1:2 – 1:1024) in FVIII deficient plasma
- Incubate 2h at 37° C
- Measure FVIII activity. Normal plasma 1:2 is 100% residual activity

# Bethesda assay



# Bethesda assay



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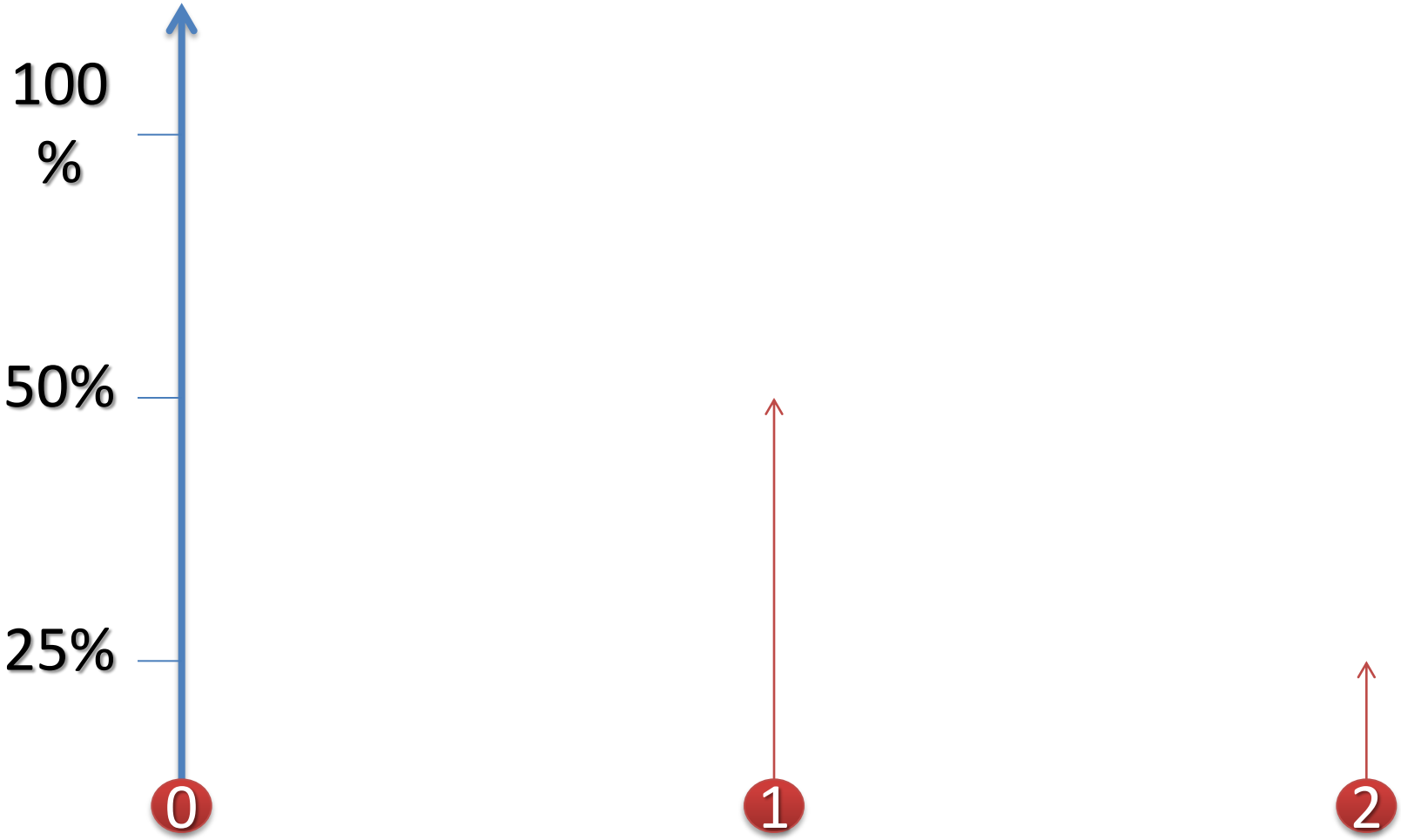

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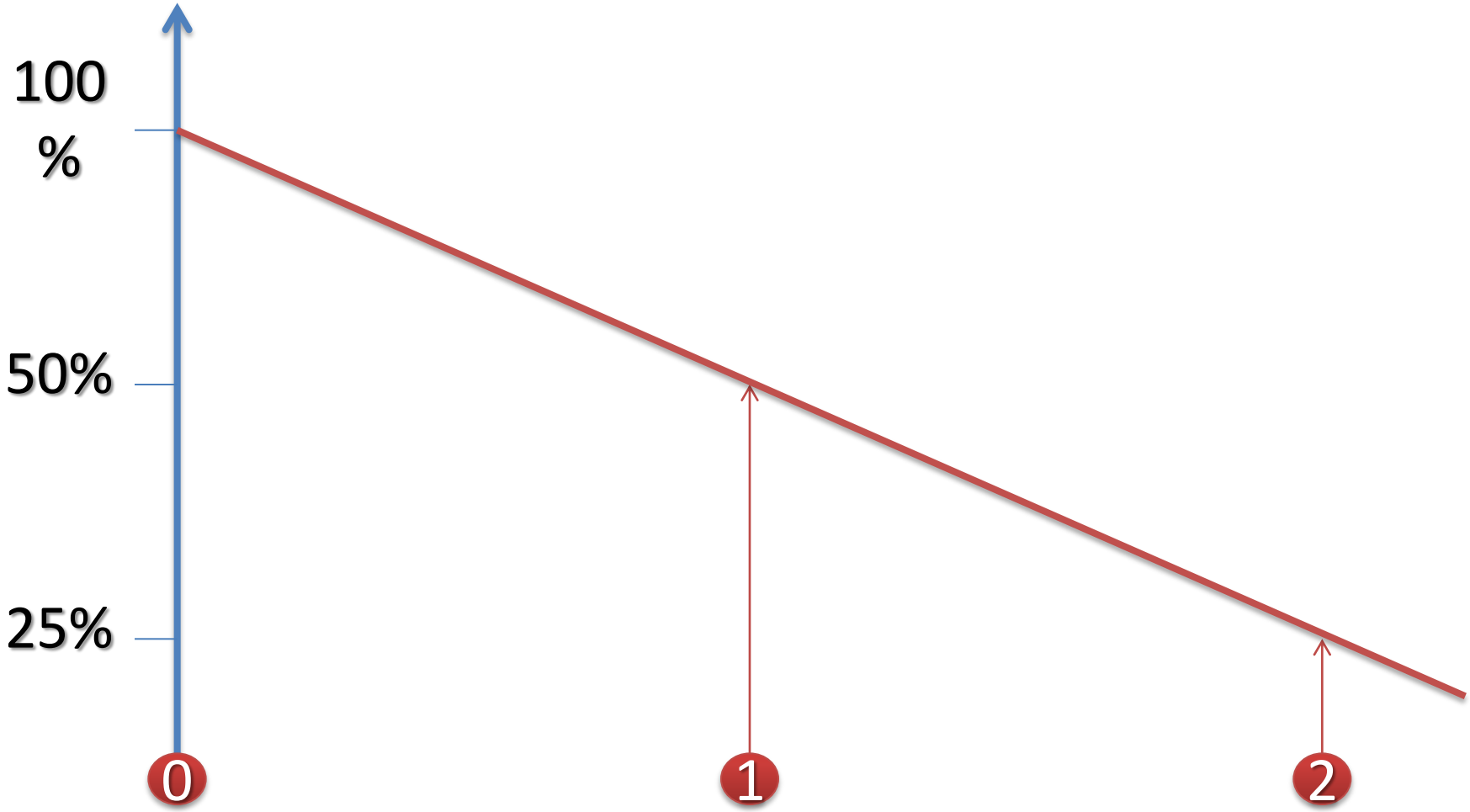

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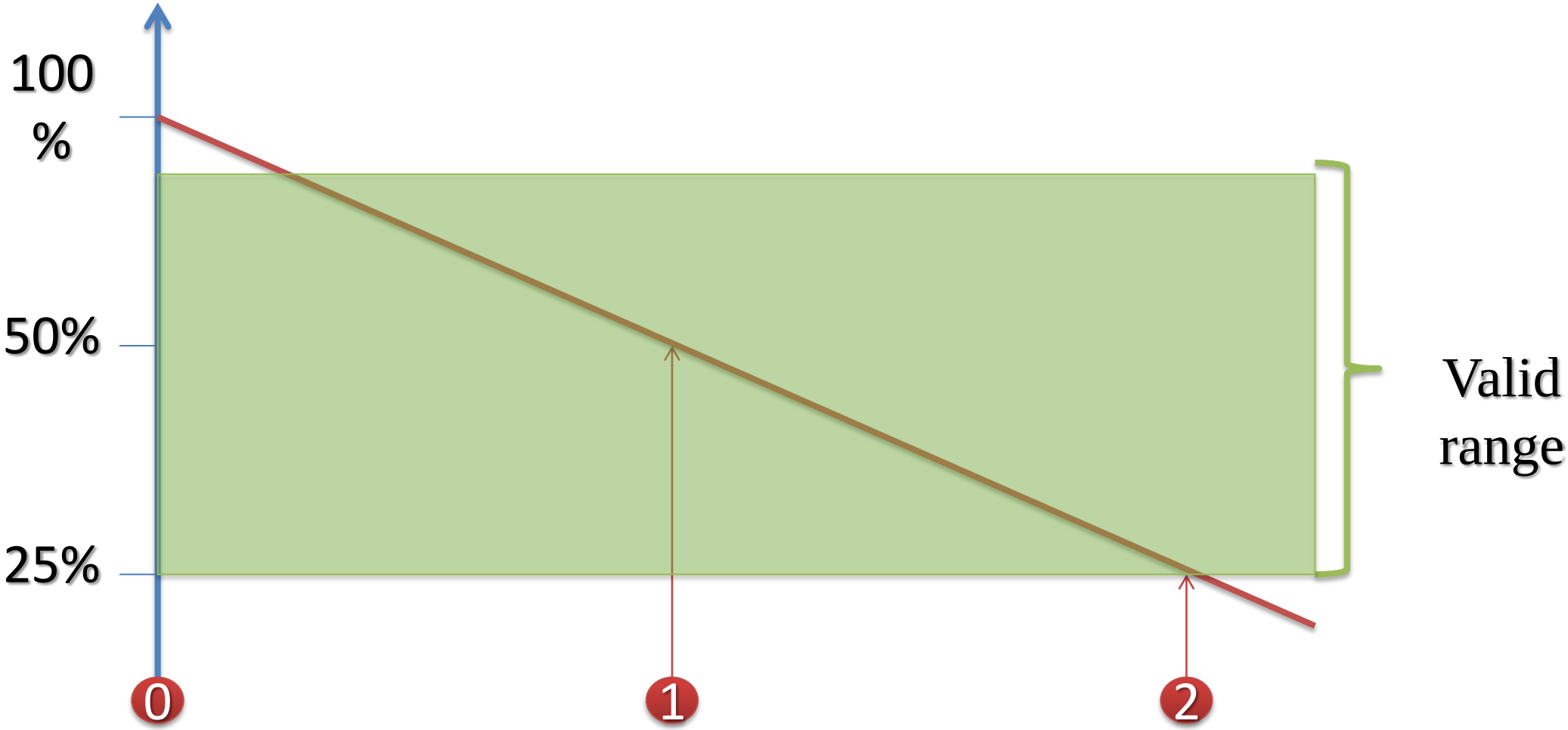

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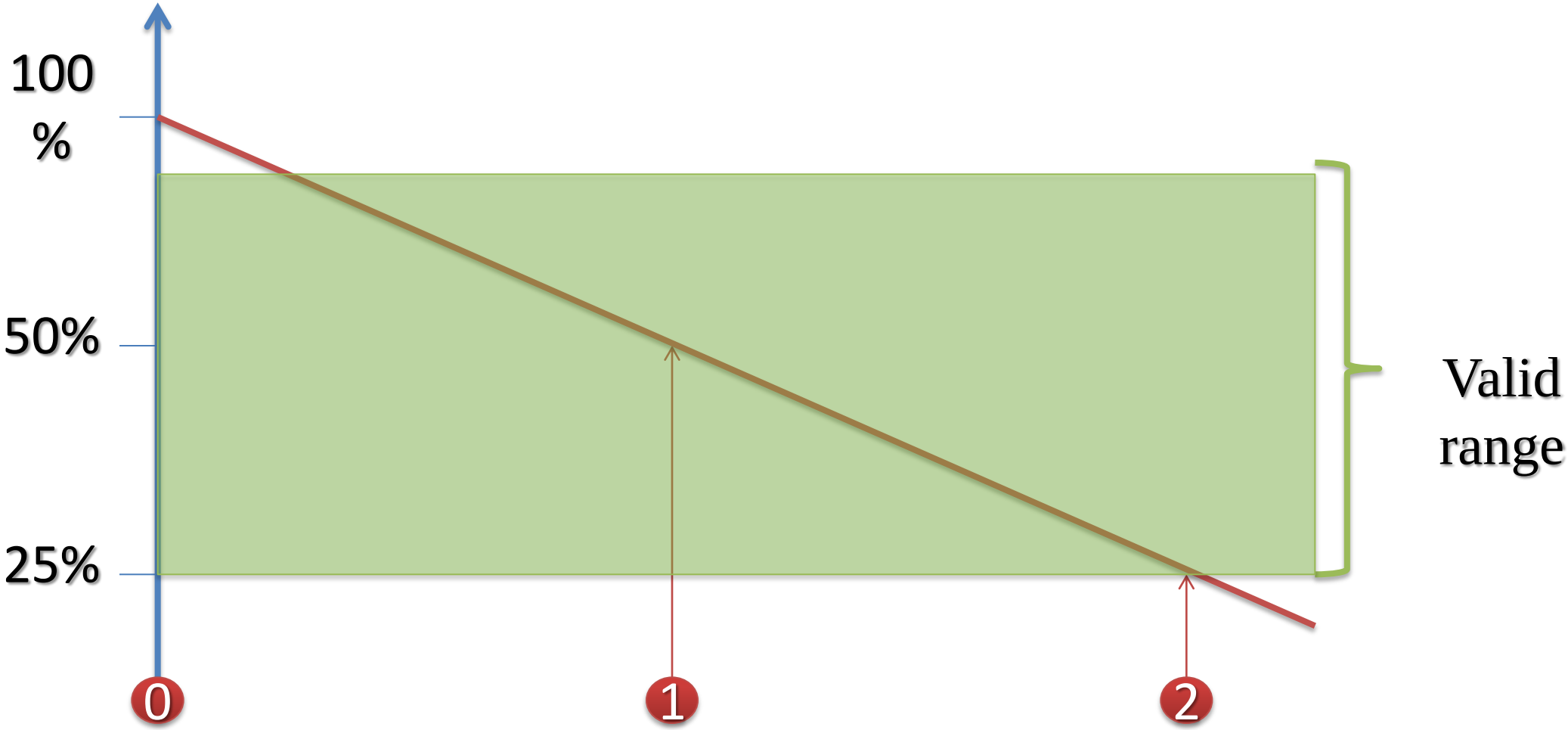
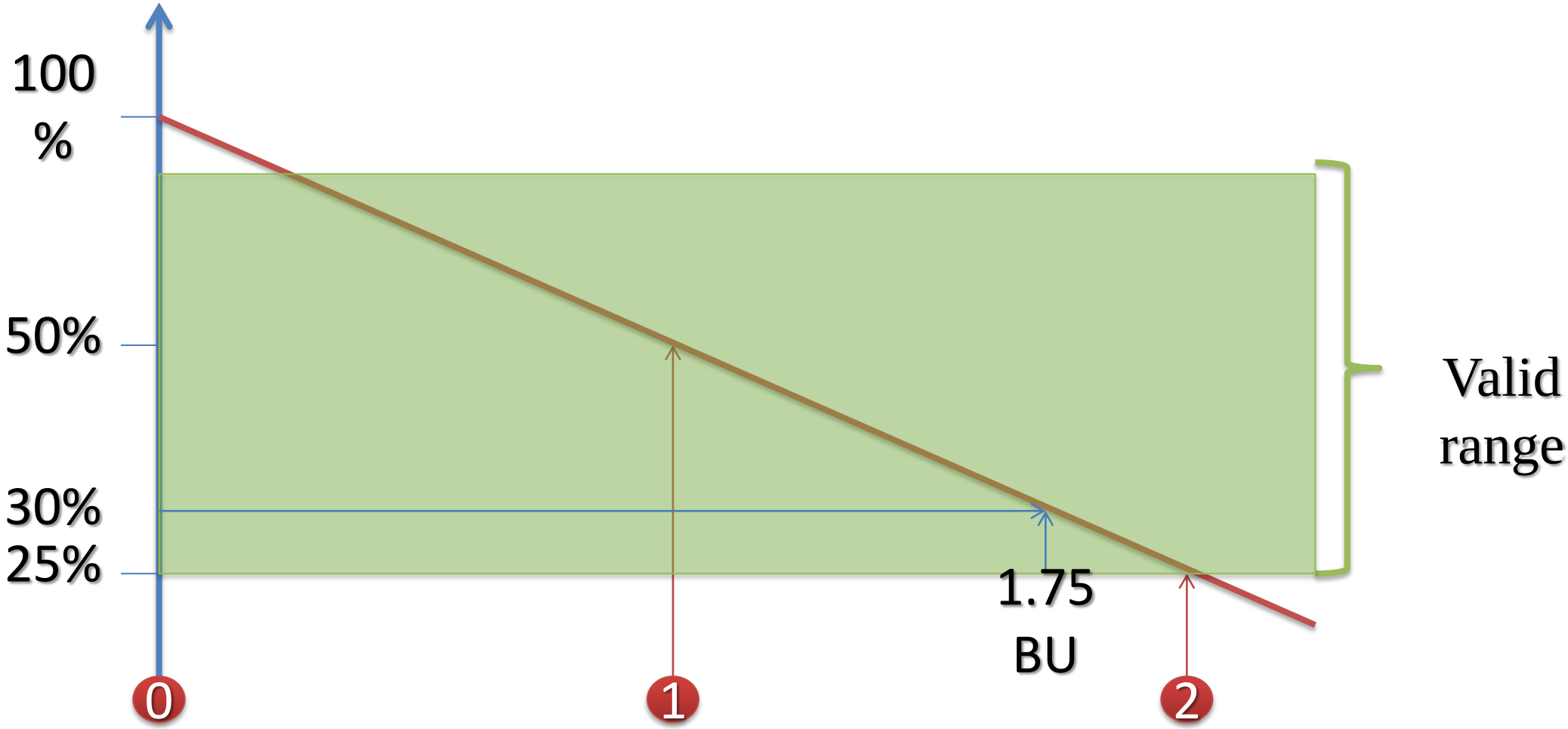



Example: 1:20 Dilution, 30%  Residual activity

# Bethesda assay




Example: 1:20 Dilution, 30%  Residual activity: 1.75 BUx20= 35 BU

# High Responding / Low Responding

- **<u>High responding</u>: >5 Bethesda Units' (BU)**
  - Benefit of FVIII/IX replacement exceptional
  - Replacement therapy triggers anamnestic response

- **<u>Low Responding</u>:**
  - **<u>True:</u>** < 5 BU even after stimulation
  - **<u>Transient:</u>** < 5 BU disappear while continuing replacement therapy within 6 – 12 months

- FVIII/FIX can be used at increased dose (neutralizing + therapeutic dose)

# Treatment according to Inhibitor titer

**Low Titer**
**< 5 BU/mL**

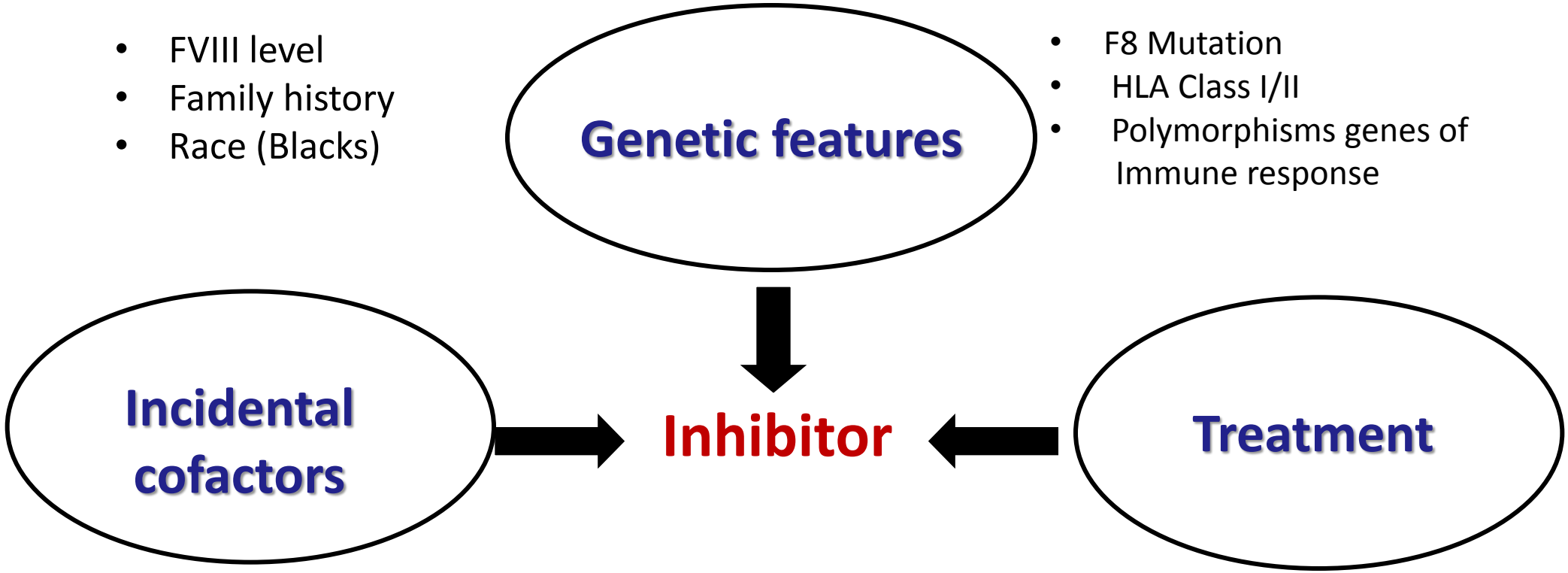
- FVIII/FIX
- APCC
- FVIIa

**High Titer**
**> 5 BU/mL**

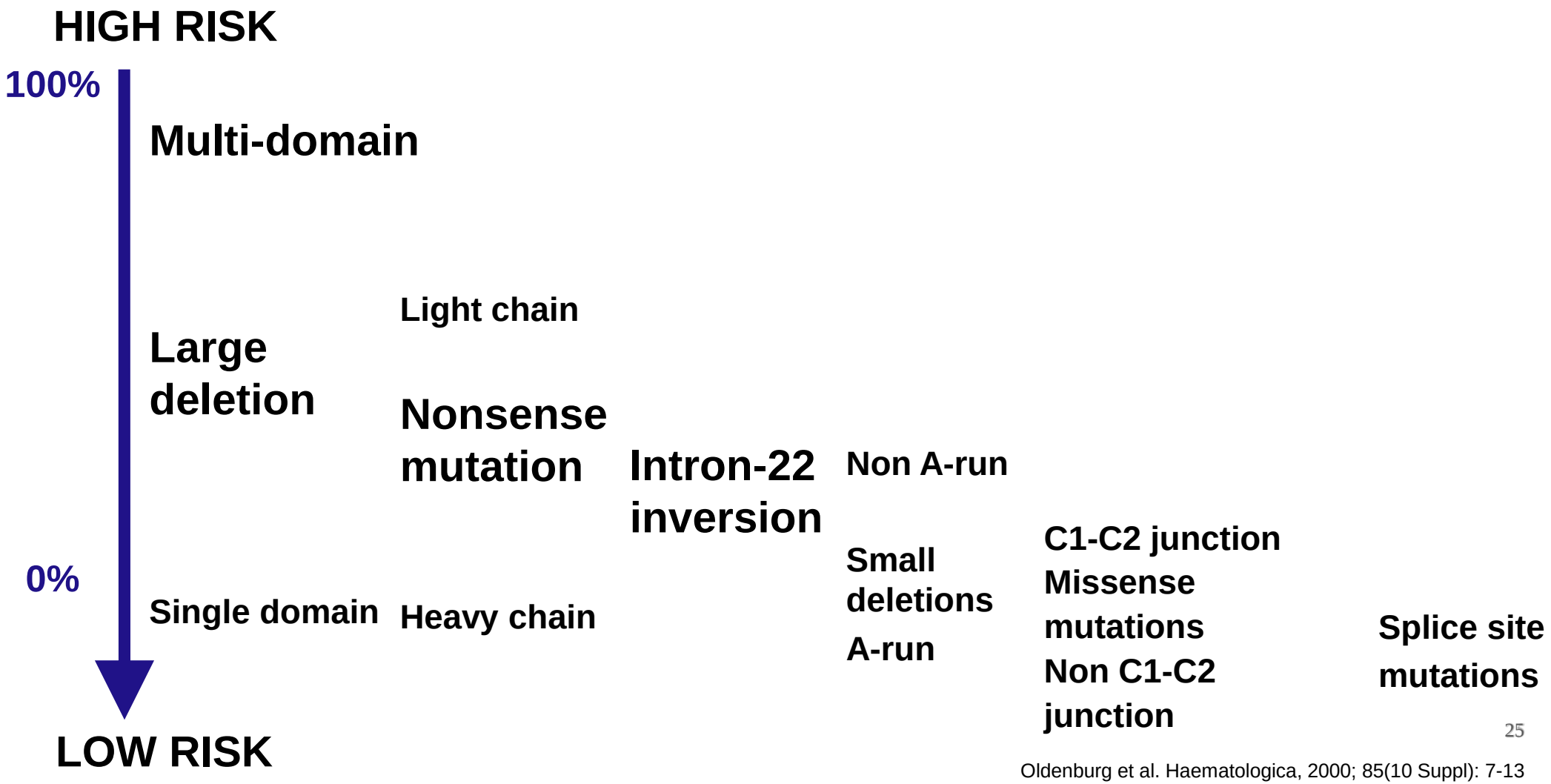
- APCC
- FVIIa

# Risk factors

# Risk factors for inhibitor development



# *F8* gene mutations and inhibitor risk in PUPs






Oldenburg et al. Haematologica, 2000; 85(10 Suppl): 7-13

# Inhibitors in Hemophilia B

- Rarer compared to hemophilia A (< 5 % vs 25-30 %)
- Only in severe
- **Correlation with gene deletion or nonsense mutations**
- Risk of allergic reactions/anaphylaxis
- Risk of nephrotic syndrome upon ITI

# ‘Genetic’ risk of inhibitor

## High-risk

- “Null” mutations
- CRM- phenotype
- HLA class II profile
- Race
- Familial occurrence

## Low-risk

- Point mutations with minimal effect on secondary and tertiary FVIII structure
- CRM+ phenotype
- Splice site (alternative splicing?)
- HLA class II profile

**Can an inhibitor be prevented in those patients with genetic high risk features?**

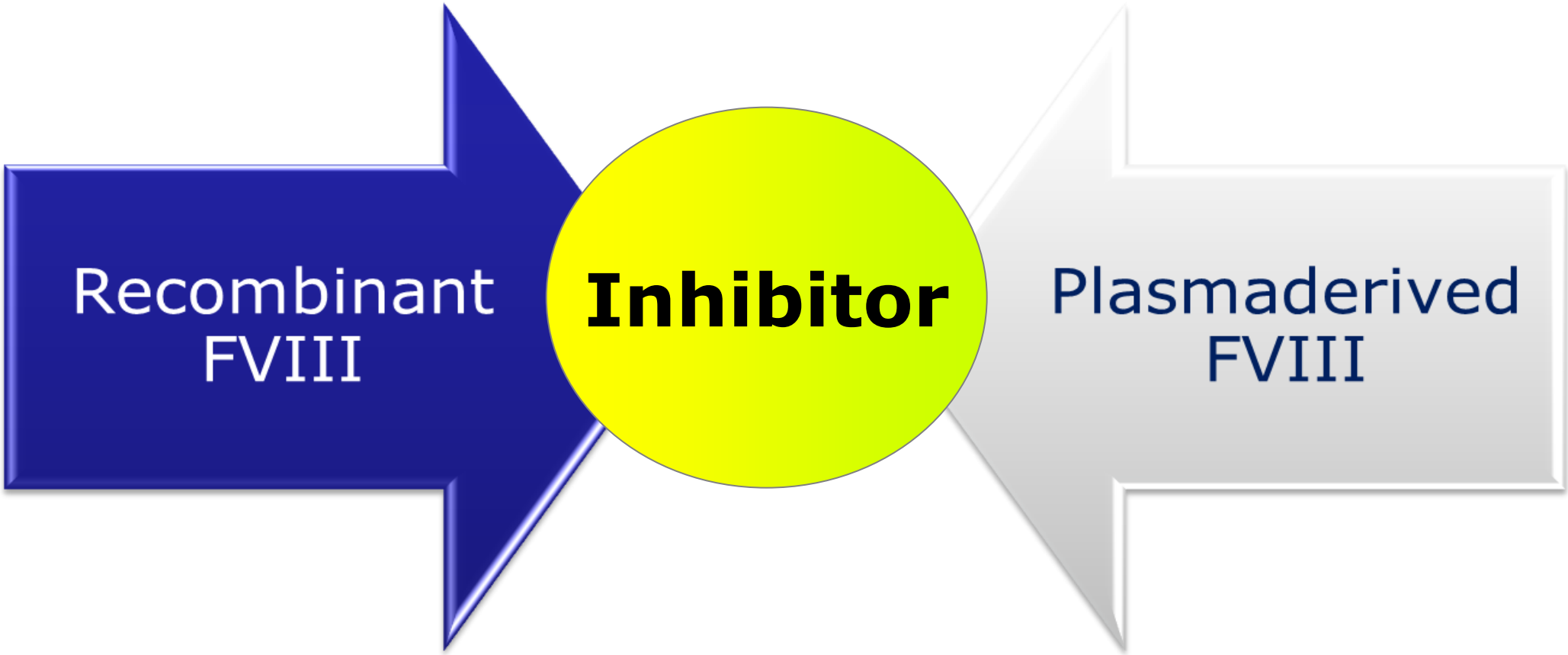
# Identifying Nongenetic Risk Factors for Inhibitor Development in Severe Hemophilia A

Samantha C. Gouw, MD, PhD[1] Karin Fijnvandraat, MD, PhD[1]

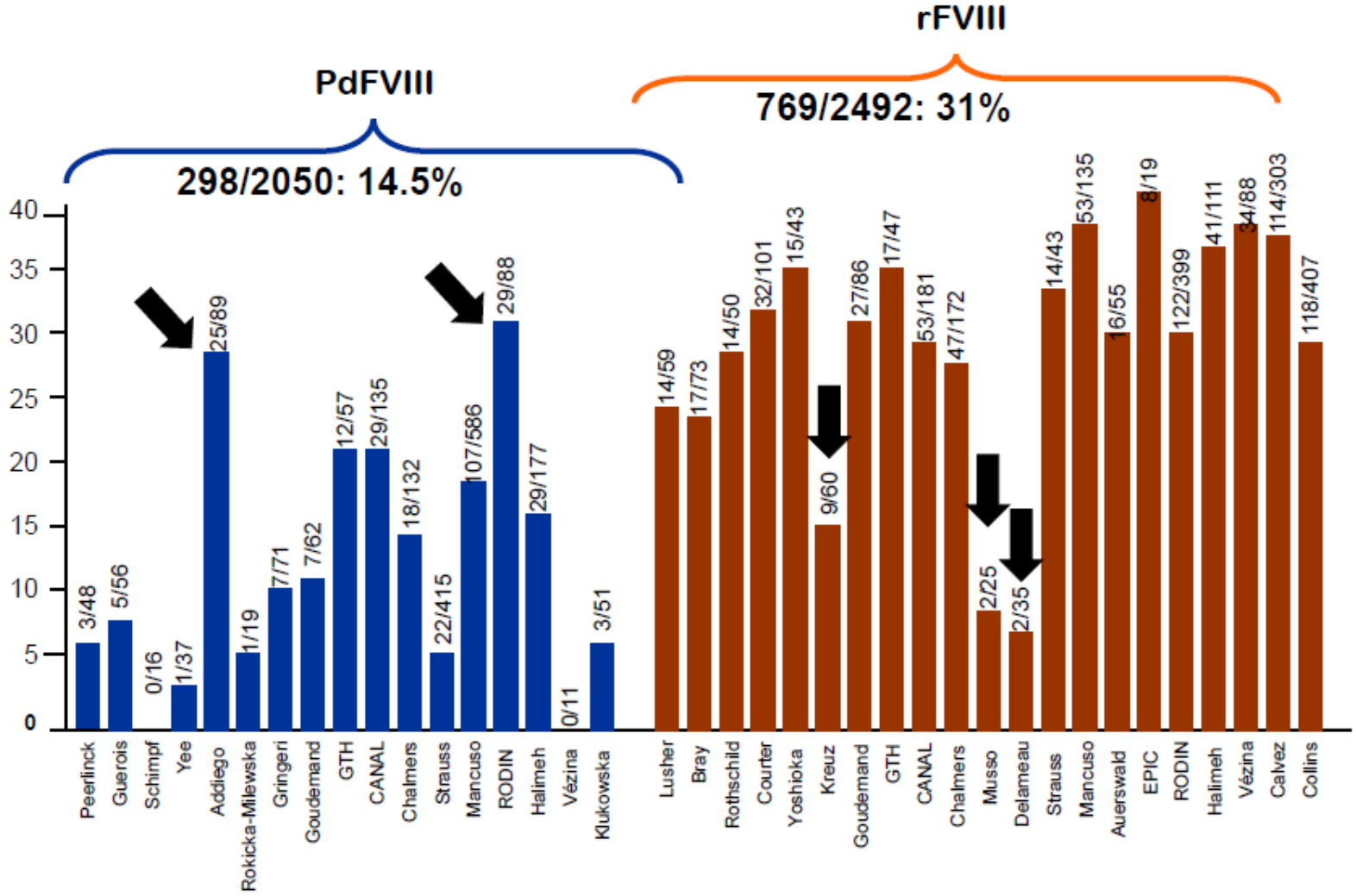
Seminars in Thrombosis & Hemostasis Vol. 39 No. 7/2013

| Non-genetic risk factors | Level of certainty |
|---|---|
| FVIII exposure | Certainly a risk factor |
| FVIII product type: specific pasteurized plasma-derived product types | Certainly a risk factor |
| Periods of intensive treatment | Certainly a risk factor |
| Prophylaxis | Likely a protective factor |
| Surgical procedures | Likely a risk factor |
| Dose of FVIII treatment | Possibly a risk factor |
| FVIII product type: certain recombinant FVIII products | Possibly a risk factor |
| Source of FVIII product: recombinant versus plasma | Unlikely a risk factor |
| Switching between FVIII products | Not a risk factor |
| Age at first FVIII exposure | Not a risk factor |
| Breastfeeding | Not a risk factor |
| Vaccinations, infections, immune modulating medication, allergic constitution | Not enough available data |
| Extravasation of FVIII product | Not enough available data |
| Mode of infusion (bolus infusion, continuous infusion) | Not enough available data |

# *Type of concentrate*

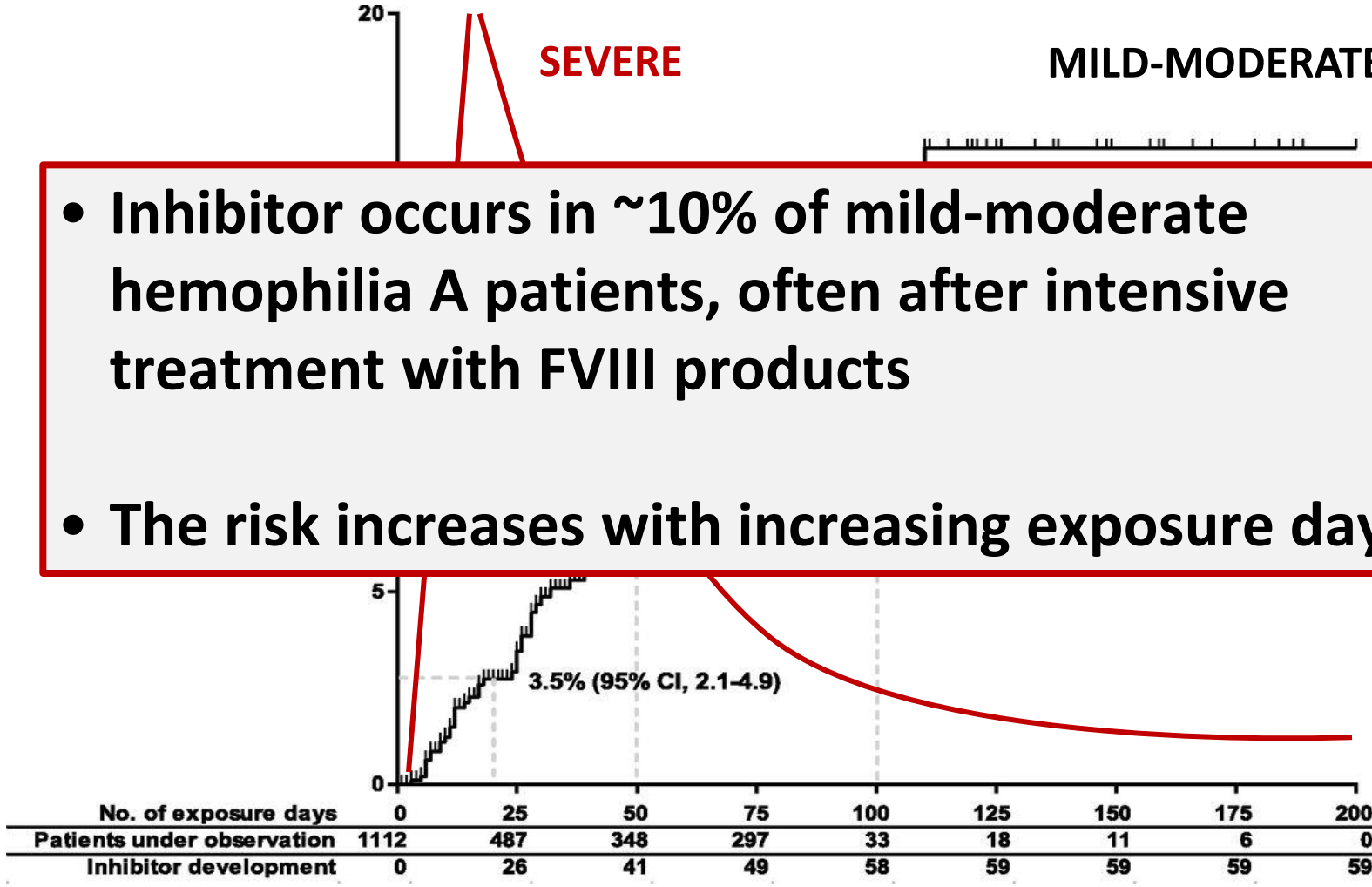


## Higher crude incidence of inhibitors in previously untreated children with severe hemophilia A (PUPs) treated with recombinant FVIII




SIPPET (ASH 2015): in PUPs, the combined risk of developing a high or low titer inhibitor within the first 50 EDs when using recombinant factors was **1.87-fold** higher

# Cumulative incidence of inhibitor in 1,112 mild-moderate hemophilia A patients




| No. of exposure days | 0 | 25 | 50 | 75 | 100 | 125 | 150 | 175 | 200 |
|---|---|---|---|---|---|---|---|---|---|
| Patients under observation | 1112 | 487 | 348 | 297 | 33 | 18 | 11 | 6 | 0 |
| Inhibitor development | 0 | 26 | 41 | 49 | 58 | 59 | 59 | 59 | 59 |

- **Inhibitor occurs in ~10% of mild-moderate hemophilia A patients, often after intensive treatment with FVIII products**
- **The risk increases with increasing exposure days**

Eckhardt C L et al. Blood 2013;122(11):1954-62

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# Prevention of inhibitors

# How to attenuate inhibitor occurrence? (I)

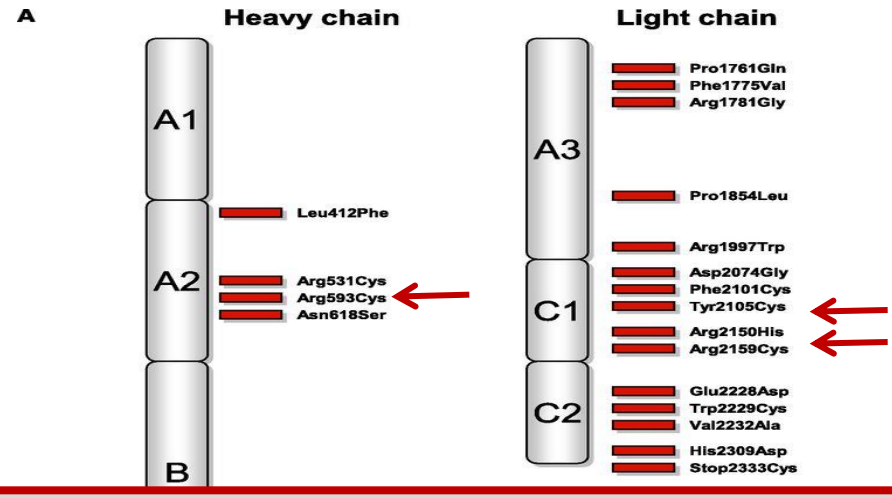
- Identify the responsible mutation
- Replacement therapy in association with a systematic inflammatory response should be avoided if possible
- Regular treatment provided at young age to prevent bleeding in presence of minimum coexisting inflammatory markers is preferable
- Low dose prophylaxis could be an advantage in high-risk patients, but not definitely proven

# How to attenuate inhibitor occurrence?[1] (II)

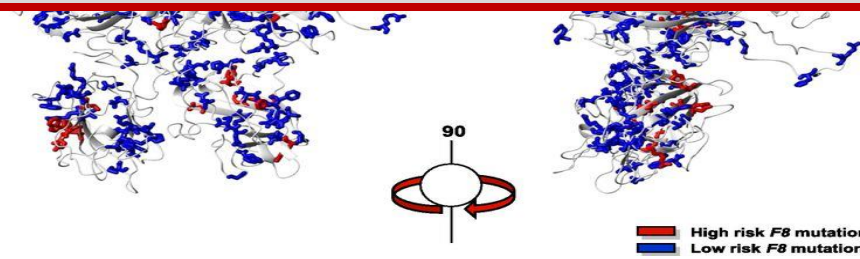
- Vaccination by subcutaneous route to avoid combined infusion of the deficient factor in a setting of "alert signal"
- Peripheral vein should be preferred first, but no risk evident with CVC placement, unless infection occurs
- Co-administration of deficient factor and immunosuppressive drugs could reduce the risk, as shown in a mouse model of hemophilia A[2]

1. Astermark, Blood 2015; 125(13): 2045-51. 2. Moorhead, JTH abs 56.6, 2013

# *F8* missense mutations associated with inhibitor risk in mild-moderate hemophilia A




- **Genotyping advisable also in mild-moderate hemophilia A**
- **Reducing the risk by using desmopressin**




Eckhardt C L et al. Blood 2013;122(11):1954-62.
Castaman , Blood 2014; 124(15): 2333-6

# CONCLUSIONS

- Inhibitor occurrence remains the most important challenge in hemophilia treatment
- Risk profiling designed at diagnosis (e.g, family history, mutation…)
- Close surveillance is recommended especially during the first days of treatment
- Testing after period of intensive treatment (e.g., surgery) should also be undertaken