Two case studies in support of an Integrated Data Approach versus *in vitro* NAb Assays

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Outline

Presence and impact of NAbs - Importance of the integrated data approach

Case study 1 – PK measurement was superior to *in vitro* NAb

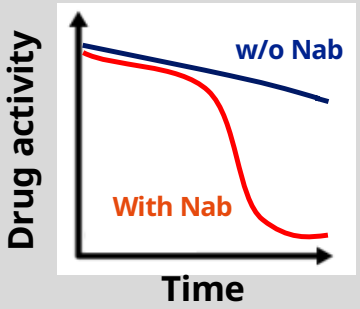
Case study 2 – Integrated data approach sufficient for BLA approval without *in vitro* NAb data, literature based

Lesson learned and feedbacks received by Regulatory Agencies

Presence and impact of neutralising antibodies NAbs

- Understanding NAb effect – crucial and required

SAFE and EFFECTIVE drugs



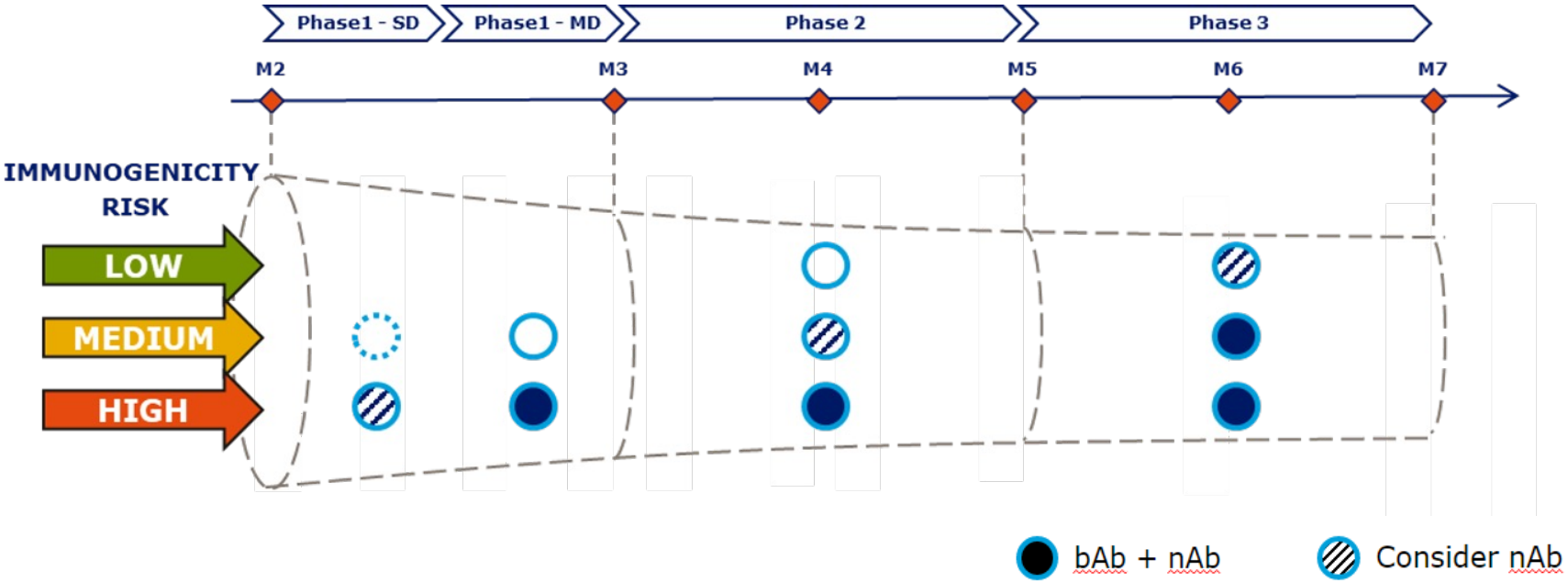
Neutralise the drug activity

Cross-react and neutralise endogenous counterpart

Risk assessment of the compound

Outline of NAb strategy:

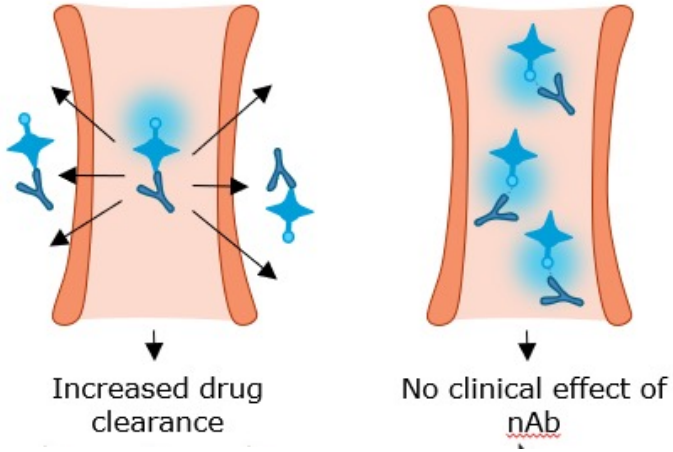
- Immunogenicity risk assessment
- Assess which tools are available for assessing the neutralising effect of ADA
- Early scientific engagement with regulators



The Nab results does not carry information of the clinical impacts

- Binding antibodies without *in vitro* neutralising activity can be *in vivo* neutralising eg by clearing the drug

- *in vitro* neutralising antibodies may not have any clinical effect



Consider if neutralising effect can be assessed by other parameters: PK, PD, safety

Presence and impact of NAbs

Standalone *in vitro* NAb assay:

- Translation to *in vivo* can be complex
- Often pre-treatment steps included to ensure drug tolerance which alters the matrix
- Drug concentration in Nab assays is very different than therapeutic levels
- Lacking knowledge about the clinically relevant levels of Nabs
- Nab negative binding antibodies can clear the drug and result in de facto neutralisation

Integrated data approach (holistic view):

- Evaluate impact on **exposure**, **efficacy** and **safety** by correlating **levels and persistence of binding ADA** to PK, PD, biomarkers and safety events
- Can also include relevant *in vitro* NAb assay

Clinically relevant NAbs to be reported in product labels –
that can guide doctors and patients

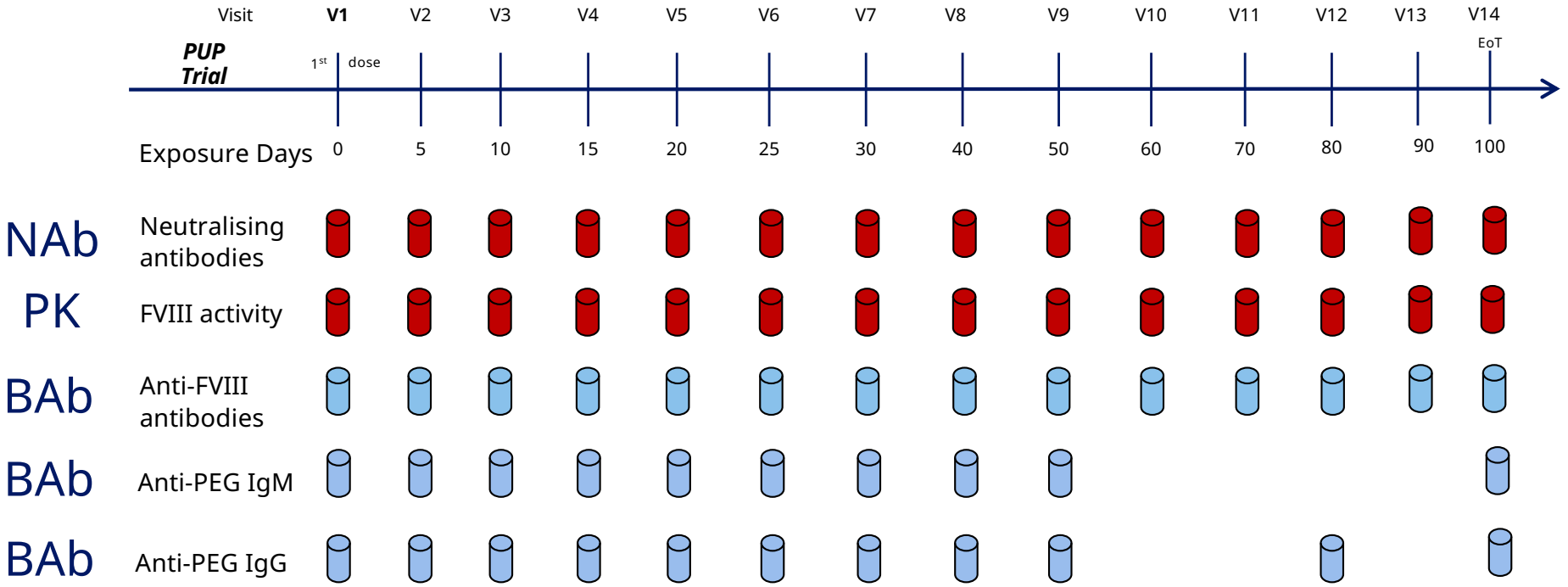
Two case studies in the haemophilia area

- Bleeding disorder caused by the lack of functional coagulation factor VIII
- Treatments restores the coagulation
- Historically many laboratory assays available and knowledge of clinical relevant cut points of neutralising assay and safety read outs

Case Story 1 – PK measurement is superior to *in vitro* NAb

Compound: rFVIII with extended half-life due to a PEG moity

Sampling schedule appropriate for an integrated data approach



Neutralizing antibody results

	Number of patients, n (%)
Number of patients	67
FVIII neutralising antibodies (inhibitors)	20 (30 %)

In hemophilia patients a clinically relevant FVIII neutralising cut point is defined in literature

70% of the patients in this trial did not have neutralising antibodies

Many patients without NAbs had decreased FVIII activity

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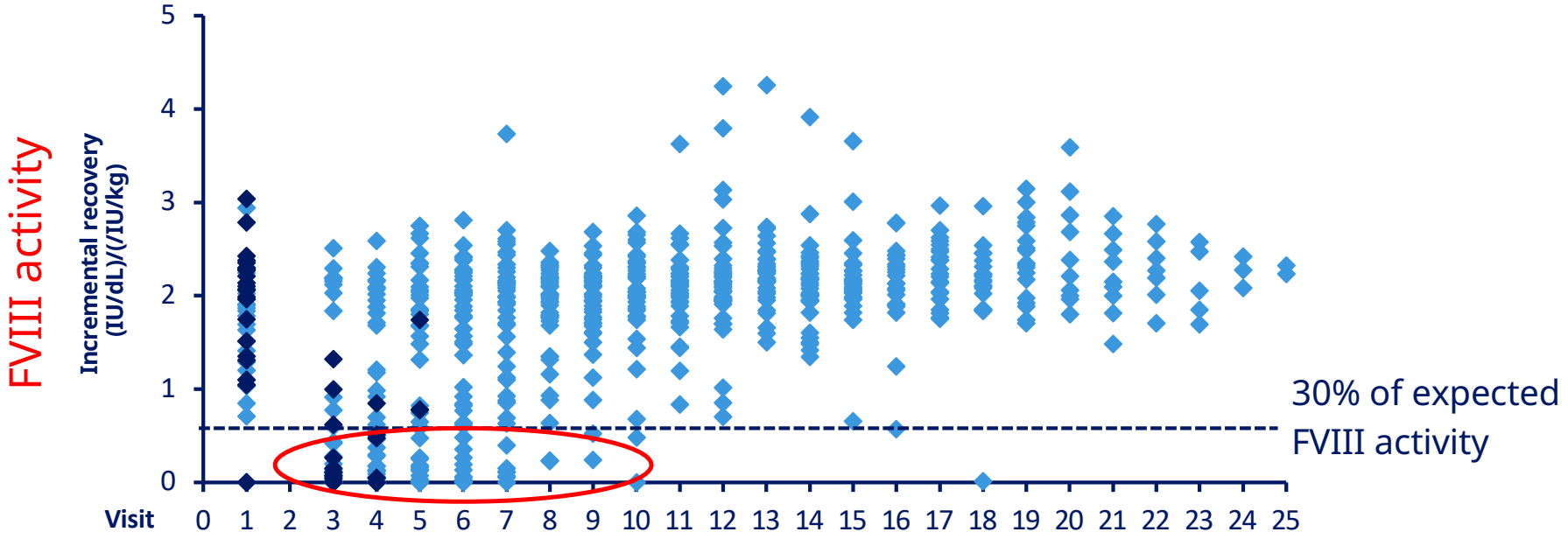
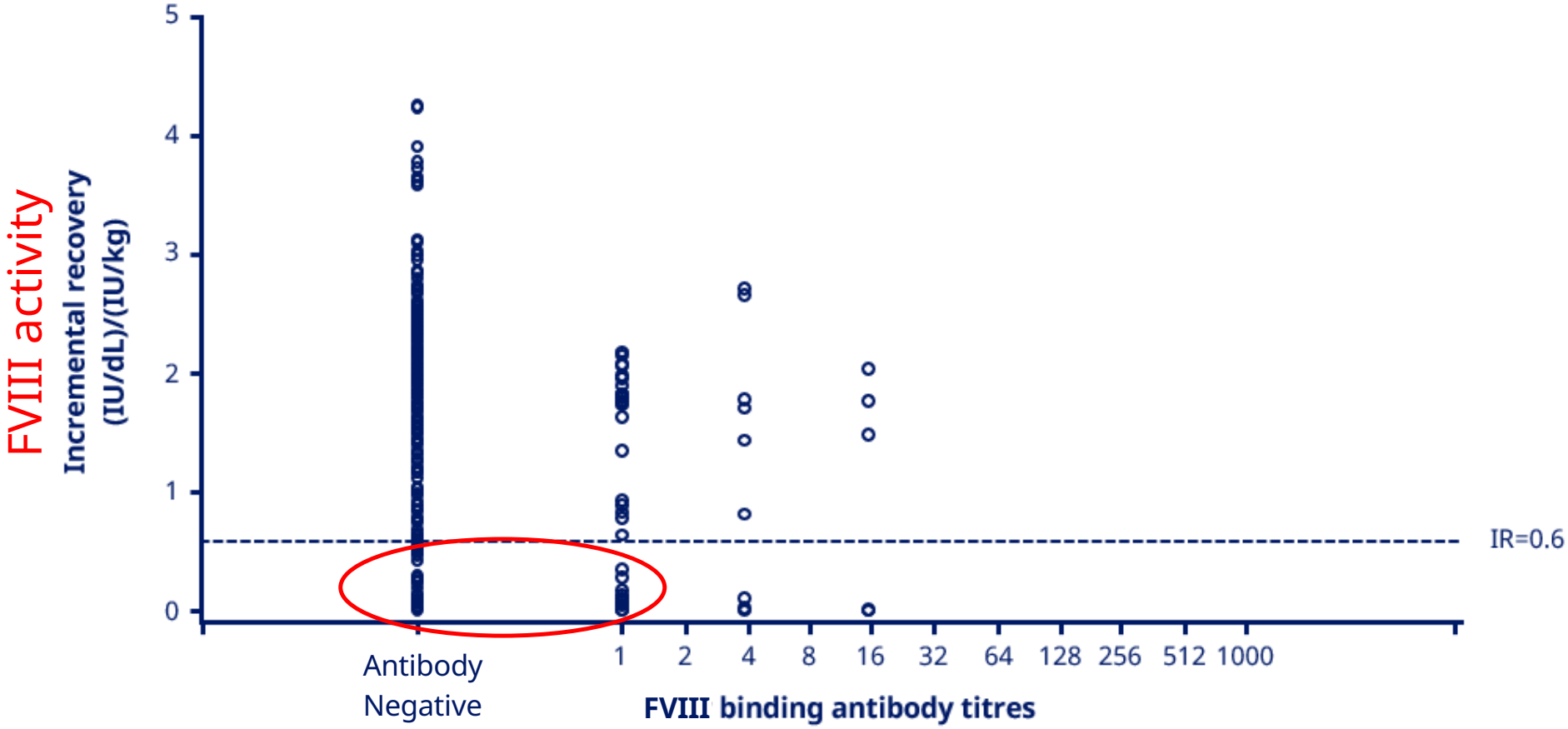
Temporarily decreased FVIII activity was observed in 17 patients negative for neutralising antibodies



Ref: Male et al., Blood Advances 2022

FVIII binding antibodies vs FVIII activity

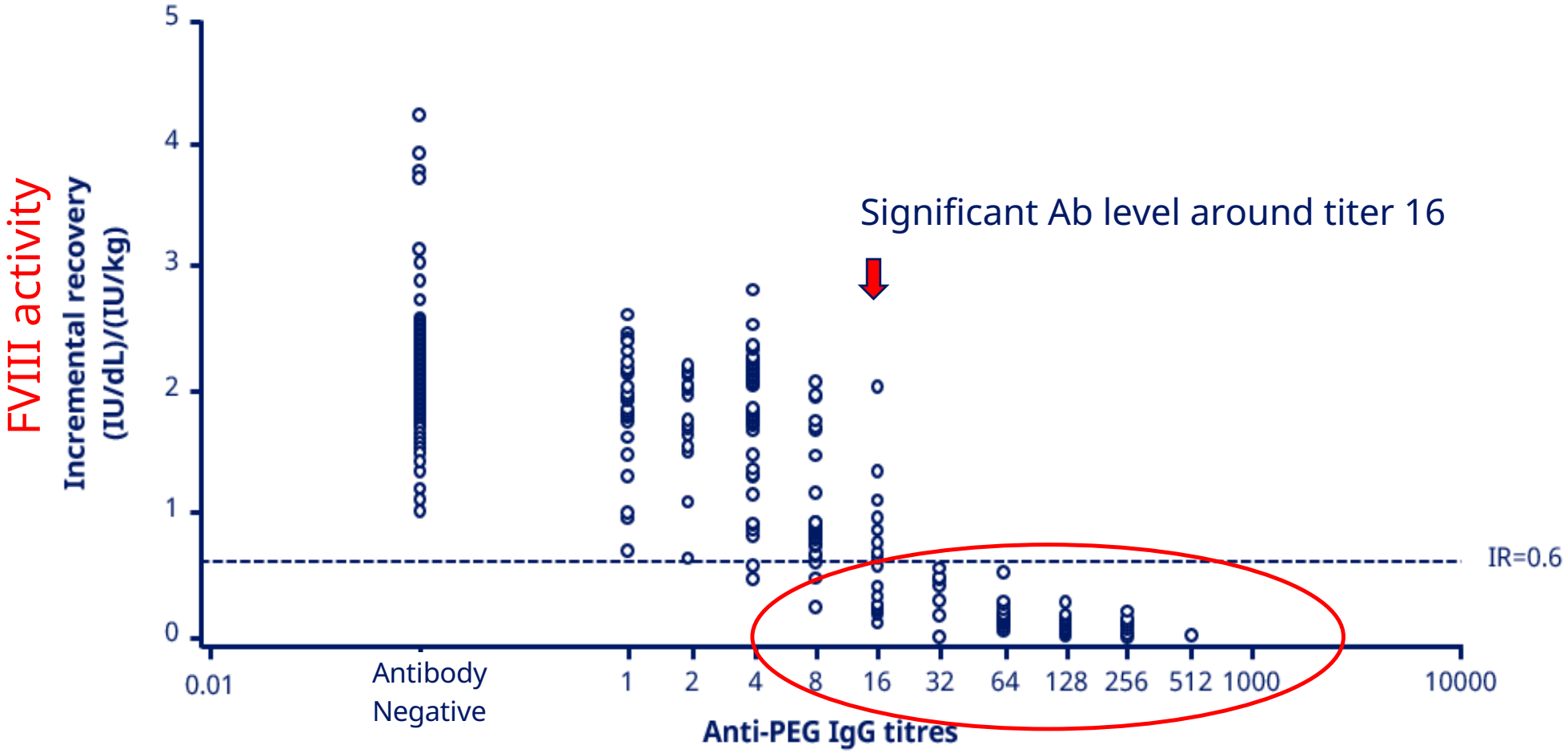


Low FVIII activity does not correlate to presence of FVIII antibodies

Titre without MRD of 7

Ref: Male et al., Blood Advances 2022

Anti-PEG IgG vs FVIII activity



Low FVIII activity correlates to presence of Anti-PEG IgG antibodies

Titre without MRD of 10

Ref: Male et al., Blood Advances 2022

Anti-PEG IgG antibodies increased clearance of the compund

In vitro experiments of Bab positive samples indicated that

- Anti-PEG IgG antibodies did not neutralize the FVIII activity
- The *in vivo* reduction FVIII activity way caused by **accelerated blood clearance** and removal of anti-PEG IgG/drug complexes from circulation

Furthermore, samples positive for neutralizing antibodies (inhibitors) also showed reduced FVIII activity measurements (PK assay)

This suggests that the PK assay (FVIII activity) was sufficient for detection of both clearing and neutralizing antibodies

Case 1 – Integrated data approach superior in understanding the lack of FVIII activity

- NAb assay detected approx. 50% of patients with decreased FVIII activity

Integrated data approach

- PK assay (FVIII activity) was a good indicator of the action of both neutralising and clearing antibodies
- Binding antibody assays helped identifying to which part of the molecule the antibodies were directed
- Clinical efficacy (bleeds) was possible affected by the transient clearing antibodies but no lasting clinical impact was observed

For doctors and patients the PK assay rather than anti-drug antibody assays was most useful for monitoring the patients

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Case 2 – Integrated data approach sufficient for BLA approval without *in vitro* nAb data, literature/data based

Drug is a bi-valent MAb for Haemophilia A

Integrated data approach for ph1-2

ADA assay is an drug tolerant ECL bridging assay

PK assays are based on:

- Concentration of drug
- Chromogenic activity of drug

PD assay is well established in the haemophlia field:

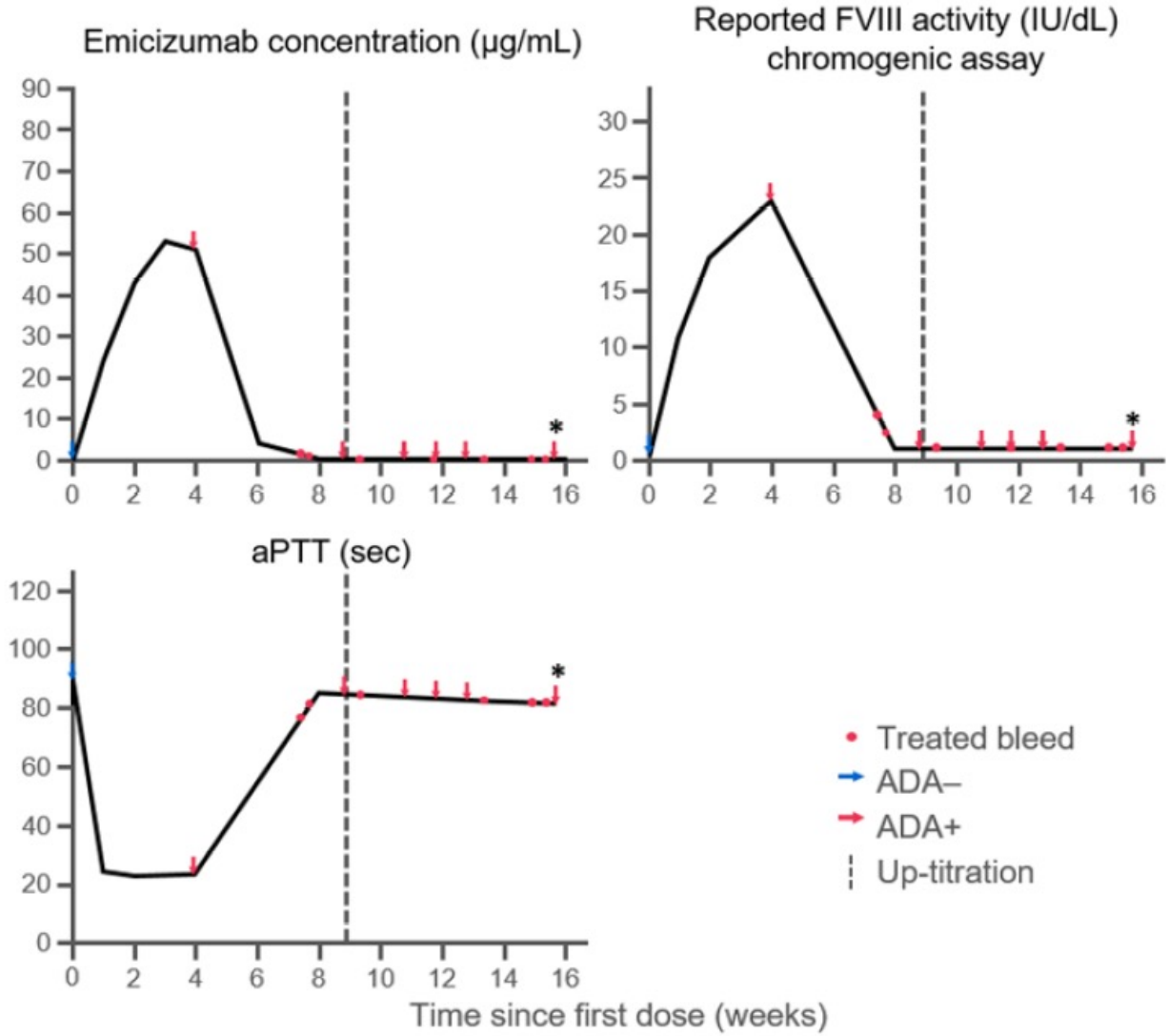
- APTT (activated partial thromboplastin time), 'clotting time'

Clinical effect: Bleeds and safety parameters

Sampling in parallel for all three assay types

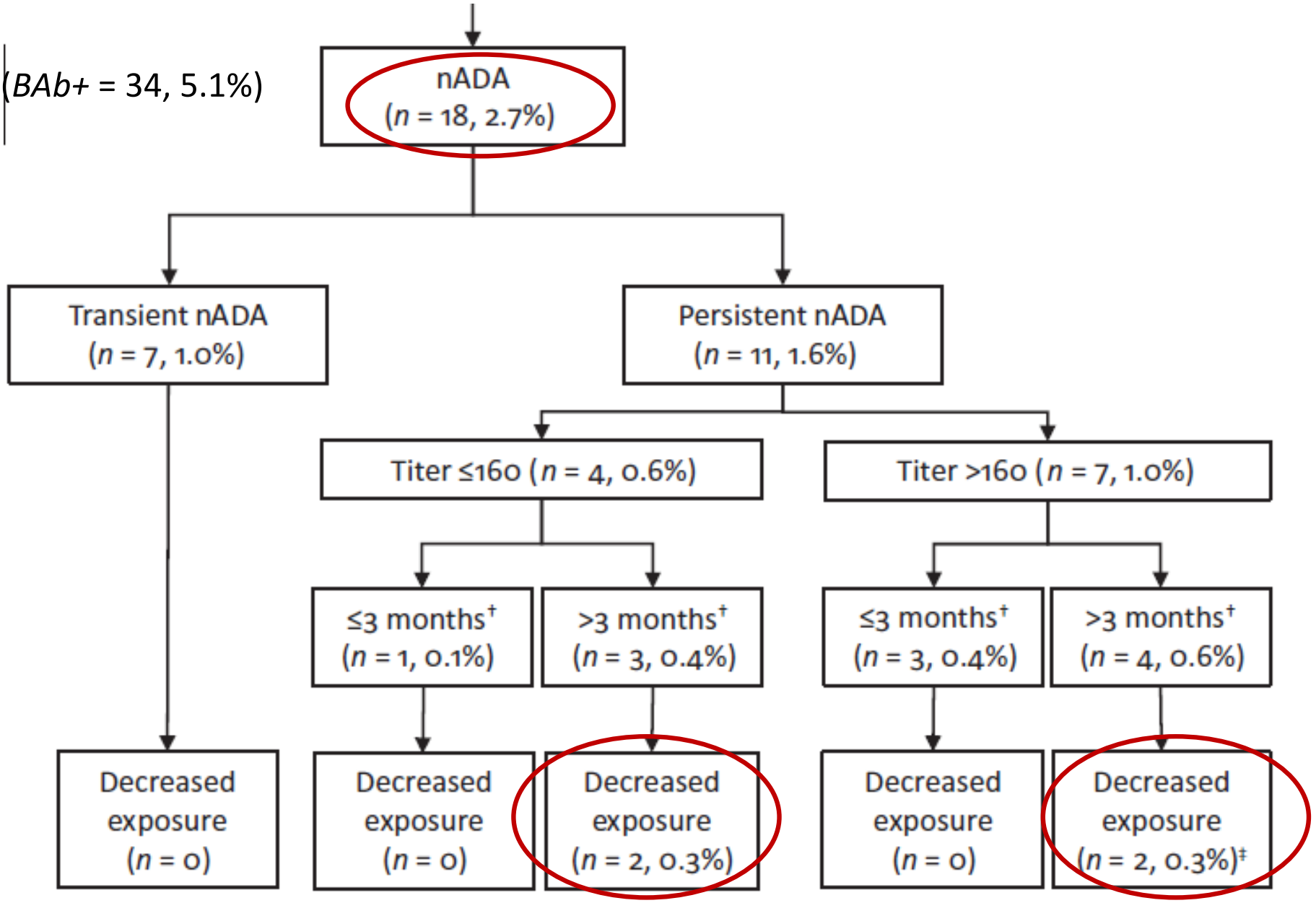
No ADAs up to 12w

Literature example with a drug with similar mode of action



Ref. 1: Schmitt et al. 2021-Heamophilia; 2021;1-9 & Ref. 2: Young et al. 2019-Blood-Dec 12;134(24):2127-2138

Only 4 of 18 *in vitro* Nab+ had reduced exposure



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Case 2– BLA submission without *in vitro* Nab was accepted

- Neutralising effect of ADAs assessed based on well established readouts for PK and PD
- Simultaneous assessment of PK, PD, ADA
  - Banking of ADA samples

- Literature describing:
  - The relationship between PK, PD, clinical relevant ADA
  - The inconsistency between *in vitro* NAb and exposure

Regulatory feedback:

The Agency agreed that evaluation of neutralizing effect based on PK and PD data was sufficient for BLA approval. The need for development of a neutralising assay post-approval will be based on the phase 3 data

Sufficient validation of the bioanalytical method for both PK and PD assays was emphasized given the critical role of PK and PD measurements in informing the neutralizing nature of ADA

Case 1 & 2 – Take Home

- In both examples, PK & PD was shown to be superior to *in vitro* NAb in determining clinical relevant ADA

- Case 2 illustrates that in the presence of strong evidence, regulators may agree to an *in vitro* NAb free BLA approval. Post commitment *in vitro* NAb analysis may be required.

- Urge everyone to present case stories to help facilitate the debate between the regulators and the industry: if, when and how *in vitro* neutralising assays are clinical relevant

Thank you for your time