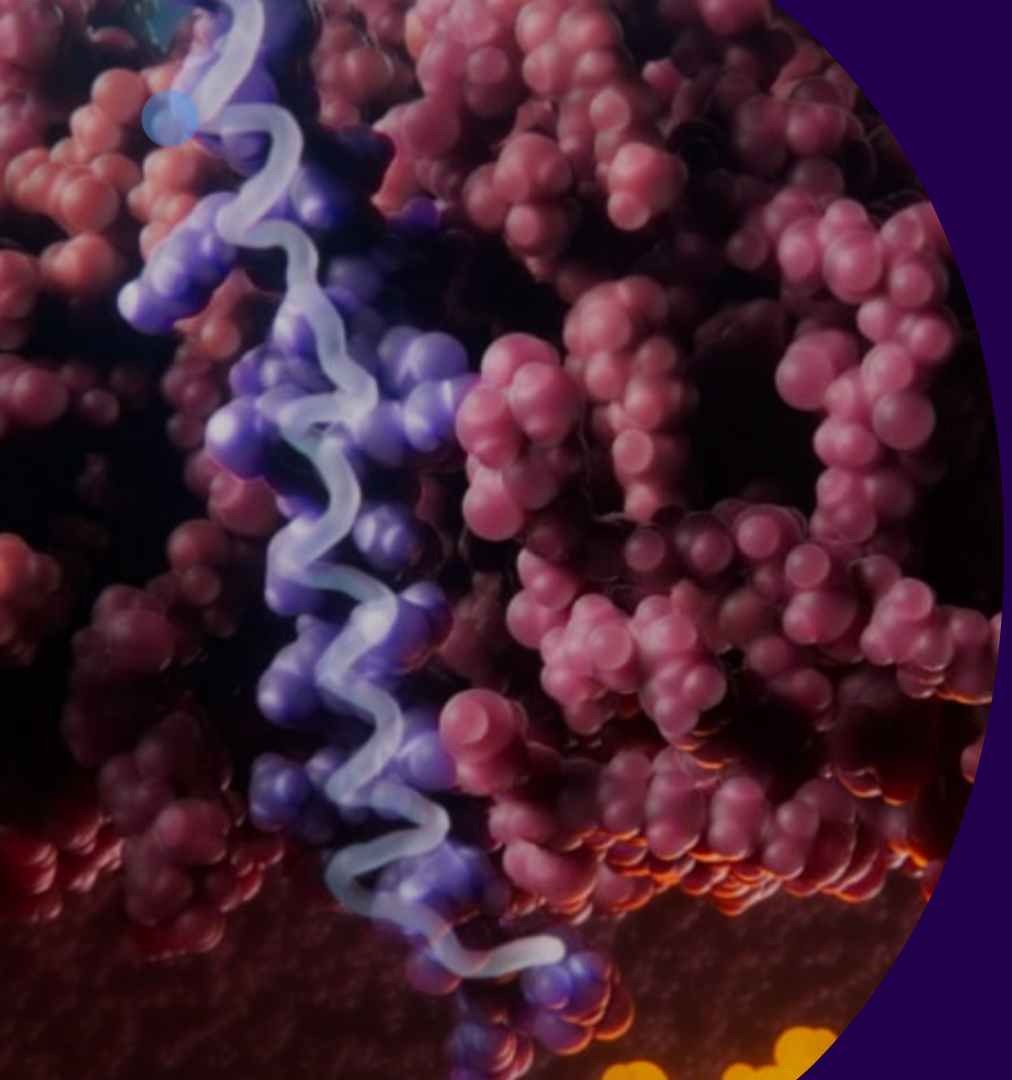


sanofi

Applying in-study cut-points in a rare-disease setting

EBF Spring Workshop 2022:

Points to Consider on Cut-Points

Brendy Van Butsel

28 APR 2022

Presentation Overview

1 What is a NANOBODY® molecule?

2 Caplacizumab

3 Applied assay strategy for immunogenicity assessment in clinical trials conducted in aTTP patients

4 Approach for cut-point assessment

5 Approach for in-study cut-point assessment

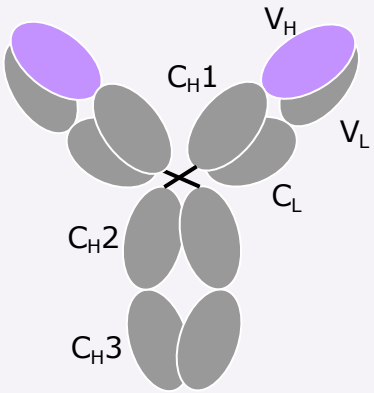
6 Case study

7 Conclusions

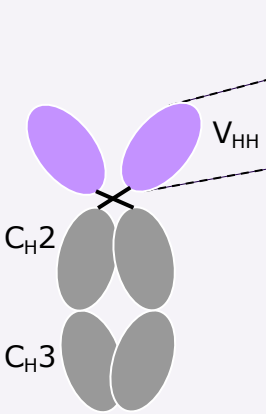
aTTP, acquired thrombotic thrombocytopenic purpura

What is a NANOBODY® molecule?

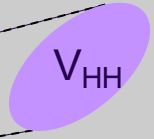
Introduction - Caplacizumab



Conventional antibodies



Heavy chain only antibodies



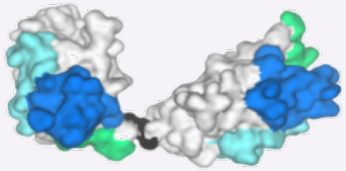
12-15kDa

Ablynx' Nanobody® molecules

- small and robust
- easily linked together
- sequence homology comparable to humanized/human mAbs
- nano- to picomolar affinities
- able to bind and block challenging targets
- multiple administration routes
- manufactured in microbial cells

Bivalent anti-vWF Nanobody® molecule (28kD) for the treatment of aTTP

Cablivi® caplacizumab



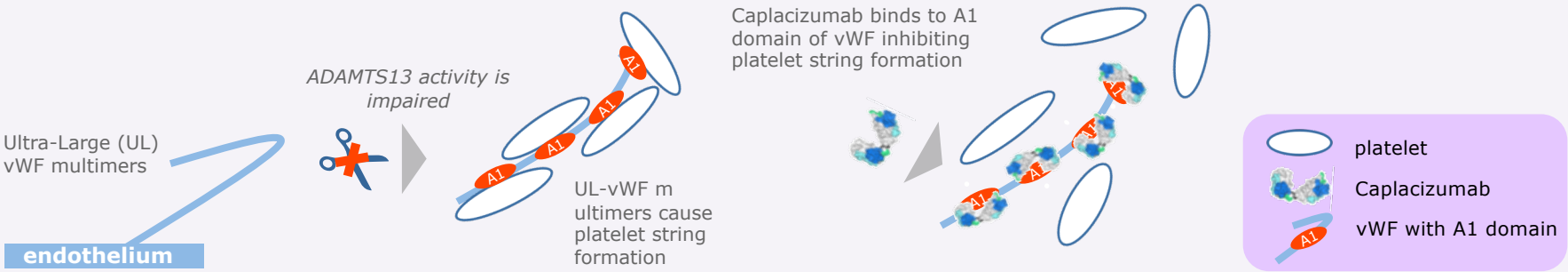
anti-vWF Nanobody® molecule — linker — anti-vWF Nanobody® molecule

vWF, von Willebrand factor; NANOBODY® is a registered trademark of Sanofi or an affiliate.

Caplacizumab

Anti-vWF activity to treat aTTP

- aTTP is an ultra-rare, life-threatening autoimmune blood clotting disorder (2-6 cases per million in Europe/US)
- High unmet medical need with no previously approved therapeutic drug
- To date 10 clinical trials have been conducted with (n=144 1:1 treatment: placebo in pivotal Ph3)

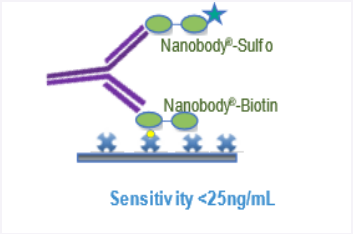
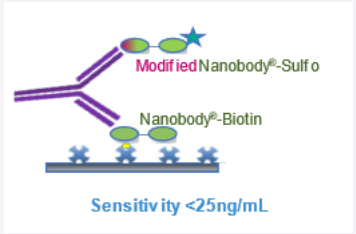
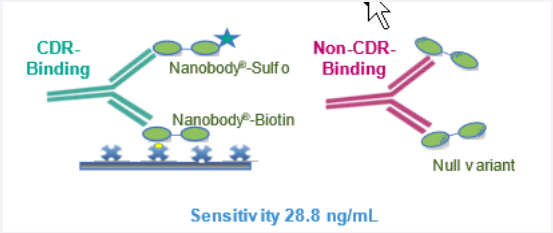
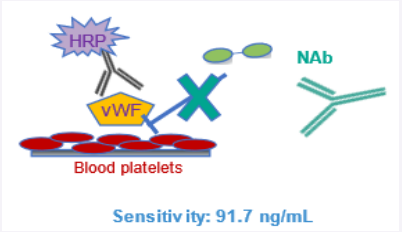


Caplacizumab's unique mode of action blocks binding of vWF to platelets which has an immediate effect on platelet aggregation and the ensuing micro-clot formation

ULvWF, Ultra-large von Willebrand Factor; ADAMTS13, a disintegrin and metalloprotease with thrombospondin type 1 motif, member 13. Figure adapted from M.L. Sargentini-Maier et al. 2019

Complex Immunogenicity Assay Strategy in Studies for aTTP

- Observed pre-existing ADA for caplacizumab ranged from 4-63% in various populations.
- Plasma exchange (SoC) complicates interpretation and required additional method(s).
- NECA introduced to increase drug tolerance and sensitivity of NAb characterization.

ADA assay	mADA assay	NECA	NAb assay
			
Sensitivity <25ng/mL	Sensitivity <25ng/mL	Sensitivity 28.8 ng/mL	Sensitivity: 91.7 ng/mL

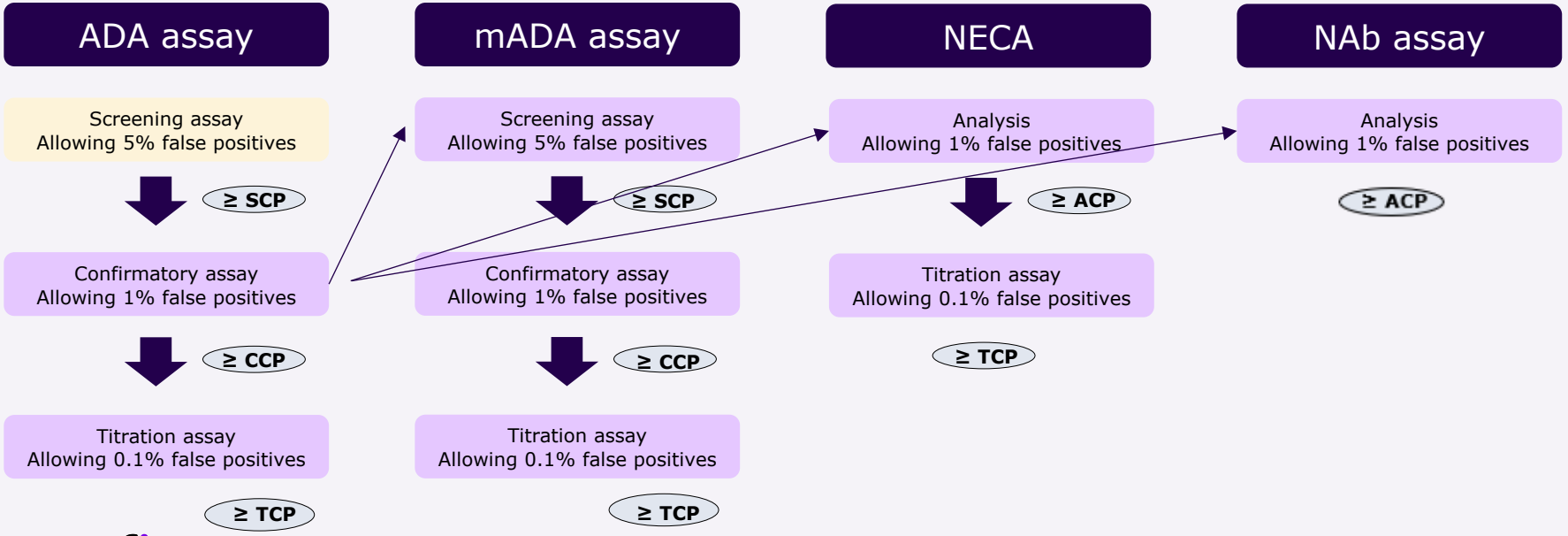
Analysis of ADA response

Characterization of ADA response (positive ADA samples)

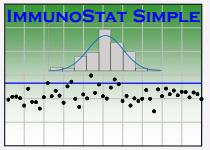
SoC: Standard of Care; ADA: anti-drug antibody; mADA: modified anti-drug antibody; NECA: neutralizing epitope characterization assay; NAb: neutralizing antibody

Full tiered approach for immunogenicity assessment

- Standard tiered approach for ADA confirmed positives → characterize (titer, mADA, NAb/NECA)
- Additional titering of mADA & NECA provides information for potential impact based on magnitude of ADA/NAb response



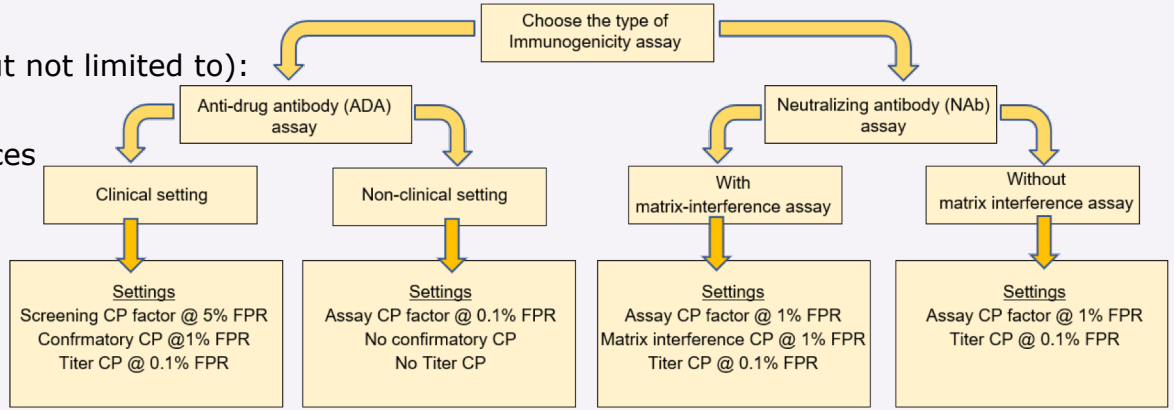
Approach for cut-point assessment



Cut-point assessment using a fully automated JMP tool – ImmunoStat Simple

- Immunostat simple was developed, validated and implemented within Sanofi (presented at 13th EBF OS)
  - Based on the latest recommendations by regulators
  - Allows harmonized and fully regulatory compliant cut-point determination within Sanofi
  - Extremely user friendly for bioanalytical scientists via tick-box menus
  - Generates a compliant PDF report containing the cut-point assessments

- Extra options for data analysis include (but not limited to):
  - Suitability of the negative control
  - Assessment on inter-operator differences
  - Different outlier removal approaches
  - Pre-exclusion of sample data based on specificity assay
  - Adaptable false positive rate (FPR)
  - Add other co-variates, e.g. plate lot



Approach for cut-point assessment

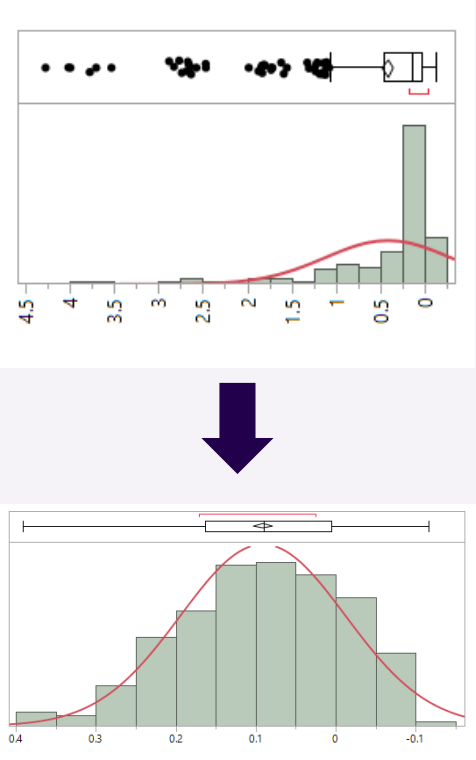
Statistical approach

- Cut-points need to be set on a treatment naïve population
  - Both analytical and biological outliers are identified and excluded based on mixed-effects model. Per default, an iterative outlier approach based on Tukey's outlier criterion is applied

- Assessment performed on both log and untransformed normalized assay responses

- The response (log or untransformed) chosen for CP determination is based on the distributional properties of the respective blank population

- CP are determined based on the normality properties of the chosen response via a parametric, robust parametric or non-parametric approach



Approach for in-study cut-point assessment

When is it needed?

- Cut-points set during validation/study may not be representative for another study
  - Demography of the subjects, medical history, disease state, race, sample collection/storage conditions,...
  - Long clinical trials → change in assay reagents/materials

- Representativeness of the validation cut-point should be demonstrated – otherwise the use of in-study cut-points should be considered
  - Generally recommended/feasible for most phase II clinical trials and later
  - However, it is advised to evaluate the representativeness of the validation CP if deemed necessary and feasible (e.g. for clinical phase I or non-clinical trials)

Approach for in-study cut-point assessment

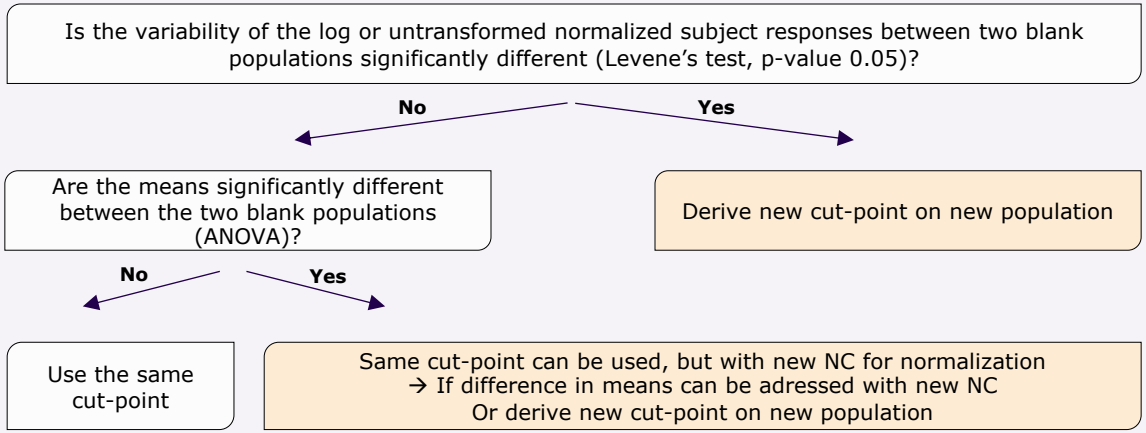
How is it done?

Pre-study validation cut-point setting	In-study cut-point setting
• Analysis of a representative dataset (ethnicity, gender, race, demography,...)	• Analysis of a representative dataset (pre-dose samples) for a specific study
• Analyze > 50 subject samples	• Preferably, analyze > 50 subject samples
• Each subject sample is analyzed 6x (i.e. 3x by 2 operators) in a balanced design	• Each subject sample is analyzed 2x, 1x by 2 operators
• Include all types of variability	• Include most important types of variability (run & analyst variation)
• Calculated using ImmunoStat Simple	• Calculated using ImmunoStat Simple

Approach for in-study cut-point assessment

How do we check whether a validation cut-point is representative for the study population?

1. Main driver: Comparison of variances and means of both blank populations (Devan et al.*)
   - Performed for both blank screening and confirmatory datasets (if applicable)
   - Comparison is to be performed on the log-transformed dataset except when both blank populations show a more normal distribution on the untransformed dataset



*Devan et al. Recommendations for Systematic Statistical Computation of Immunogenicity Cut-Points, *AAPS Journal*, Vol. 19, No. 5, September 2017

Approach for in-study cut-point assessment

How do we check whether a validation cut-point is representative for the study population?

2. Supportive: Assessment of the FPR of a cut-point towards the study population
   - Assessed on the blank population (without outliers)
   - The FPR of a SCP targeted at 5% false positives can vary between 2 – 11% (Devan/Monte-Carlo simulation):
     - FPR calculated, falling outside these limits can trigger study specific CP setting
   - In case of a screen & confirmatory tier, solely performed for the screening dataset
   - FPR range does not apply for a CP targeted at 1% false positives (i.e. NECA & NAb assay)

Approach for in-study cut-point assessment

Consequence

- When in-study cut-points are applied, critical assay characteristics defined during method validation must be re-evaluated.

Validation parameter	Non-clinical	Clinical		
	ADA assay	ADA/mADA assay	NAb assay	NECA
Assay sensitivity	x	x	x	x
Selectivity	N/A	x	x	x
Precision of PC and NC samples in the screening assay set-up	x	x	x	x
Precision of PC and NC samples in the confirmatory assay set-up	N/A	x	optional	N/A
Drug tolerance	x	x	x	X
Target interference	N/A	x	x	x

Case study – Immunogenicity assays CP determination for a Phase II/III clinical trial

- Outline
  - A phase II/III clinical trial was conducted in 21 Japanese aTTP patients
  - A CP was set during validation on healthy commercial Japanese subject samples for the different immunogenicity assays to be applied during the clinical trial
    - ADA, mADA, NECA and NAb assay
  - For in-study justification, pre-dose samples were analyzed twice by 2 different operators over 2 analyses

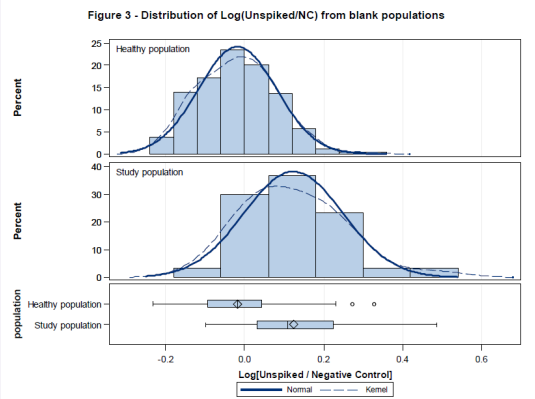
- Aim
  - Evaluation on representativeness of the validation CP towards the study population
    - Comparison of the blank validation and study population in terms of means & variances
    - In-study CP were calculated simultaneously
    - Performed for both log-transformed screening and confirmatory (if applicable) dataset

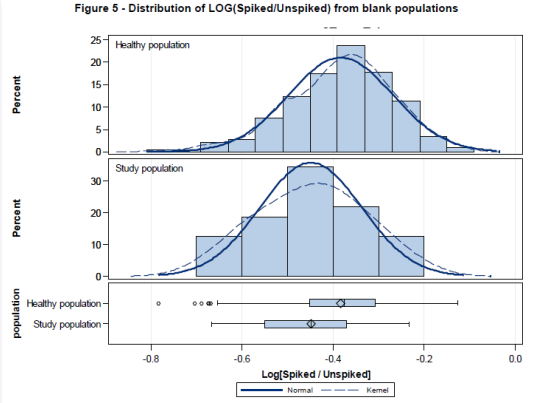
Case study – Immunogenicity assays CP determination for a Phase II/III clinical trial

- Representativeness of the **ADA assay** validation CP(F) on the study population

Assay	ADA Screening assay	ADA confirmatory assay
Variances (p-value 0.05)	0.0591	0.9436
Means (p-value 0.05)	<0.0001	0.0021
FPR* (Validation CPF)	46.7%	NA
Validation CP(F)	1.150	49.22%
In-study CP(F)	1.391	50.78%

*FPR preferably between 2 and 11% (Devan/Monte-Carlo simulation)





Case study – Immunogenicity assays CP determination for a Phase II/III clinical trial

- Representativeness of the validation CP(F) towards study population

Assay	ADA Screening assay	ADA confirmatory assay	mADA screening assay	mADA confirmatory assay	NECA assay	NAb assay
Variances (p-value 0.05)	0.0591	0.9436	0.1219	0.0164	<0.0001	0.5316
Means (p-value 0.05)	<0.0001	0.0021	<0.0001	<0.0001	<0.0001	<0.0001
FPR* (Validation CPF)	46.7%	NA	11.8%	NA	0%	21.1%
Validation CP(F)	1.150	49.22%	1.054	28.47%	1.262	1.243
In-study CP(F)	1.391	50.78%	1.083	38.85%	1.216	2.057

*FPR of SCP preferably between 2 and 11% (Devan/Monte-Carlo simulation). Not applied for NECA and NAb assay

Conclusions

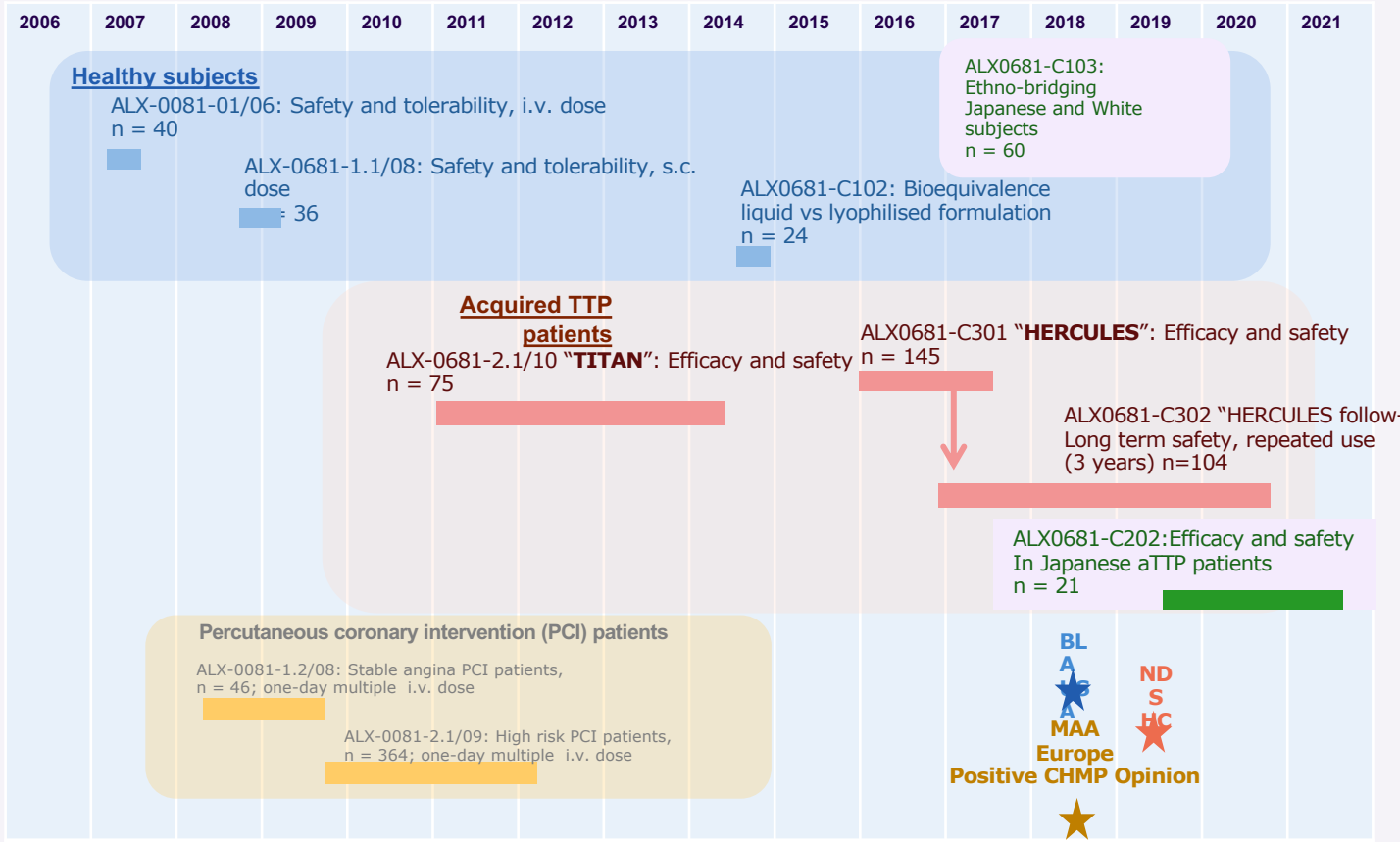
- Representativeness of the validation cut-points should also be demonstrated in clinical studies with a limited number of samples.

- In case in-study cut-points need to be applied:
  - Applicable for the whole tier (i.e. screening, confirmatory and titration; if applicable)
  - Critical assay characteristics need to be re-assessed

- A big thank you to all the study participants and the whole caplacizumab team.

Back-up slides

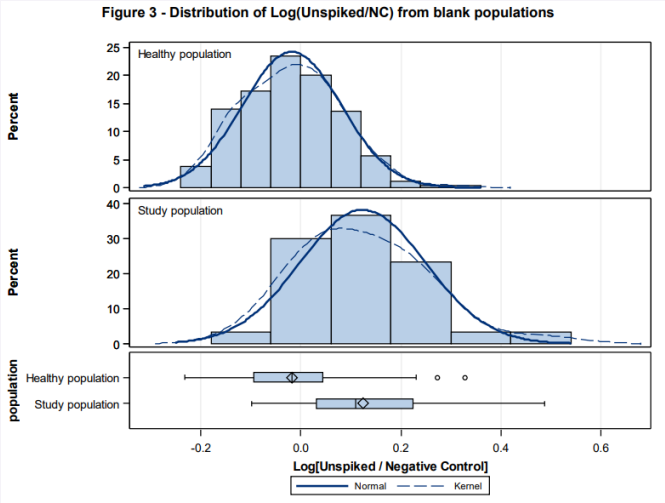
Caplacizumab clinical development program



* Study timelines: first subject in – last patient out

ADA screening assay CP determination for a Phase II/III clinical trial

Assessments	Validation	In-study
CPF (5% FPR)	1.150	1.391
CPF (0.1 % FPR)	1.298	1,667
CCP (1 % FPR)	49.22	50.78
FPR on study population	46.7%	
Variances	0.0591	
Means	<0.0001	
Population	Healthy Japanese	aTTP Japanese

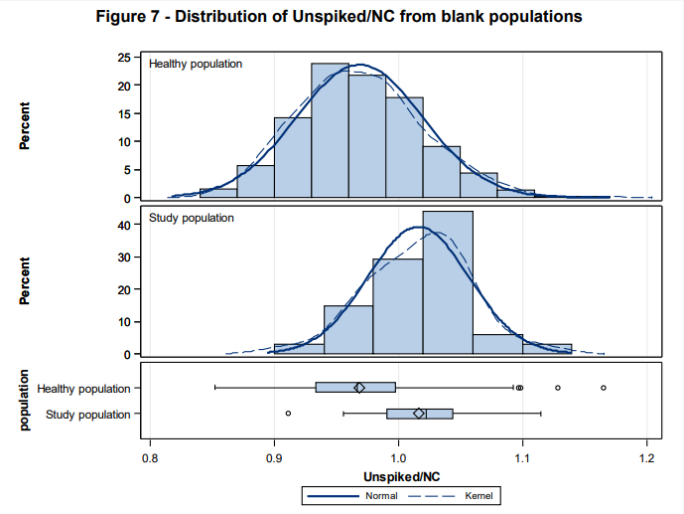


- Significant differences in means noted
- → Use in-study CPF

  Consequence: Re-calculation of all validation parameters (assay sensitivity, selectivity, …) using the new in-study specific CPs

mADA screening assay CP determination for a Phase II/III clinical trial

Assessments	Validation	In-study
CPF (5% FPR)	1.054	1.083
CPF (0.1 % FPR)	1.136	1.142
CCP (1 % FPR)	28.47	38.85
FPR on study population	11.8%	
Variances	0.1219	
Means	<0.0001	
Population	Healthy Japanese	aTTP Japanese

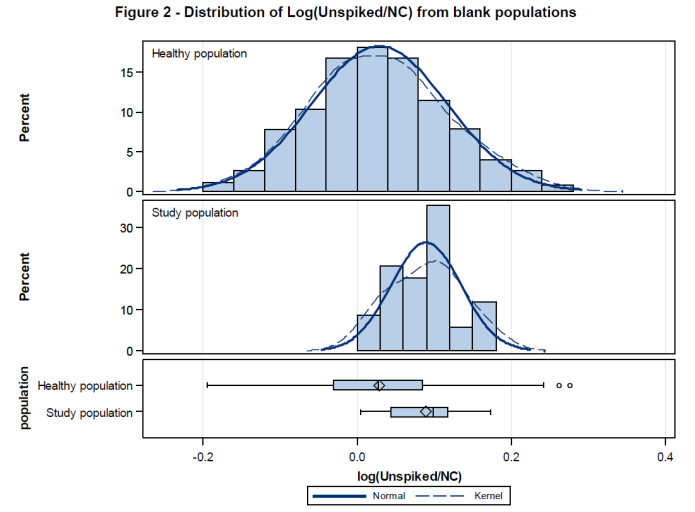


- Significant differences in means noted
- → Use in-study CPF (for the different tiers)

  Consequence: Re-calculation of all validation parameters (assay sensitivity, selectivity, …) using the new in-study specific CPs

NECA CP determination for a Phase II/III clinical trial

Assessments	Validation	In-study
CPF (1% FPR)	1.262	1.216
CPF (0.01 % FPR)	1.349	1.259
FPR of study population	0.0 %	-
Variances	< 0.0001	
Means	< 0.0001	
Population	Healthy Japanese	aTTP Japanese

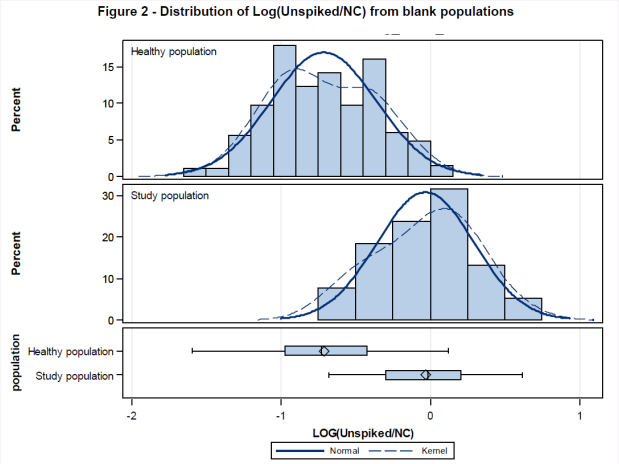


- Significant differences in variances and means noted
- → Use in-study CPF (for the different tiers)

  Consequence: Re-calculation of all validation parameters (assay sensitivity, selectivity, …) using the new in-study specific CPs

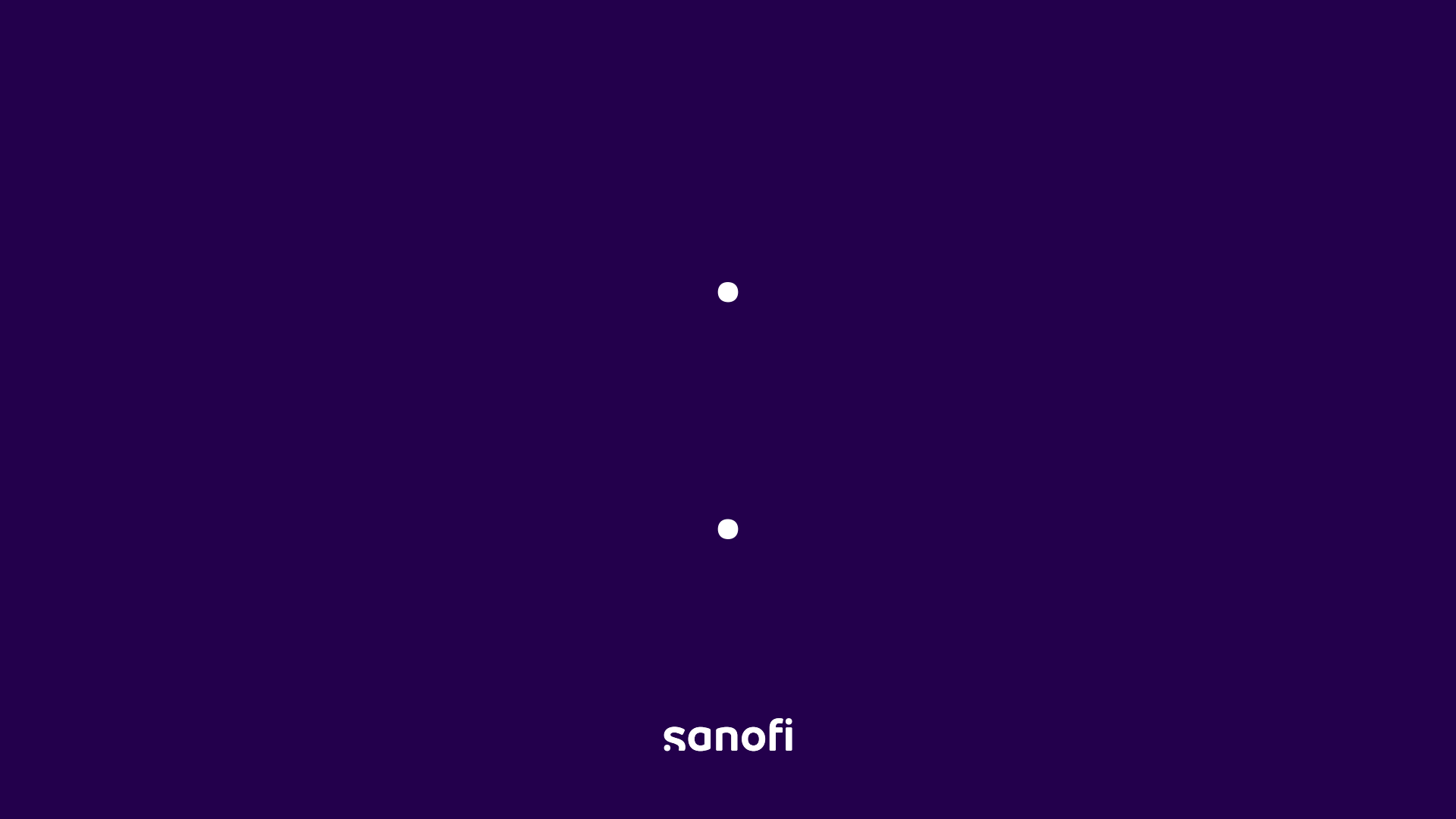
NAb assay CP determination for a Phase II/III clinical trial

Assessments	Validation	In-study
CPF (1% FPR)	1.243	2.057
FPR of study population	21.1%	-
Variances	0.5316	
Means	< 0.0001	
Population	Healthy Japanese	aTTP Japanese



- Significant differences in means noted
- → Use in-study CPF (for the different tiers)

  Consequence: Re-calculation of all validation parameters (assay sensitivity, selectivity, …) using the new in-study specific CPs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