NUVISAN

Cut-Point Limbo - Low Cut-Points and Their Challenges

21-Sep-2023

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Introduction

Cut-Point Limbo

From a bioanalytical point of view, a lot of effort has been put into improving the sensitivity and drug tolerance of ADA assays, which is often achieved by pre-treatment processes

→ pre-treatment processes make the assay cleaner but this reduces the variance and leads to increasingly lower cut-points close to background noise

→ low cut-points lead to higher positivity rates

In this case study from a biosimilar development program the challenges and consequences of low cut-points will be highlighted

1) **Challenges for the bioanalysis of study samples**
2) **Impact in clinical studies**

Background Information

Clinical Study AVT04-GL-301

- **A Randomized, Double-blind, Multicenter Study to Demonstrate Equivalent Efficacy and to Compare Safety and Immunogenicity of a Proposed Biosimilar Ustekinumab (AVT04) and Stelara® in Patients With Moderate to Severe Chronic Plaque-type Psoriasis**
- **This study compared efficacy, safety, tolerability, pharmacokinetics (PK) and immunogenicity between AVT04 and the reference product**
- **Clinical Phase: 3**
- **Sponsor: Alvotech Swiss AG**
- **Publication: https://doi.org/10.1080/14712598.2023.2235263**

Background Information

Ustekinumab (Stelara) / AVT04

- **Fully human IgG1k monoclonal antibody**
- **Binds to IL-12 and IL-23 via their common p40 protein subunit; inhibits IL-12 and IL-23 by preventing p40 from binding to the IL-12R1 receptor protein expressed on the surface of immune cells and modulating the Th1 and Th17 cytokine pathways**
- **IL-12 and IL-23 dysregulation has been linked to psoriasis and other inflammatory diseases**
- **AVT04: proposed biosimilar to Stelara**

Bioanalysis of Study Samples

Summary of Assay Performance – Binding Antibodies (LBA)

Screening / Titration Cut Point

- Ratios of the signals were used for cut point calculations; one round of outlier exclusion was performed
- Data showed normal distribution, means were different, variances were similar
- Correction Factor MCF (Screening): 1.04; observed FPR: 8.0%
- Correction Factor MCF (Titration 0.1%): 1.10

Confirmatory Cut Point

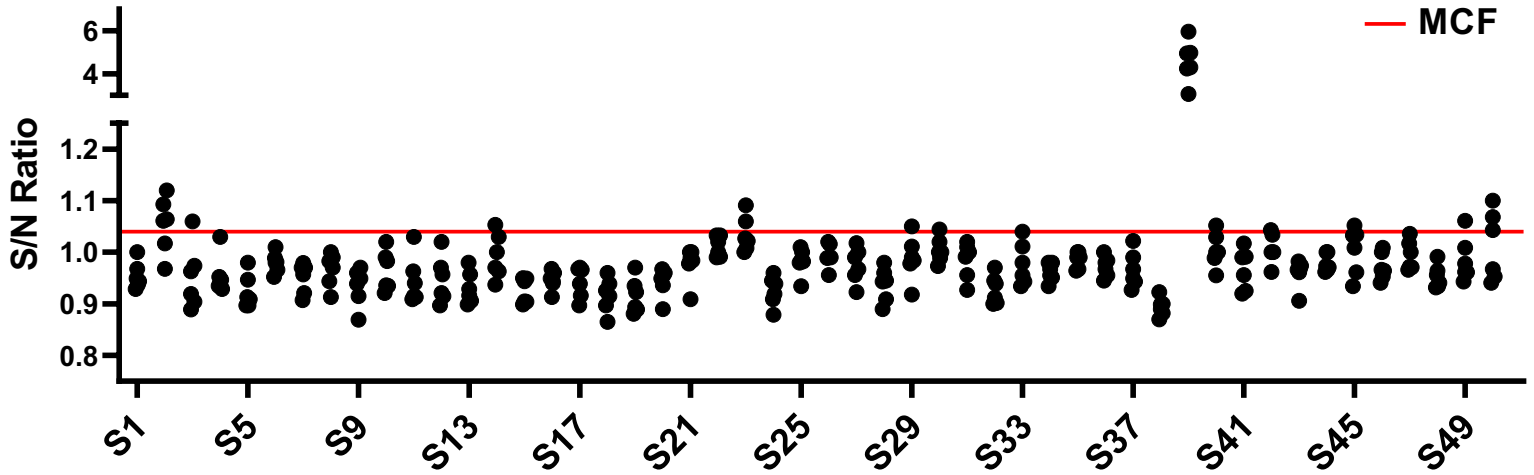
- Inhibition rates were used for calculating the confirmatory cut point; one round of outlier exclusion was performed
- Inhibition data showed normal distribution.
- Confirmatory Cut Point: 12.24%; observed FPR: 4.3%

Drug Tolerance

- 22.9 µg/mL at 500 ng/mL PC
- 7.2 µg/mL at 100 ng/mL PC

Sensitivity

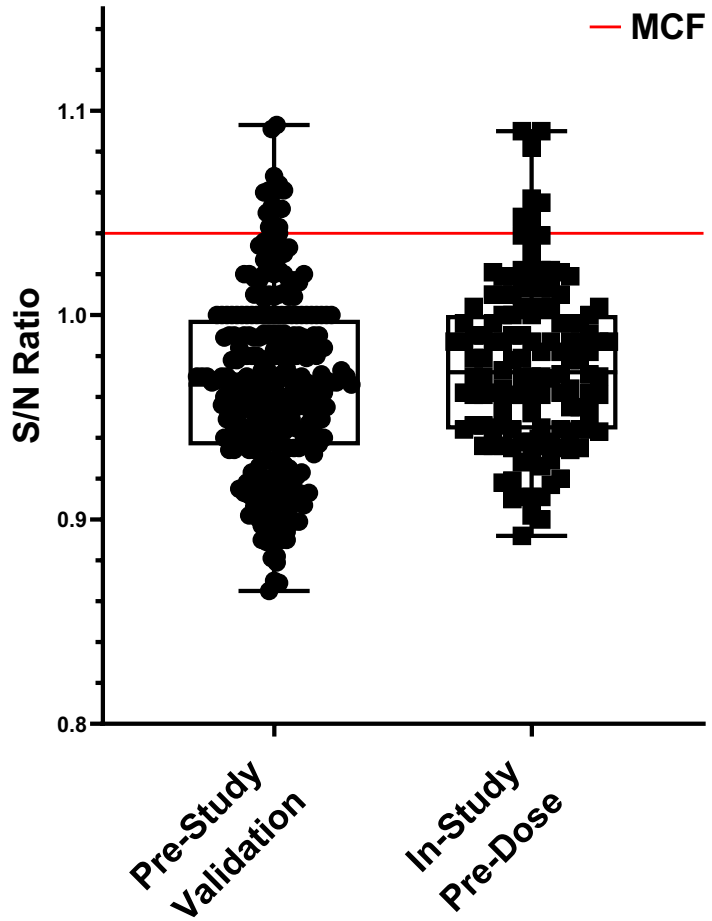
1.5 ng/mL



Calculation of an In-Study Cut Point

	Pre-Study Validation	In-Study Pre-Dose
n	292 (8 OL excluded)	134 (15 OL excluded)
Indication	serum from patients with PSO	serum from patients with PSO
Measurements	6x	1x
Runs	18	6
Analysts	2	2
Design	Balanced	Unbalanced
CF	**1.04**	**1.04**
FPR	8.0 %	14.8 %

(M)CF: (Multiplicative) correction factor
FPR: False positive rate



Summary of Sample Analysis – Screening / Confirmatory Assay

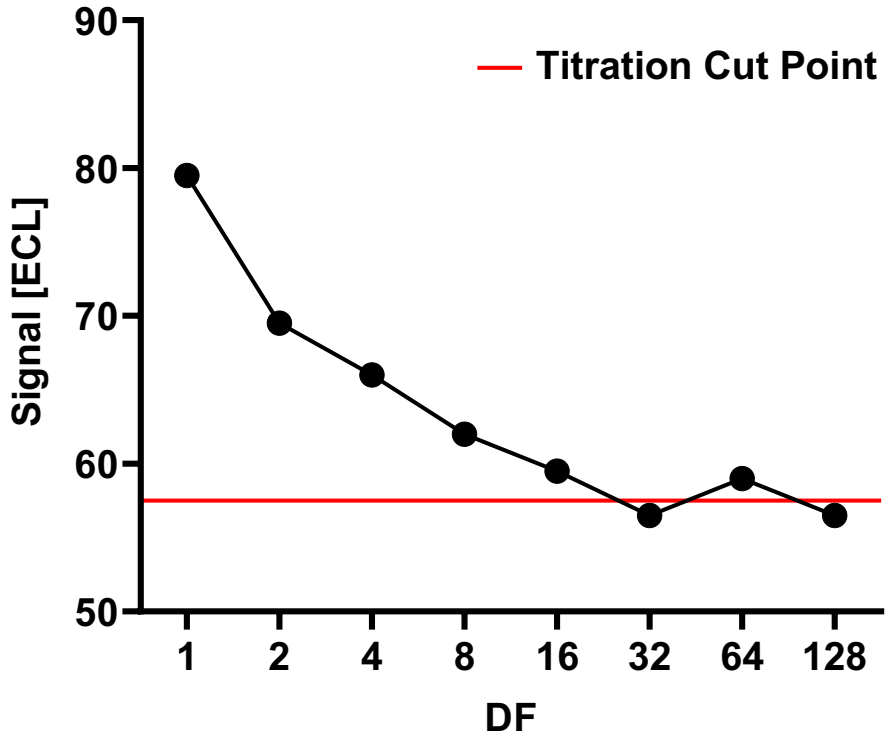
Visit #	Total number (n) of samples	n screening positive samples	% screening positive samples	n confirmed positive samples	% confirmed positive samples
Day 1	582	192	33.0	14	7.3
Week 4	581	373	64.2	102	27.3
Week 12	578	408	70.6	190	46.6
Week 16	575	421	73.2	233	55.3
Week 28	556	335	60.3	179	53.4
Week 40	551	303	55.0	164	54.1
Week 52	558	321	57.5	151	47.0
Unscheduled	8	6	75.0	2	33.3
All Visits	**3989**	**2359**	**59.1**	**1035**	**43.9**

Titration of Confirmed Positive Study Samples

MCF Titration 0.1%: 1.1

DF	Signal [ECL]	> TTR-CP 57.5
1	79.5	Yes
2	69.5	Yes
4	66.0	Yes
8	62.0	Yes
16	59.5	Yes
32	**56.5**	**No**
64	59.0	Yes
128	**56.5**	**No**

Titer indeterminate



MCF Titration < Assay Precision

Adjustment of MCF Titration

- Calculation of the precision limit from the validation data of the screening assays to the 99% level

- Adjustment of the MCF titration:
  MCF Titration ≥ Assay Precision

New MCF Titration: 1.2

Run	Set	NC [ECL Signal]	NC Intra-Run CV [%]
019	1	90	0.8
	2	91	
020	1	90	1.7
	2	91	
	3	88	
022	1	86	2.5
	2	83	
023	1	89	5.8
	2	82	
024	1	83	3.5
	2	79	
025	1	89	6.7
	2	81	
026	1	83	0.9
	2	82	
027	1	79	3.7
	2	75	
		Mean	**3.2**
		SD	**2.18**
		n	**8**
		t value (df, p=0.01)	**2.998**
		Precision limit [%]	**20.9**

Summary of Assay Performance – NAb (cLBA)

Cut Point

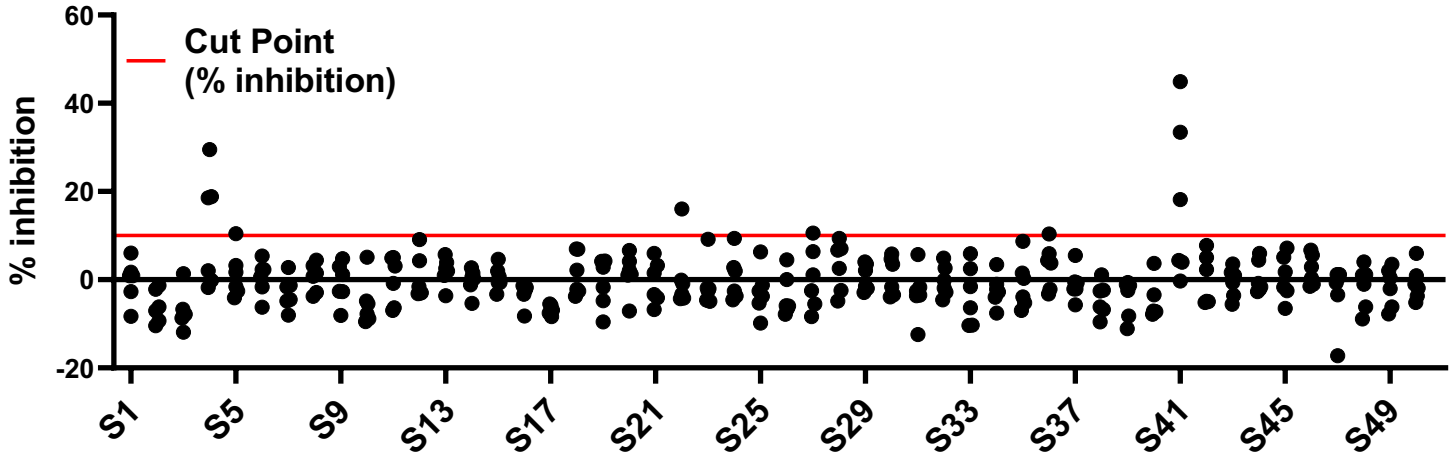
- % inhibition data was used for cut point calculations; one round of outlier exclusion was performed
- Data showed normal distribution, means were different, variances were similar
- Cut point (% inhibition): 10.02; observed FPR: 3.3 %
  (M)CF: 0.900

Drug Tolerance

- > 160 µg/mL at 1000 ng/mL PC
- > 160 µg/mL at 500 ng/mL PC
- 74.7 µg/mL at 100 ng/mL PC

Sensitivity

20.1 ng/mL



Titration of Confirmed Positive Study Samples

MCF Titration 0.1%: 1.1

DF	Signal [ECL]	> TTR-CP 57.5
1	79.5	Yes
2	69.5	Yes
4	66.0	Yes
8	62.0	Yes
16	59.5	Yes
32	56.5	No
64	59.0	Yes
128	56.5	No

Titer indeterminate

MCF Titration < Assay Precision

MCF Titration 0.1%: 1.2

DF	Signal [ECL]	> TTR-CP 62.7
1	79.5	Yes
2	69.5	Yes
4	66.0	Yes
8	62.0	No
16	59.5	No
32	56.5	No
64	59.0	No
128	56.5	No

Reported Titer: 4

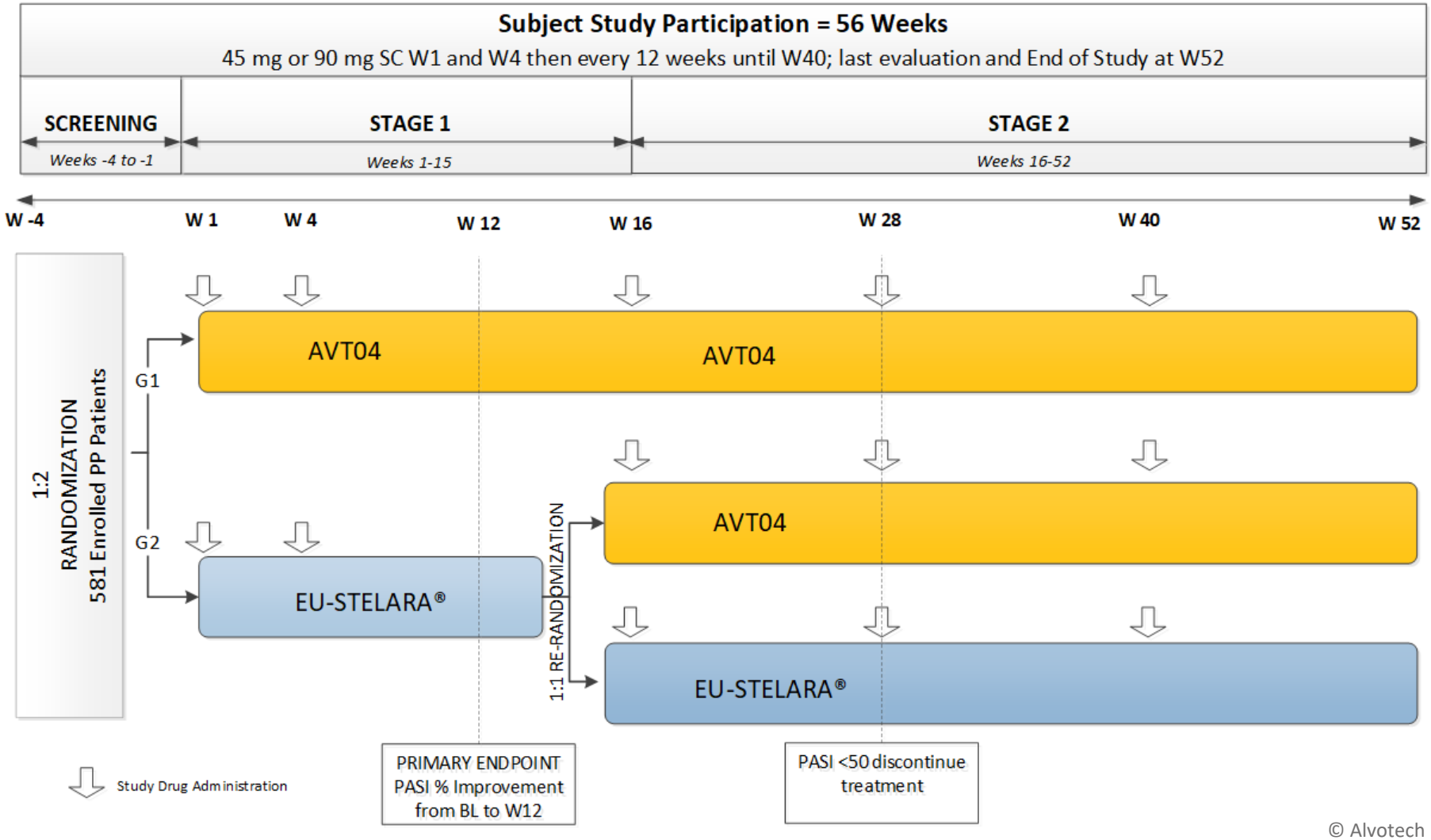
MCF Titration = Assay Precision

Summary of Sample Analysis – NAb Assay

Visit #	n confirmed positive samples	NAb positive samples	% NAb positive samples	Range for % inhibition min	Range for % inhibition max
Day 1	14	0	0.0	-3.18	3.93
Week 4	102	8	7.8	-10.25	48.88
Week 12	190	61	32.1	-10.99	96.92
Week 16	233	70	30.0	-10.38	95.65
Week 28	179	47	26.3	-16.93	96.23
Week 40	164	33	20.1	-13.10	97.79
Week 52	151	37	24.5	-15.01	97.86
Unscheduled	2	0	0.00	-0.85	2.52
All Visits	**1035**	**256**	**24.7**	--	--

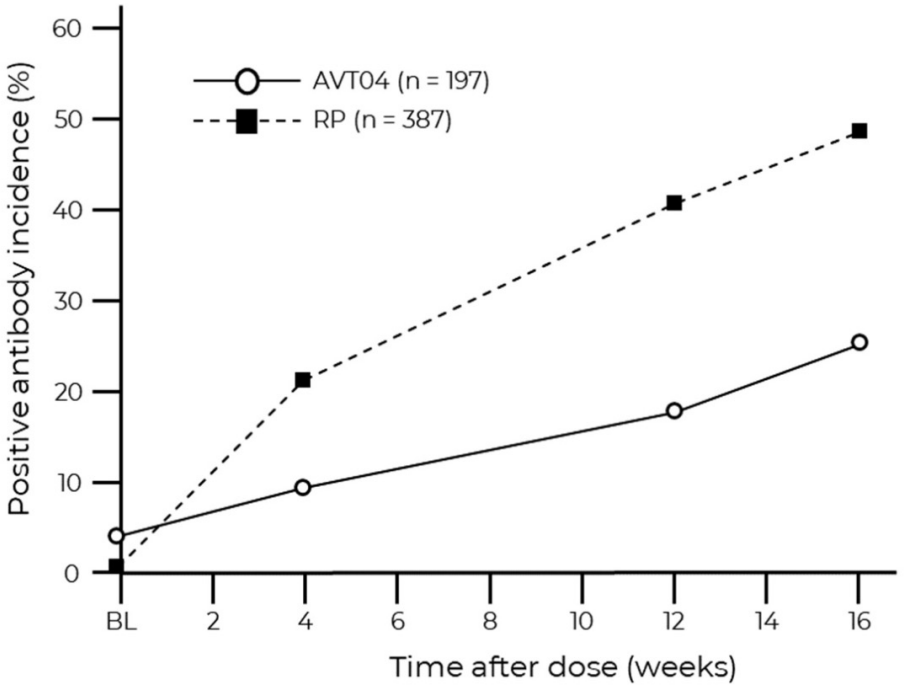
Impact in Clinical Studies

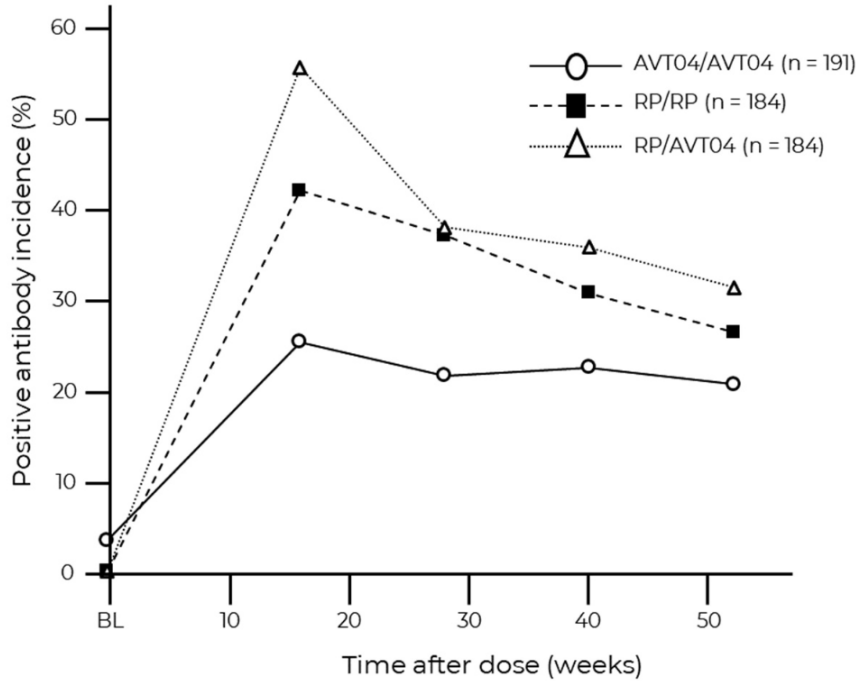
Study Design AVT04-GL-301



ADA / NAb Incidence of Ustekinumab in Previous Studies

ADA Incidence in Study AVT04-GL-301





- Lower ADA frequency in the AVT04 group was more marked up to Week 16
- At EOS, differences became more balanced between the treatment groups: AVT04/AVT04, EU-Stelara/AVT04 and EU-Stelara/EU-Stelara
- Median ADA titers were comparable between the groups at all time points up to EOS
- **Immunogenicity showed no clinical impact on efficacy, safety or PK**

Where Do These Differences in BAb / NAb Incidence Come From?

	Initial Submission	Study AVT04-GL-301
BAb Assay	Enzyme immunoassay, no acid dissociation	Bridging Assay (MSD), acid dissociation
Sensitivity	125 ng/mL	1.5 ng/mL
Drug Tolerance	0.007 µg/mL at 50 ng/mL PC	7.2 µg/mL at 100 ng/mL PC
NAb Assay	cell-based	cLBA (MSD) acid dissociation
Sensitivity	?	20.1 ng/mL
Drug Tolerance	?	> 160 µg/mL at 500 ng/mL PC

The reason for inconsistency in the reported drug immunogenicity in different studies is the difference in sensitivity / cut-points of the assays used!

Comparing the ADA / NAb status from the AVT04-GL-301 study to previous studies and the originator would be misleading because ADA detection is highly dependent on the cut point of the method used.

Summary

Strong effort in bioanalytic to maximize immunogenicity method performance, especially on two parameters: sensitivity and drug tolerance, translates into low assay cut-points

(e.g. 2016 US FDA recommendation to increase assay sensitivity from 250 – 500 ng/mL to 100 ng/mL)

→ Challenges for the Bioanalysis of Study Samples

- May lead to MCF < assay precision
- Low cut-points result in false positive rates above the regulatory expectation of 2 to 11 %
- Even very low positive samples are **identified as screening positive** and need to undergo the next tiers which makes sample analysis very time-consuming and expensive

  => Is the tiered approach still suitable when such sensitive assays are used?

→ Impact in Clinical Studies:

- results in an increased ADA / NAb incidence

  => comparison with previous studies may be misleading (problem for Biosimilar candidates!)

  => majority of antibodies detected by the higher sensitivity of the assay have no clinical relevance

Thank You For Your Attention!

Any questions? Feel free to ask!

Acknowledgement:

Alvotech Swiss

Dr. Hendrik Otto

Eveline Schurink, MD MPH

Nuvisan GmbH

Dr. Michaela Golob

Dr. Uwe Kärcher

Department Immunoassays