In-study CP setting in NAb assays - a case study

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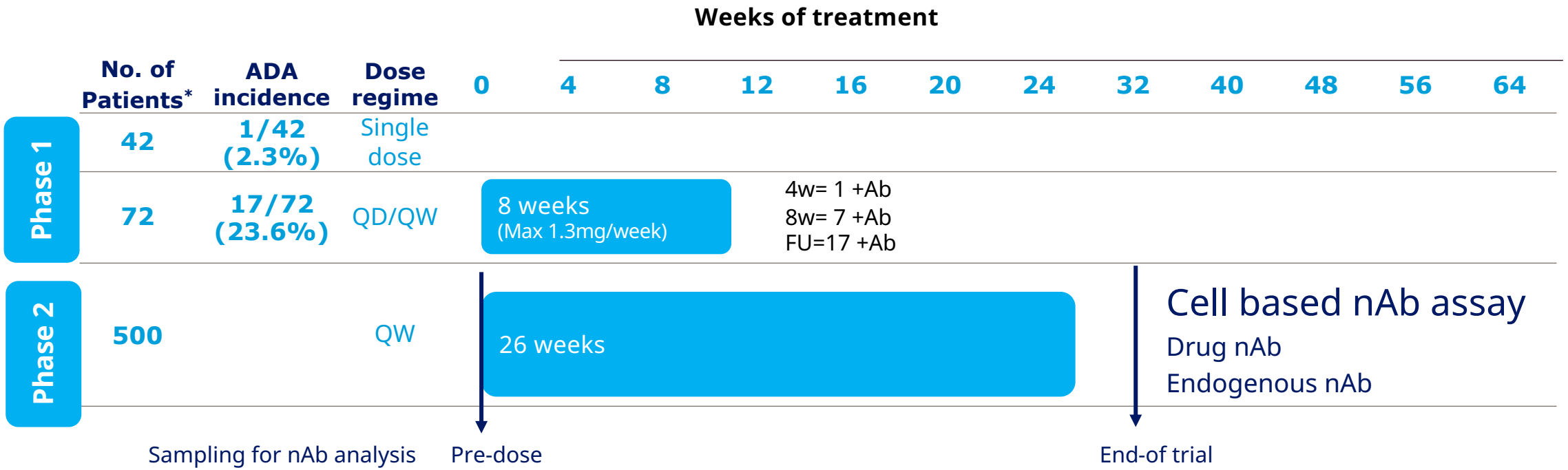
Agenda

- Introduction
- Cut point in a phase 2 trial – drug nAb and endo nAb
- Cut point in a follow up analysis
- Do we need a nAb in a phase 2?
- Points for discussion and conclusions

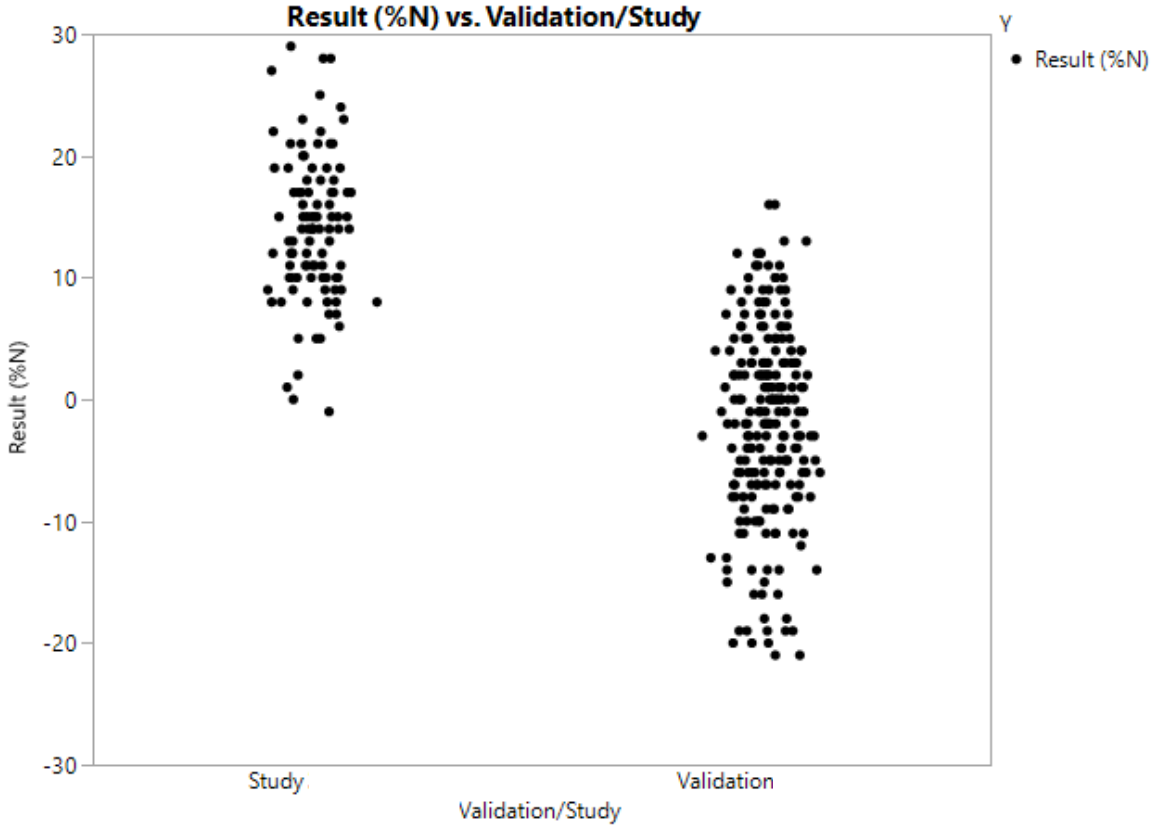
Peptide drug – Medium risk
20% Ab positives before planning of Phase 2 trial

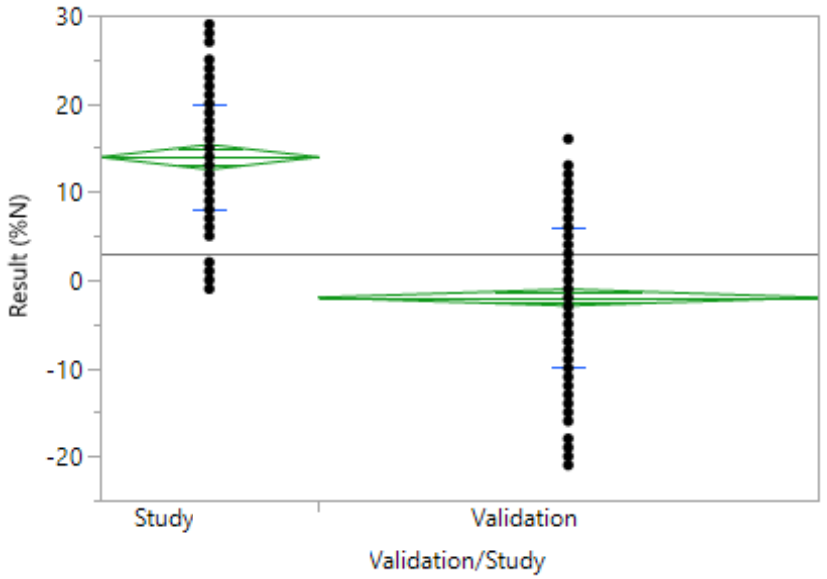


FDA recommendation for phase 2 trial

We recommend continued assessment of ADA in patients positive for ADA at the follow-up visit until ADA becomes negative.

Cut point evaluation drug nAb



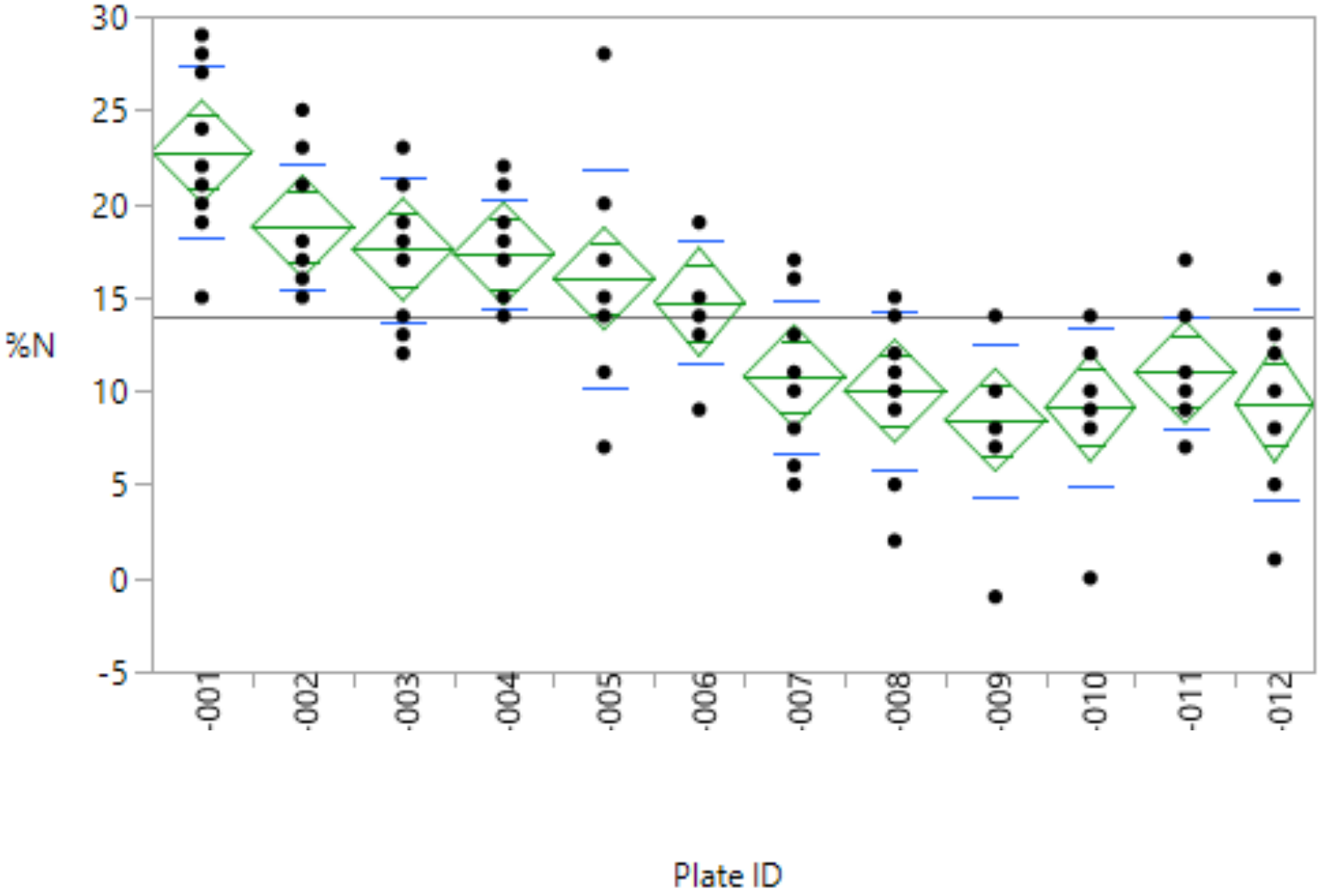


Validation samples: Obese drug naïve

Study samples: Overweight and obese

		False positive rate
NF validation	15	50%

In-study cut point calculation in drug nAb

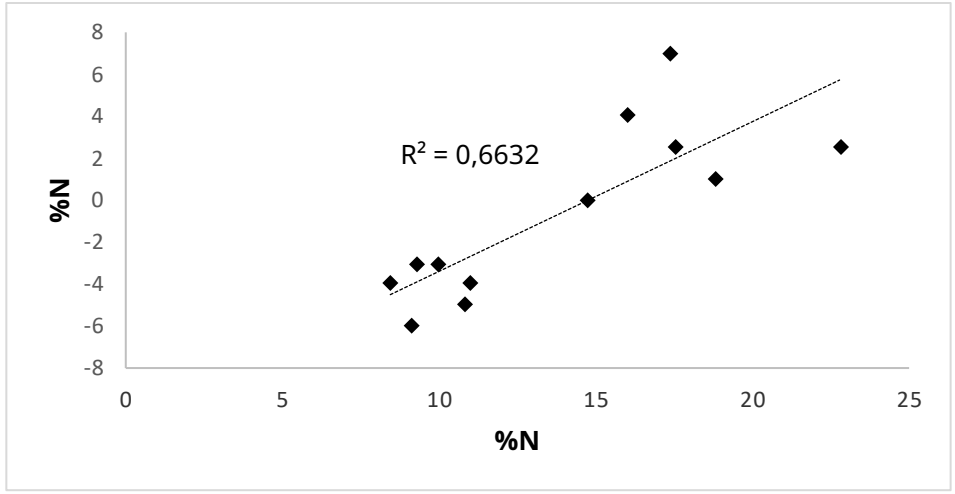


104 pre-dose samples were analysed once in 12 plates by 3 different analysts = **3 runs**

In-study cut point calculation in drug nAb

Are results for pre-dose samples normally distributed and/or non-skewed	Yes
Has log transformation been necessary	No
Have outliers been removed (yes/no):	No

Test for equal means and variance	
Does the 3 set-ups have equal means:	No
Does the 3 set-ups have equal variances:	Yes
Type of cut point:	Floating



Correlation between the results of the QC neg and the mean of pre-dose samples per set-up.

In-study cut point calculation in drug nAb

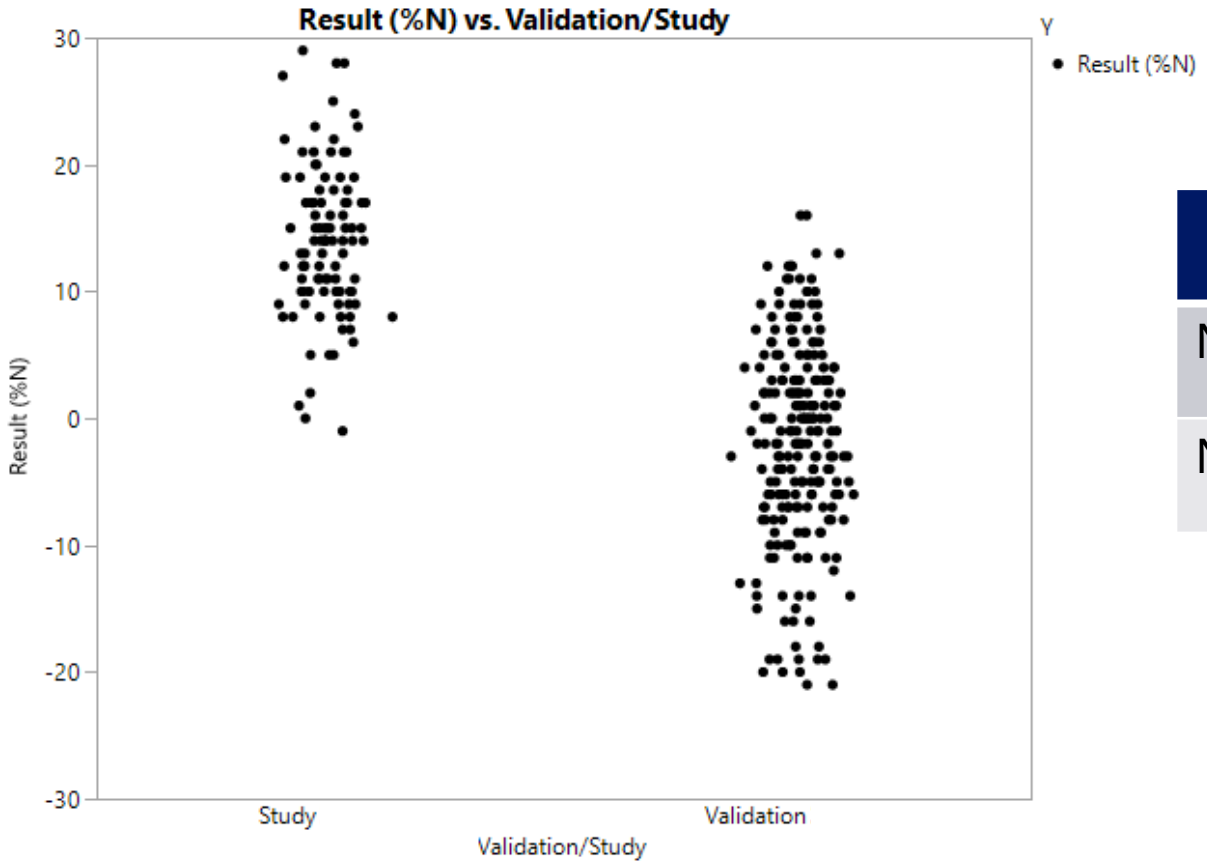
Plate ID	-001	-002	-003	-004	-005	-006	-007	-008	-009	-010	-011	-012
Mean	22.77	18.77	17.55	17.33	16	14.75	10.77	10	8.44	9.13	11	9.29
SD	4.63	3.42	3.88	2.87	5.85	3.24	4.11	4.18	4.06	4.25	2.96	5.09
N	9	9	9	9	9	8	9	9	9	8	9	7
Median QC neg	2.5	1	2.5	7	4	0	-5	-3	-4	-6	-4	-3
T value	2.8964	2.8964	2.8964	2.8964	2.8964	2.99795	2.8964	2.8964	2.8964	2.99795	2.8964	3.14266
NCP (1% false positive)[A]	36.19	28.68	28.78	25.65	32.95	24.46	22.70	22.12	20.22	21.89	19.57	25.28
Average NCP	26											
Normalisation factor[B]	33.7	27.7	26.3	18.7	29.0	24.5	27.7	25.1	24.2	27.9	23.6	28.3
Average NF = NF_{study}	26											

NCP = Mean (individuals, %N) + (one-sided, t(0.01;df) x SD

NF_{study} = Neutralising cut point (NCP) - median QC neg

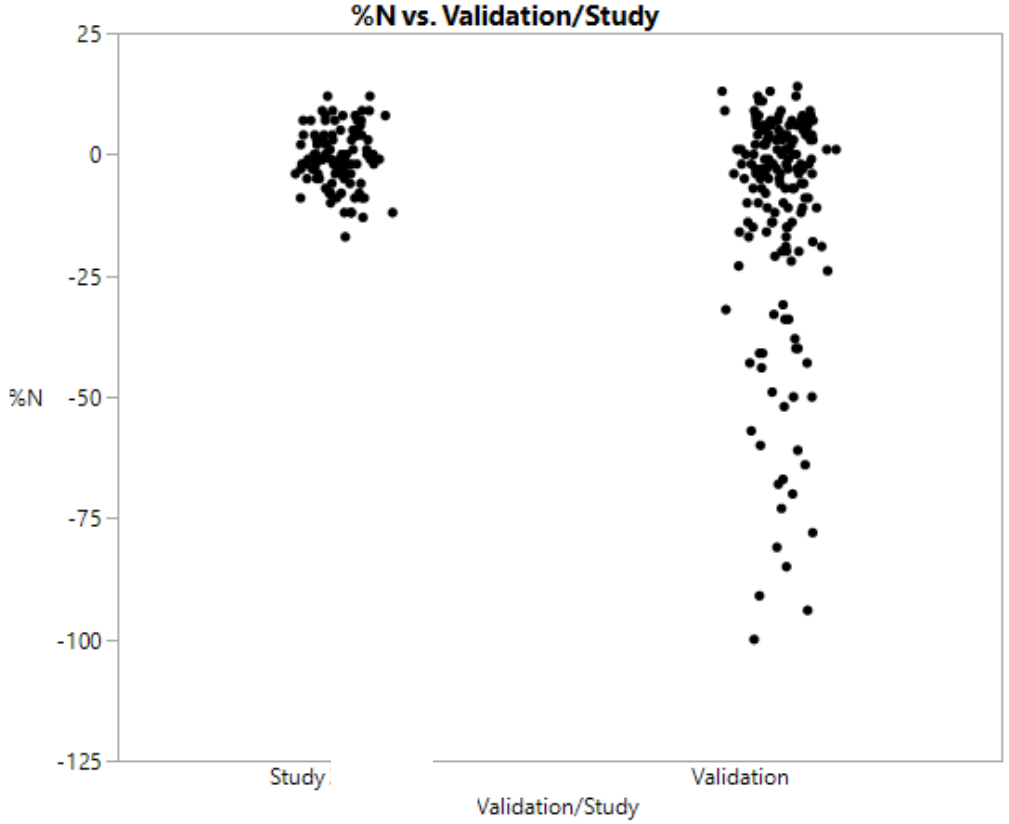
nNCP = Median QC neg plate + NF_{study}

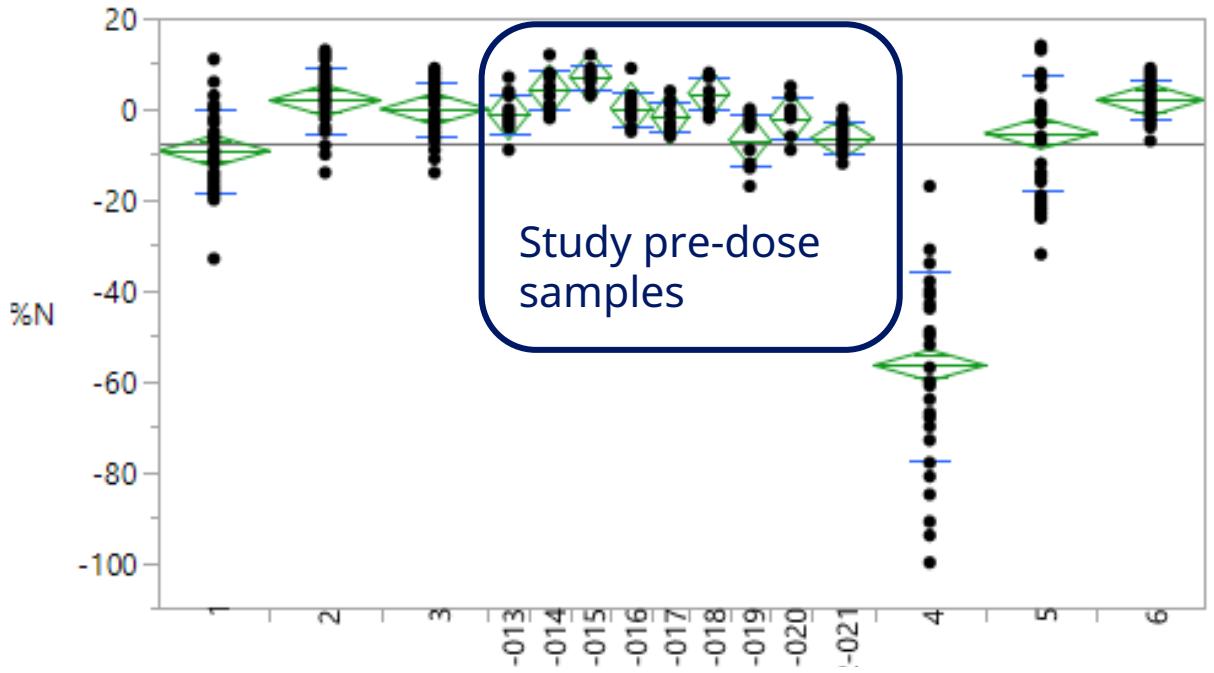
Cut point evaluation drug nAb



		False positive rate
NF validation	15	50%
NF study	26	1%

Cut point evaluation endogenous nAb

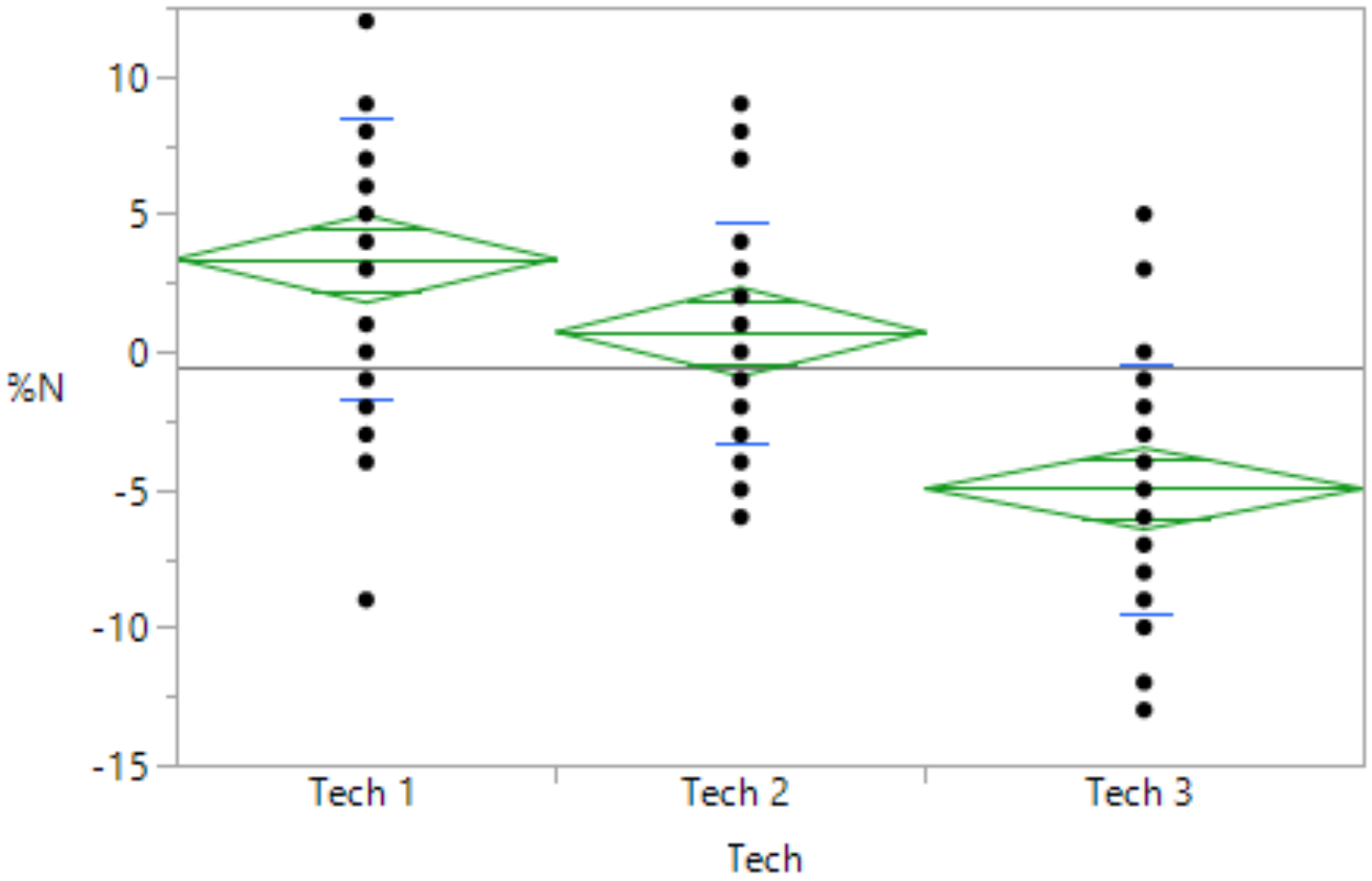




	False positive rate	
NF validation	20	0 %

The pre-dose samples from the study have mean signals different from the samples used for the validation of the assay.

In-study cut point calculation in endo-nAb

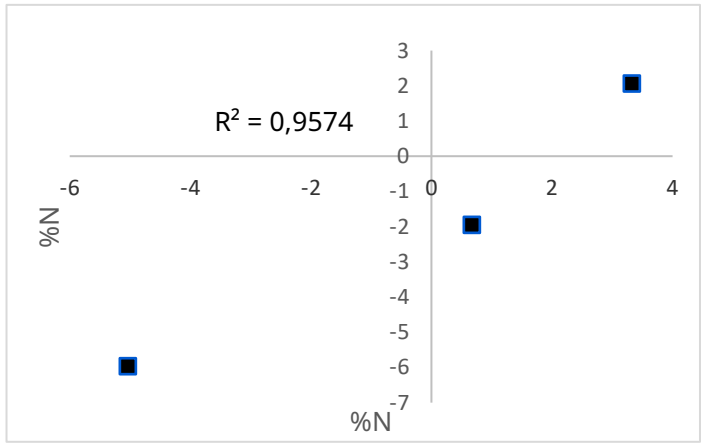


104 pre-dose samples were analysed once in 9 plates by 3 different analysts = 3 runs.

In-study cut point calculation in endo-nAb

Are results for pre-dose samples normally distributed and/or non-skewed	**Yes**
Has log transformation been necessary	**No**
Have outliers been removed (yes/no):	**Yes**

Test for equal means and variance	
Does the 3 set-ups have equal means:	**No**
Does the 3 set-ups have equal variances:	**Yes**
Type of cut point:	**Floating**



Correlation between the results of the QC neg and the mean of pre-dose samples per set-up.

In-study cut point calculation in endo-nAb

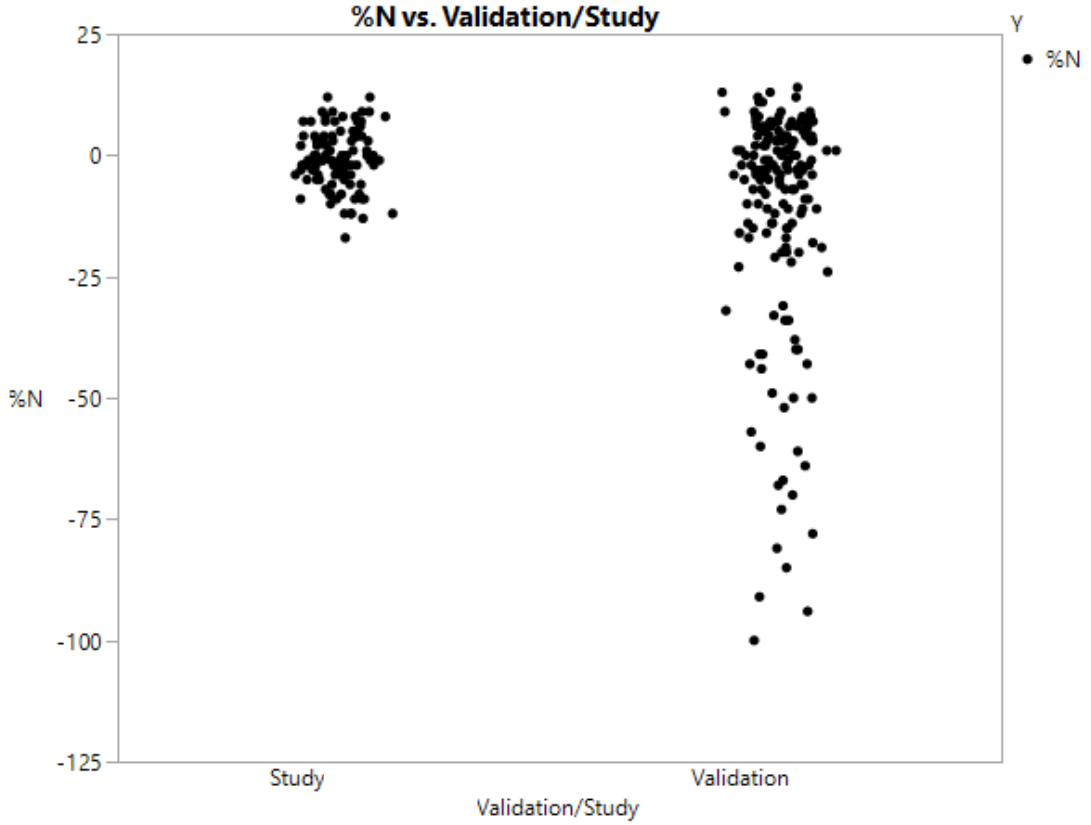
Set-up	1	2	3
Plate ID	319172-013-015	319172-016-018	319172-019-021
Mean	3.36	0.71	-4.97
SD	5.10	4.01	4.55
N	33	32	38
Median QC neg	2	-2	-6
T value	2.448677634	2.452824193	2.4314474
NCP (1% false positive)	15.86	10.57	6.10
Average NCP	11		
Normalisation factor	13.9	12.6	12.1
Average NF = NF_{study}	13		

NCP = Mean (individuals, %N) + (one-sided, t(0.01;df) x SD

NF_{study} = Neutralising cut point (NCP) - median QC neg

nNCP = Median QC neg plate + NF_{study}

Cut point evaluation endo-nAb



		False positive rate
NF validation	20	0 %
NF study	13	0 %

nNCP = Median QC neg + NF

60% ADA incidence in Phase 2 trial

	No. of Patients*	ADA incidence	Dose regime	Weeks of treatment: 0	4	8	12	16	20	24	32	40	48	56	64
Phase 1	42	1/42 (2.3%)	Single dose												
	72	17/72 (23.6%)	QD/QW	8 weeks (Max 1.3mg/week)											
Phase 2	506	307/506 (60.7%)	QW	26 weeks											

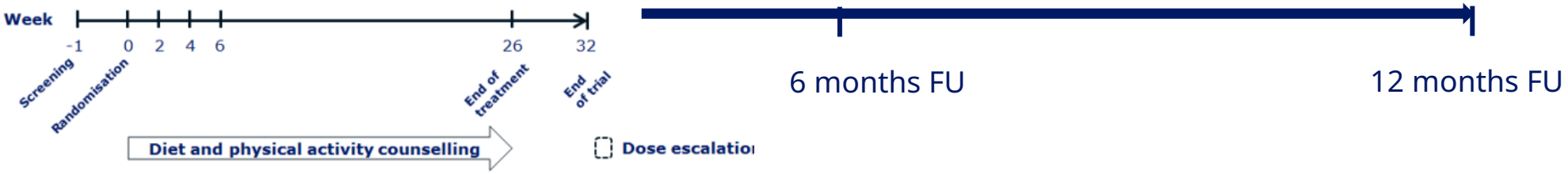
4w= 1 +Ab
8w= 7 +Ab
FU=17 +Ab

Dose dependent Ab formation
(90% of titres <260)
6 *in vitro* anti-drug nAbs
5 *in vitro* endogenous nAb
No effect in PD / small effect in PK (sustaining Abs)

FDA recommendation for phase 2 trial

We recommend continued assessment of ADA in patients positive for ADA at the follow-up visit until ADA becomes negative.

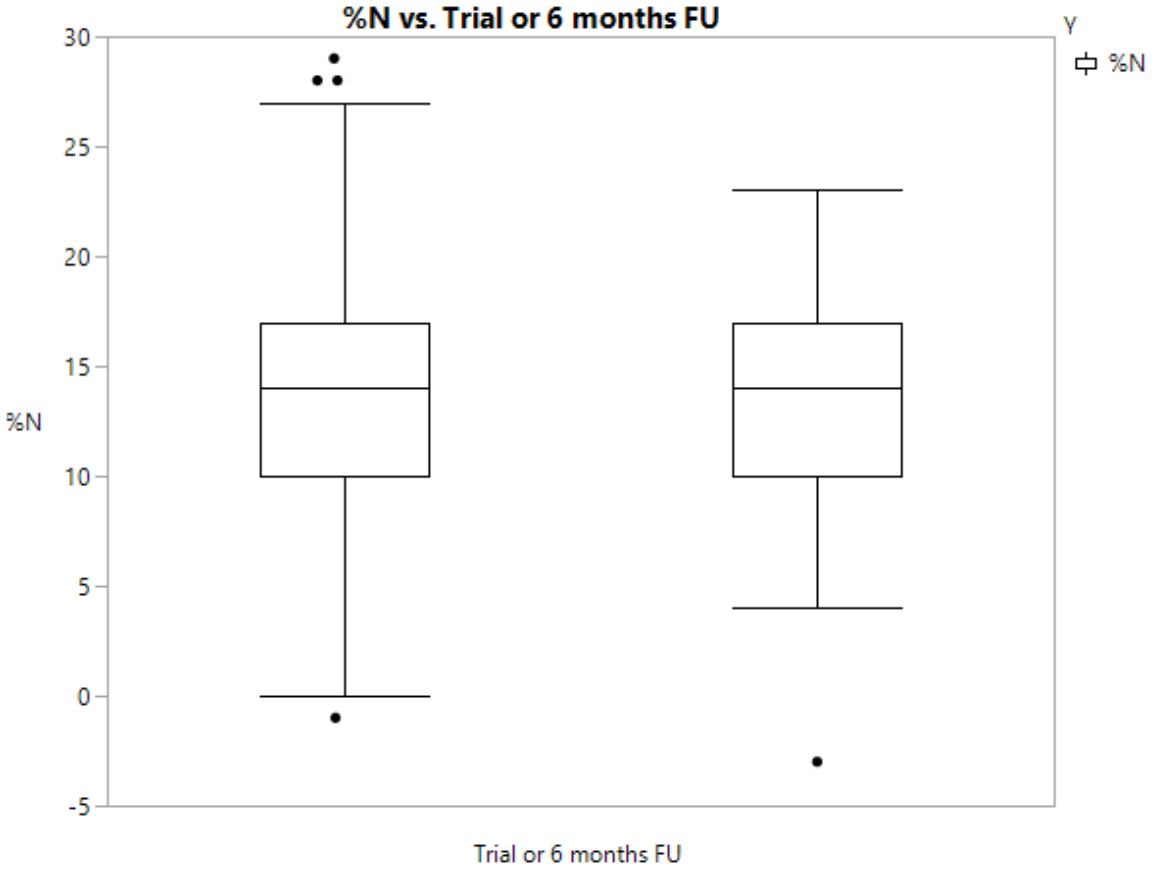
Follow up analysis in Phase 2 trial



Subjects who have tested positive for antibodies against drug (high titre antibodies and/or in vitro neutralising antibody response) at visit 12 ('end of trial' visit) will be requested to have a **follow-up analysis performed 6 months after visit 12**.

If the follow-up analysis is positive for antibodies against drug (high titre antibodies and/or in vitro neutralising antibody response), the subject will be requested to have an additional follow-up analysis **performed 12 months after visit 12**.

Cut point evaluation in follow up analysis

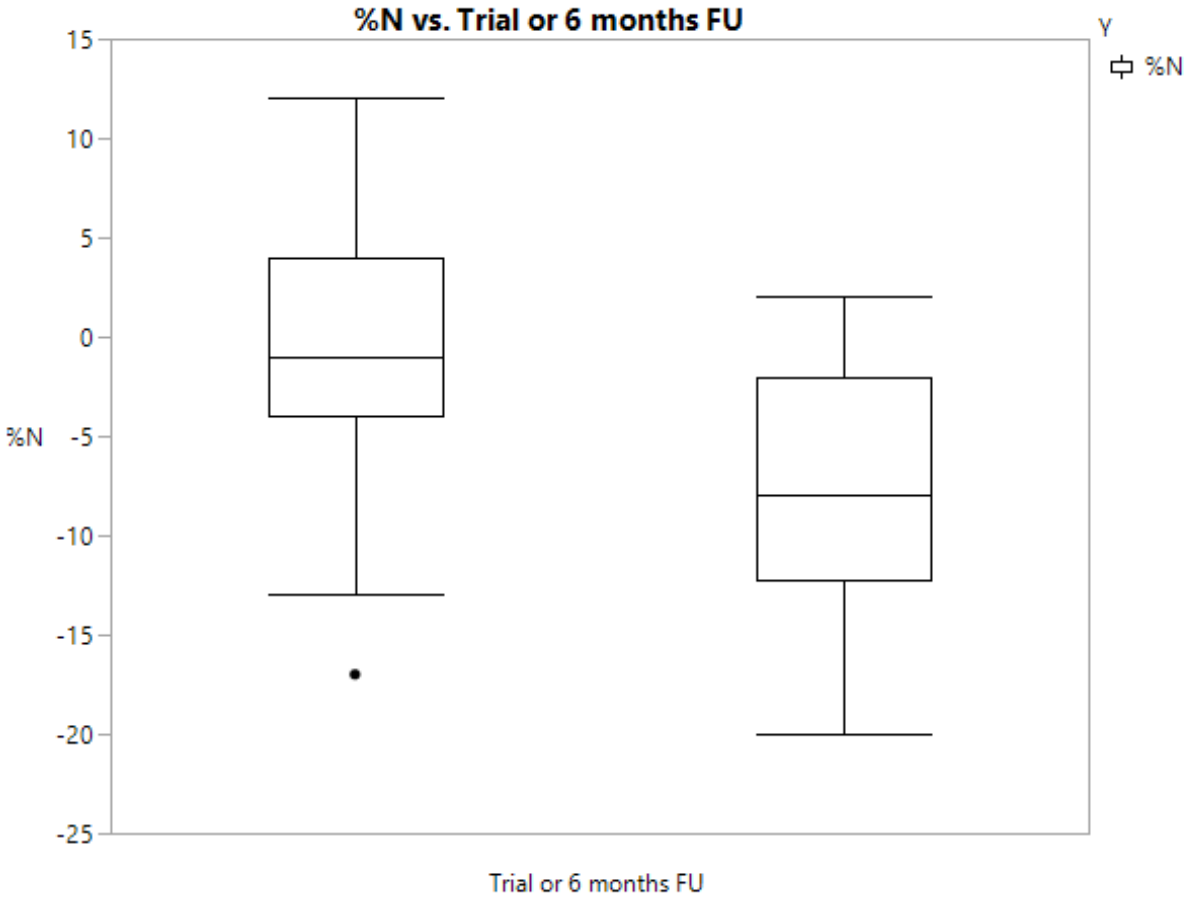


43 pre-dose samples were analysed again in 3 different plates for cut point evaluation

		False positive rate
NF study	26	1%

Drug nAb

Cut point evaluation in follow up analysis



43 pre-dose samples were analysed again in 3 different plates for cut point evaluation.

		False positive rate
NF study1	13	0%
NF study2	9	0%

Endogenous nAb

Immunogenicity assessment strategy in this project

Peptide drug with 20% bAb positives in phase 1 MD

Summary of Risk Assessment		Immunogenicity assessment strategy				
Consequence of ADA	**Overall Safety Risk**[1]	**Sampling**	**Assays**			**Reporting of data**
			Phase 1 - SD	**Phase 1 -MD**	**Phase 2**	
Moderate	Medium	During trial	bAb	bAb + nAb	bAb + nAb	After LPLV

SD = Single dose
MD = Multiple Dose
LPLV = Last Patient Last Visit

bAb = binding antibody
nAb = neutralising antibody

Immunogenicity assessment strategy

Risk level impacts the final analysis strategy

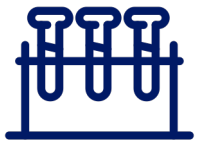
Summary of Risk Assessment		Immunogenicity assessment strategy				
Consequence of ADA	**Overall Safety Risk**[1]	**Sampling**	**Assays**			**Reporting of data**
			Phase 1 - SD	**Phase 1 -MD**	**Phase 2**	
None/Mild	Lower	During trial	- [2]	(bAb)- [2]	bAb	After LPLV
Moderate	Medium	During trial	(bAb)- [2]	bAb	bAb + (nAb)[3]	After LPLV
Severe	Higher	During trial (+ post-trial)	bAb (+ nAb)[4]	bAb + nAb	bAb + nAb	During trial (decision tree)

[1]The rated consequence of ADA decides overall safety risk. In some cases, high ADA incidence (likelihood) may increase the overall safety risk (e.g. in cases with known hypersensitivity).
[2]Consider not to analyse bAb and only bank samples (William Hallet, FDA summit conf. 2018: Approx. 50% of submissions do not measure bAb in phase 1)
[3]Exclude nAb in phase 1 and consider if drug/endogenous nAb is needed in phase 2 or if PK/PD can be used instead.
[4]Only nAb if long PK, pre-existing ADA etc.

SD = Single dose
MD = Multiple Dose
LPLV = Last Patient Last Visit

bAb = binding antibody
nAb = neutralising antibody

Discussion and Conclusions



In-study cut-point needed to be calculated in both assays
104 samples analysed in 3 runs was appropriate
Variation in same pre-dose samples analysed 6 months after

2 nAb assays at the phase 2 trial were useful to confirm that although the ADA incidence was high there was low effect in the efficacy (only 2% *in vitro* nAb and no effect in PD)

Time and focus on the nAb assays that are challenging assays
- Robustness of the assay
- Increase drug tolerance

Thank you

Questions ? Comments ?

What would you do differently?