Number of Donors Needed to Adequately Assess *in-vitro* Assays

APRIL 27TH 2023, LISBON

SOFIE DENIES

Introduction

No clear guidelines on
how to determine sample size for
in-vitro immunogenicity
screening

= **uncertainty in how to decide on sample size**

Basic statistical principles that can guide **evidence-based sample size decisions**

Overview

Theory: what is important in determining sample size, and what is not?

Examples: sample size calculations for specific research questions

Resources: online freely available tools to perform sample size calculations

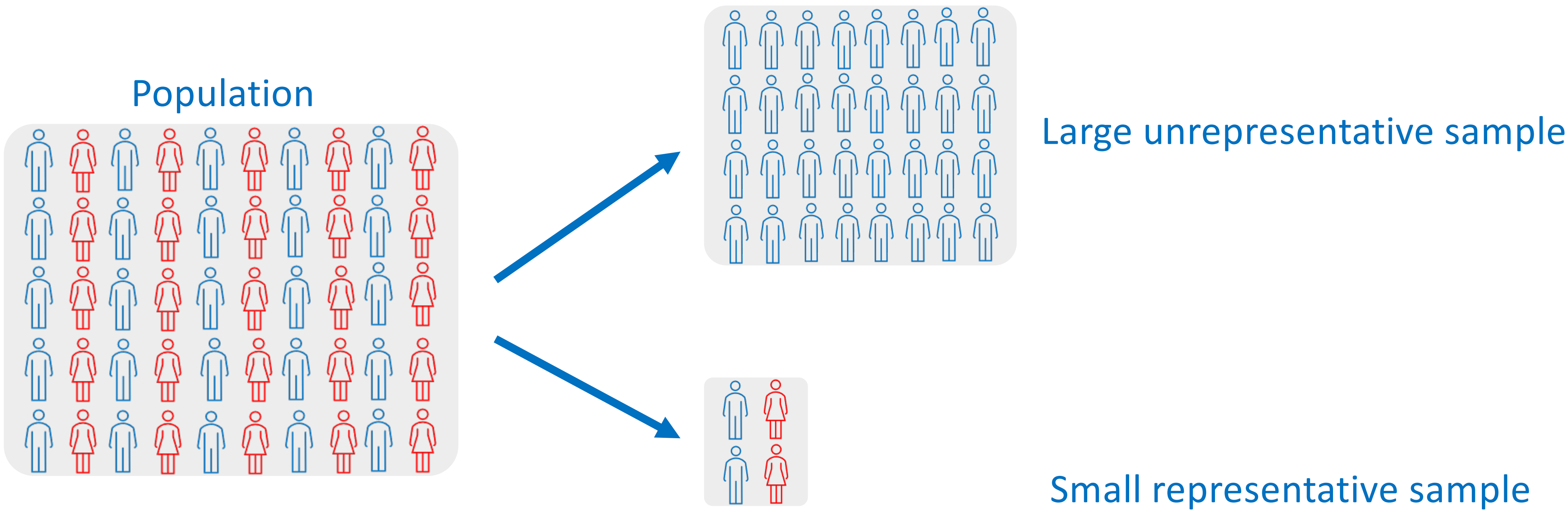
Overview

Theory: what is important in determining sample size, and **what is not**?

Examples: sample size calculations for specific research questions

Resources: online freely available tools to perform sample size calculations

What sample size do I need to get a representative sample?



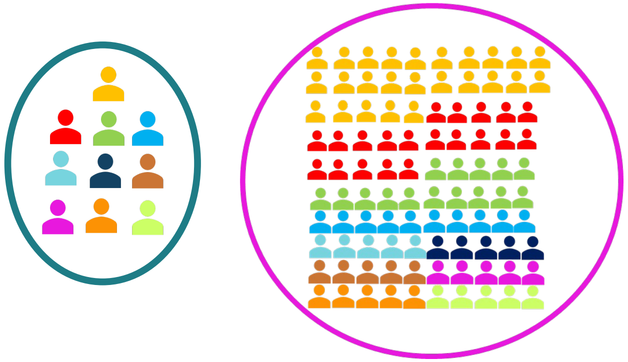
What sample size do I need to get good HLA coverage?

Variant of a similarly flawed question!

What sample size do I need to get good HLA coverage?

Coverage
=
The % of the **population** that has the same alleles as included in the **sample**

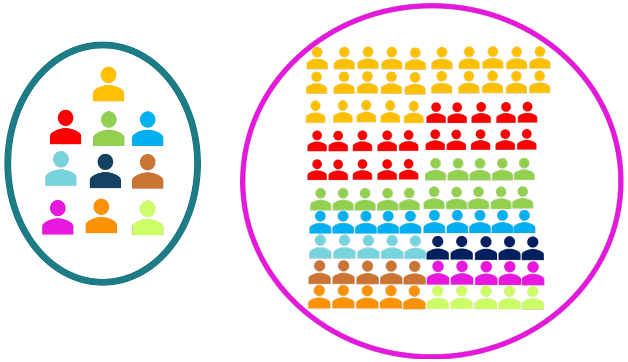
Coverage cannot determine sample size!



Sample includes all alleles present in the **population**

= 100% coverage

Coverage cannot determine sample size!

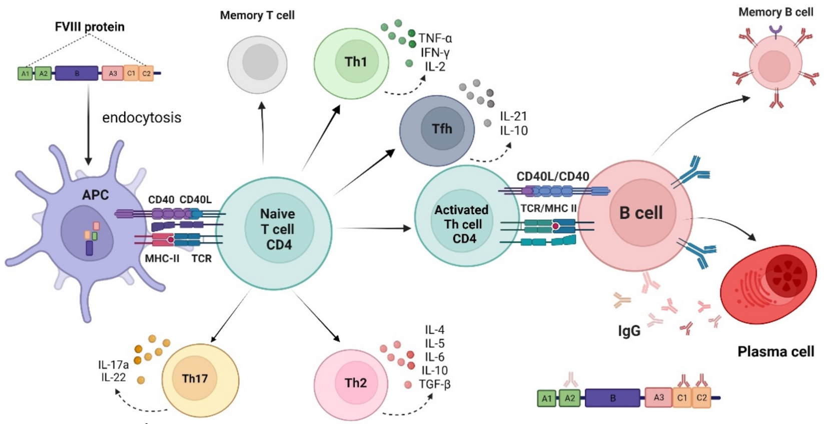


Sample includes all alleles present in the **population**

= 100% coverage

≠ Sufficient to characterize response in population:

HLA not only factor determining immunogenicity risk



Prezotti et al. 2022

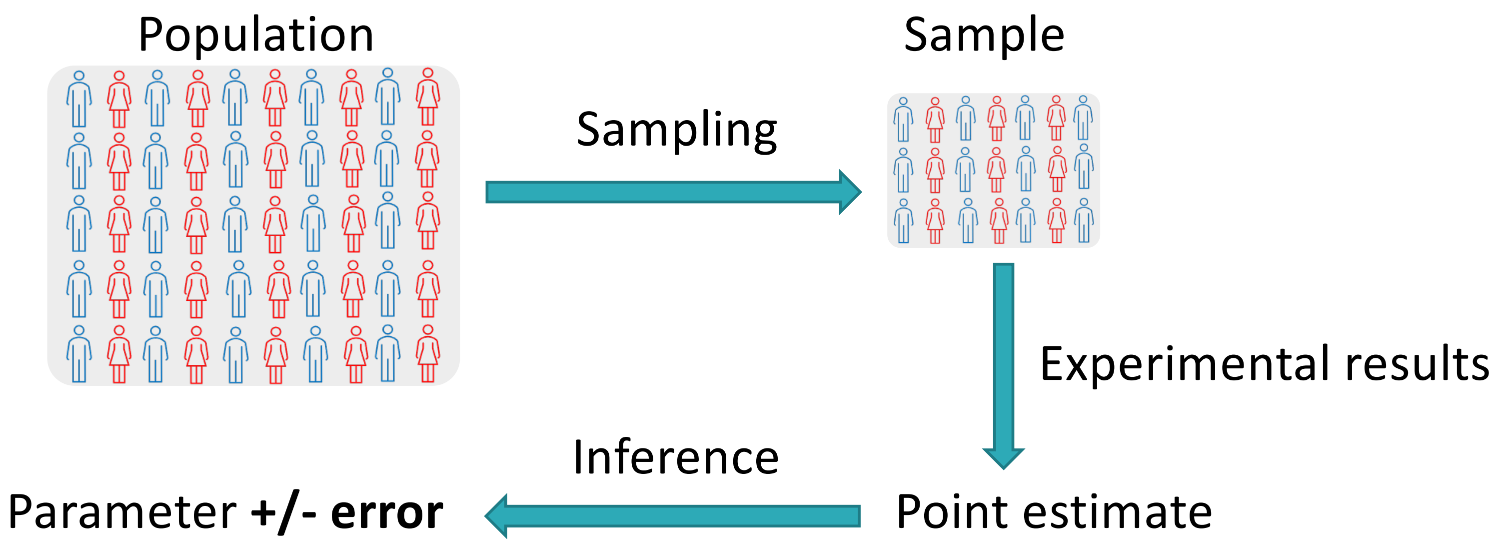
Overview

Theory: what is important in determining sample size, and what is not?

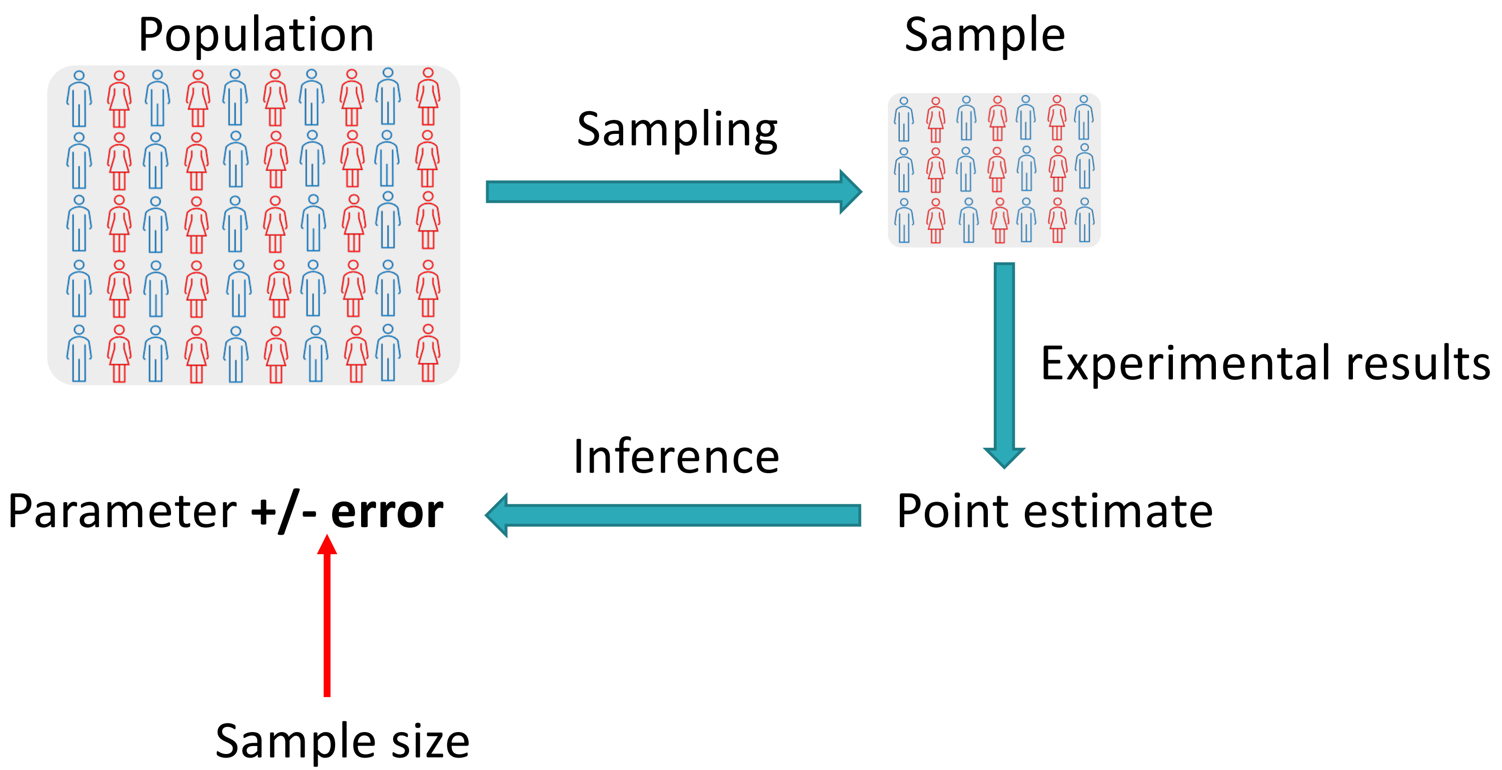
Examples: sample size calculations for specific research questions

Resources: online freely available tools to perform sample size calculations

What does determine sample size?

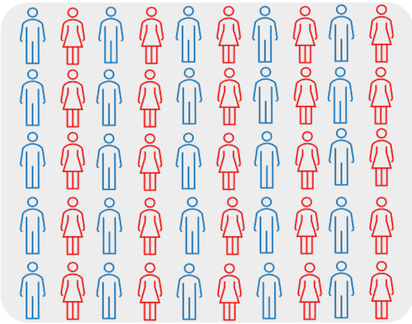


What does determine sample size?



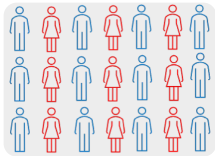
What does determine sample size?

Population



Sampling

Sample



Experimental results

Inference

Parameter **+/- error**

Point estimate

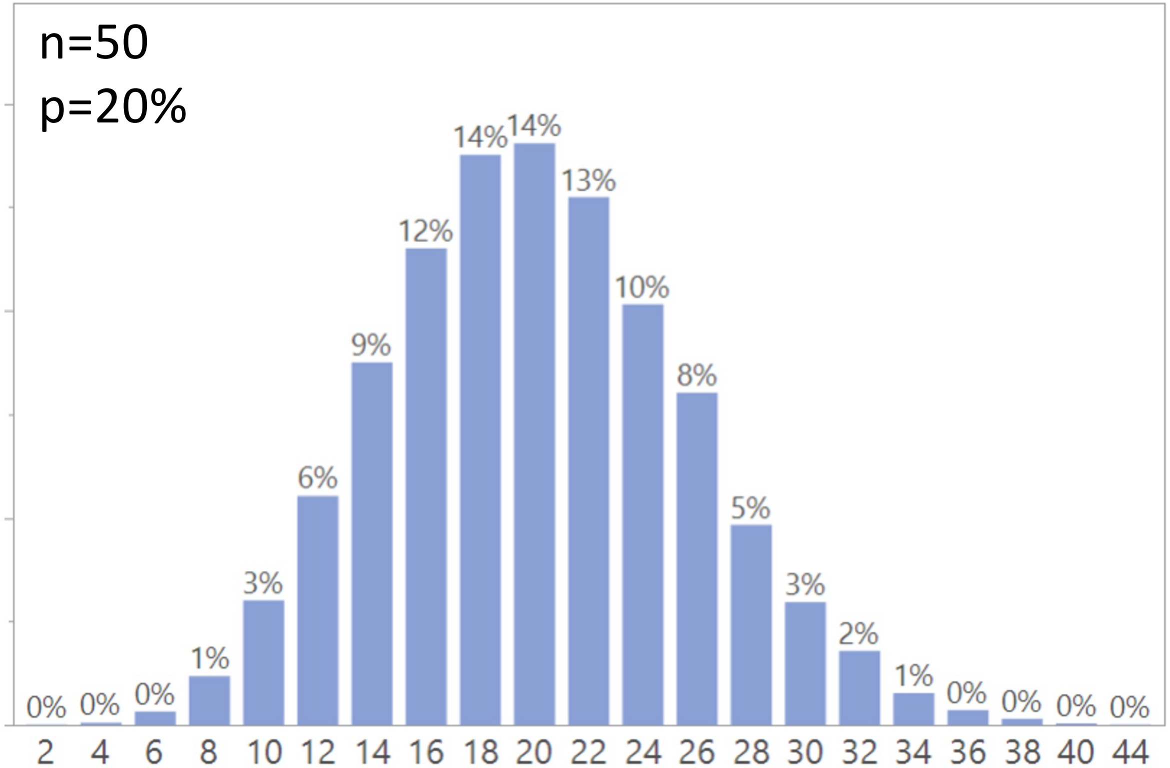
Binomial distribution*

Sample size

(*assuming representative sample: all subsequent calculations assume test sample with similar HLA distribution as population)

Binomial distribution

Binomial distribution describes how likely observed response rates in individual experiments occur with sample size n and true response rate p in population



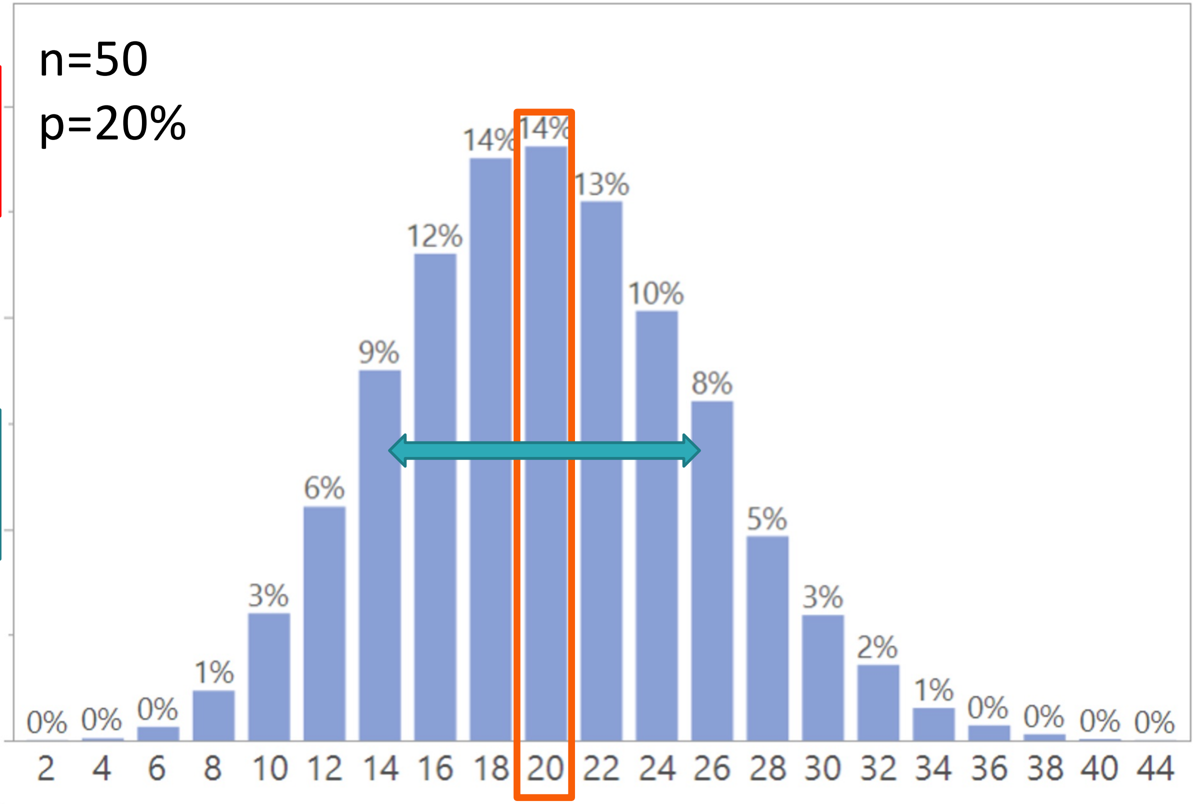
Binomial distribution

Most likely outcome: response rate in target population

=> Not dependent on sample size

Likelihood of deviating response rates in individual experiments

=> Directly dependent on sample size



Sample size criteria

- Based on the binomial distribution, it can be evaluated which sample sizes result in sufficient:

  - **Sensitivity**: detect responses in a sample if a relevant proportion of the population shows a response

  - **Specificity**: possibility to discriminate compounds with a relevant response rate from compounds with non-relevant response rate

Sensitivity: probability of 0 responses

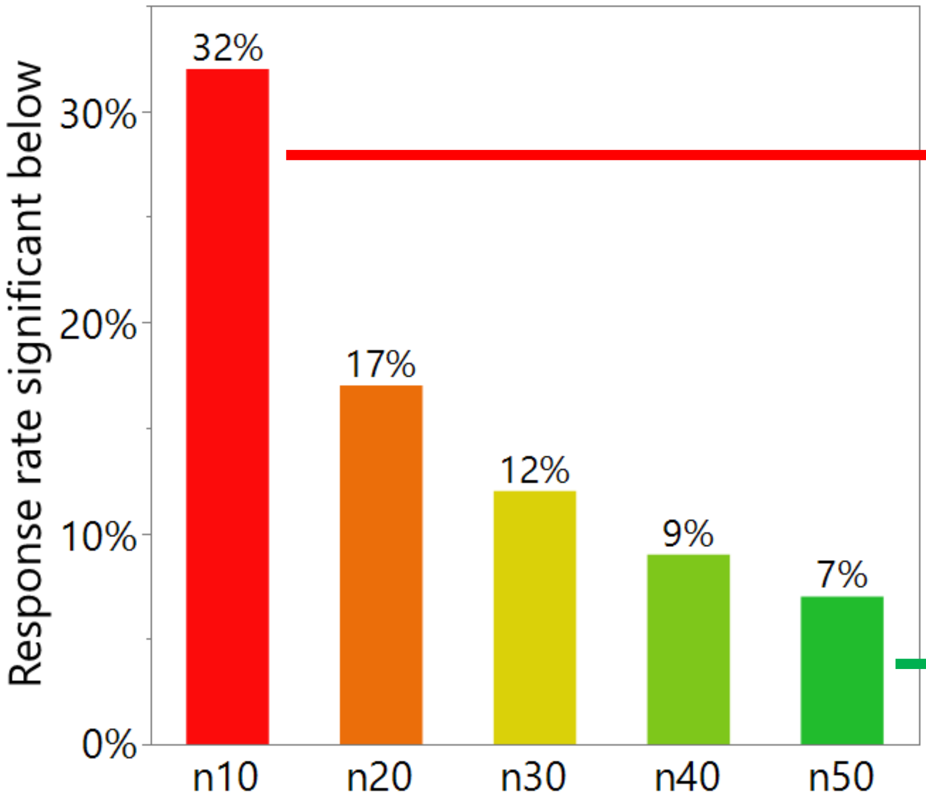
Response rate population →

n test sample ↓

n test sample	10%	20%	30%	40%
10	43%	17%	6%	2%
20	19%	3%	<1%	<1%
30	8%	<1%	<1%	<1%
40	4%	<1%	<1%	<1%
50	2%	<1%	<1%	<1%

Probability of 0 responses in test sample in function of sample size and response rate in the population, assuming 80% assay sensitivity

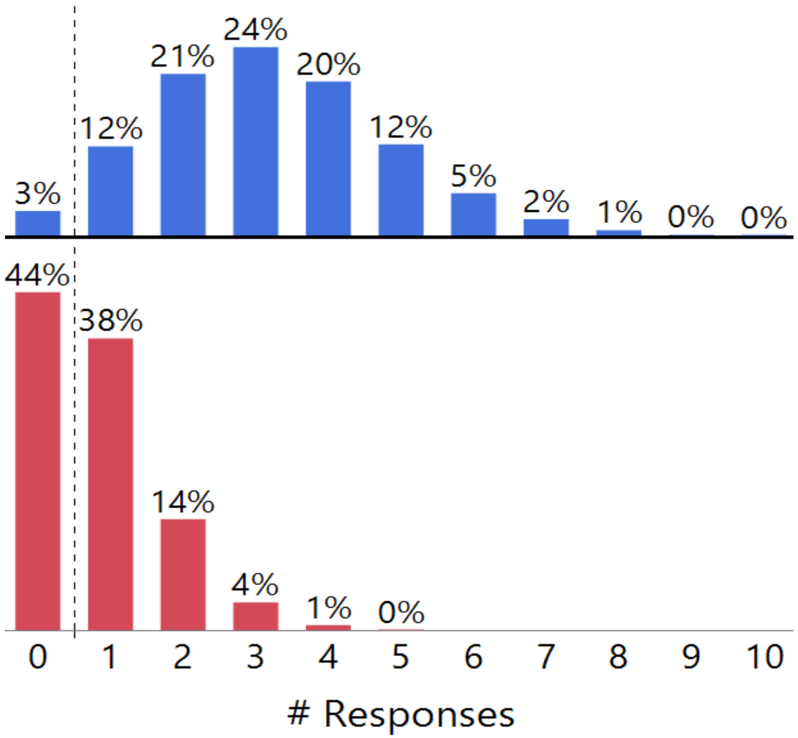
Sensitivity: conclusions with 0 responses



Even with 0 responses it can only be concluded with 95% confidence that the response rate in the population is <32%
(assuming 80% assay sensitivity)

With 0 responses it can be concluded with 95% confidence that the response rate in the population is <7%
(assuming 80% assay sensitivity)

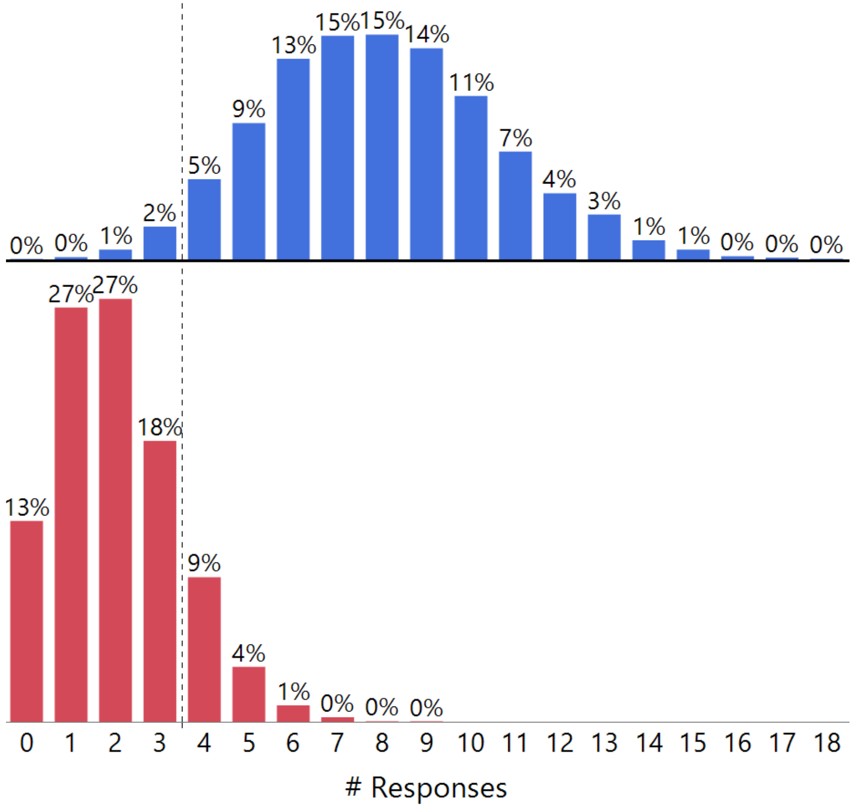
Specificity: example n=20



Although a compound with 20% response rate will result in at least 1 response in >95% of experiment…

…a compound with 5% response rate will do this as well in 56% of experiments.

Specificity: example n=50



A compound with 20% response rate will result in at least 4 responses in >95% of experiment with n=50…

…a compound with 5% response rate only in 14% of experiments.

⇒Specificity versus a response rate of 5% is improved to **86%** (**44%** for n=20).

Overview

Theory: what is important in determining sample size, and what is not?

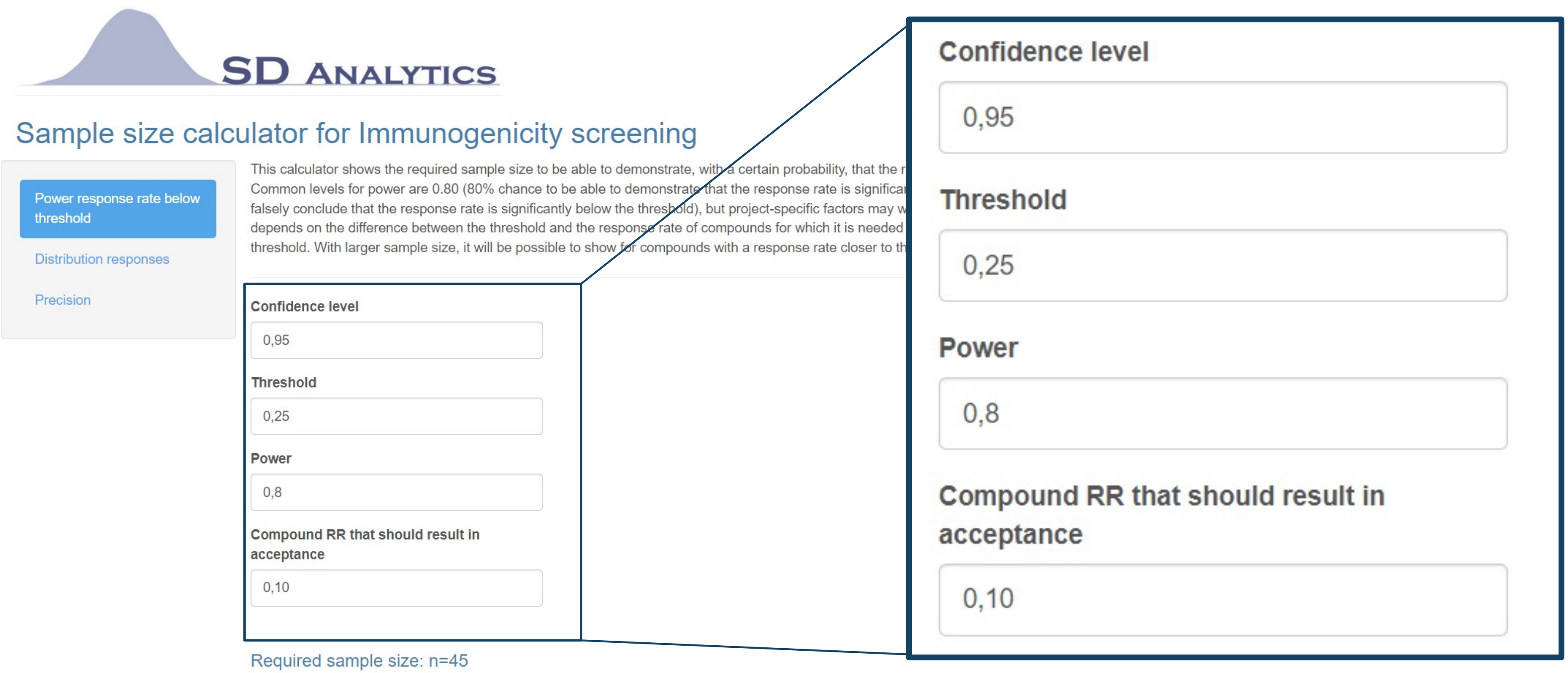
Examples: sample size calculations for specific research questions

Resources: online freely available tools to perform sample size calculations

Case study 1: Design

- Disease context/risk analysis: target profile <25% positive responses.
- Prior information: product is not strongly immunogenic.
- Required end conclusion: to confirm with sufficient confidence, that the response rate is <25%.
- Sponsor would like to be able to show a response rate significantly <25% if the true response rate of the product is up to 10%.
- To have (at least) 80% power to demonstrate that the response rate is significantly <25%, if the true response rate is 10% (or lower), **the required sample size = 45**.

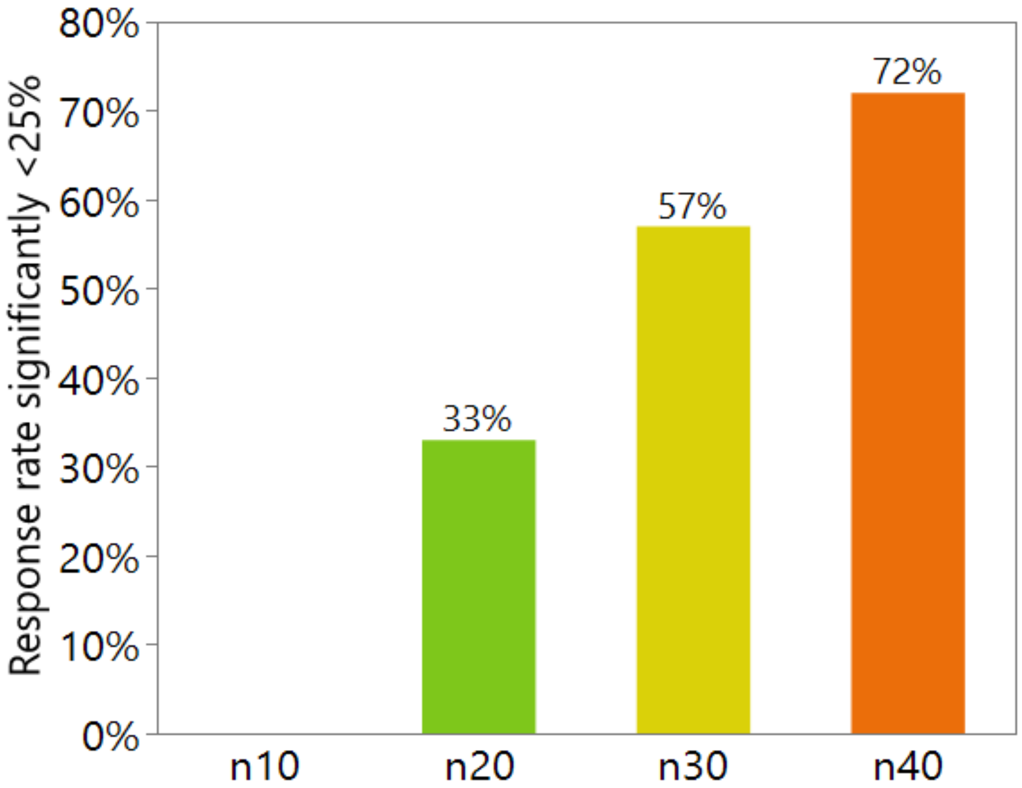
Case study 1: Design



Case study 1: Results

- Experiments with a lower number of donors were simulated by repeatedly taking a different subset of 45 donors evaluated for Nivolumab (11% response rate)

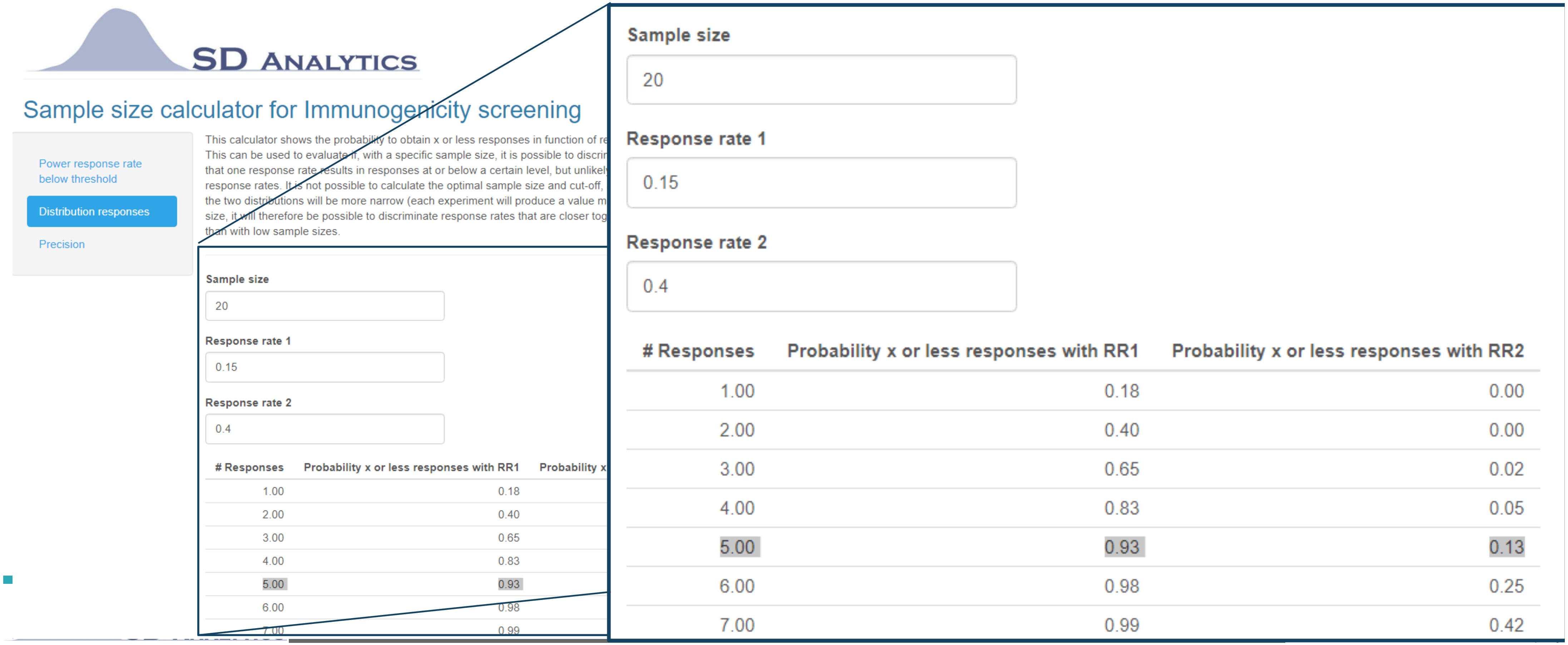
- Results confirm that lower sample sizes would not have had sufficient power to reach the desired end conclusion



Case study 2: Design

- Disease context/risk analysis: target profile <15% positive responses.
- Prior information: none.
- Required end conclusion: **filter out** compounds that are **far away** from this target (>40% response rate). It is important to **keep potentially interesting** compounds (compounds with a response rate <15%).
- With n=20 and continuing with only compounds showing 5 or less responses:
  - 90% of the compounds with a response rate of 15% (*and more with a response rate below 15%*) will be selected,
  - 90% of compounds with a response rate of 40% (*and more with a response rate above 40%*) will be filtered out.

Case study 2: Results



Sample size

20

Response rate 1

0.15

Response rate 2

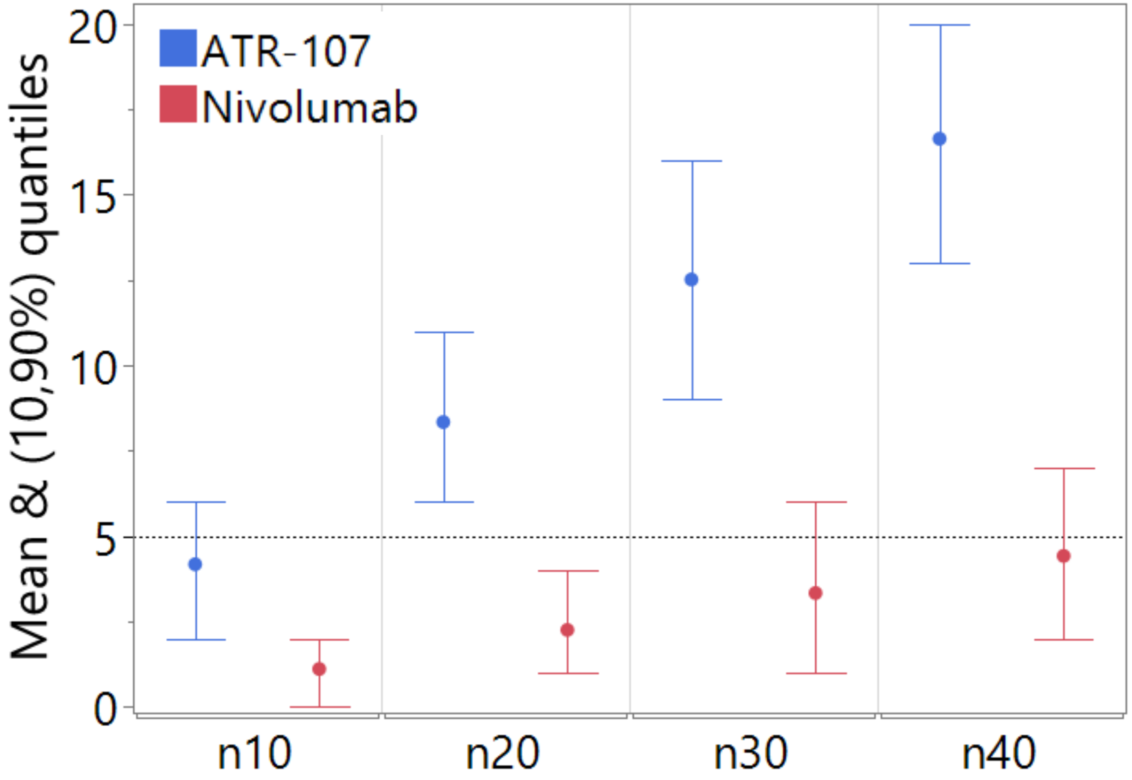
0.4

# Responses	Probability x or less responses with RR1	Probability x or less responses with RR2
1.00	0.18	0.00
2.00	0.40	0.00
3.00	0.65	0.02
4.00	0.83	0.05
5.00	0.93	0.13
6.00	0.98	0.25
7.00	0.99	0.42

Case study 2: Design

- Experiments with lower sample size were simulated by repeatedly taking a different subset of 45 donors, evaluated for ATR-107 (42%) and Nivolumab (11%)

- Range of responses in function of sample size, excluding the 10% most extreme responses at each end

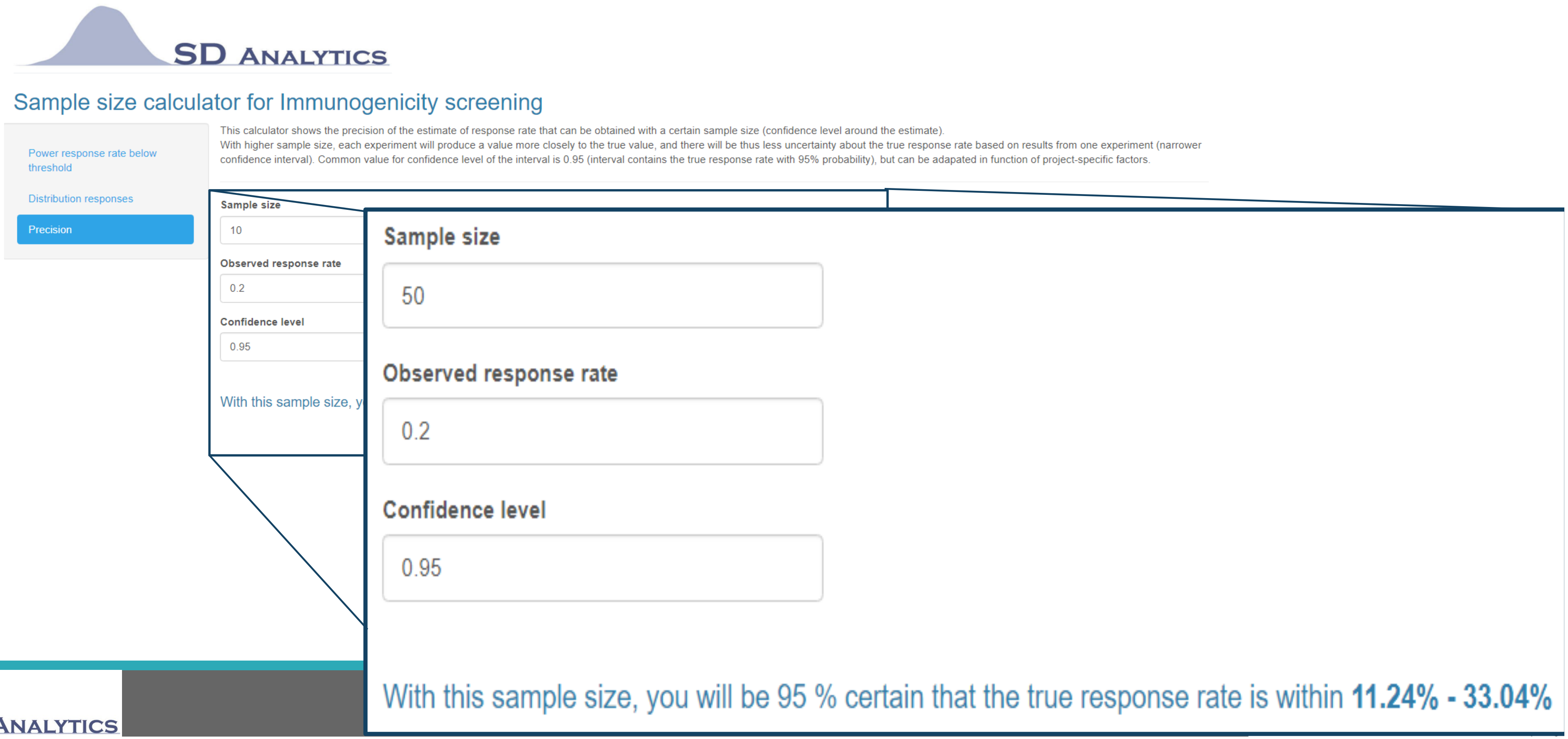


Case study 3: Design

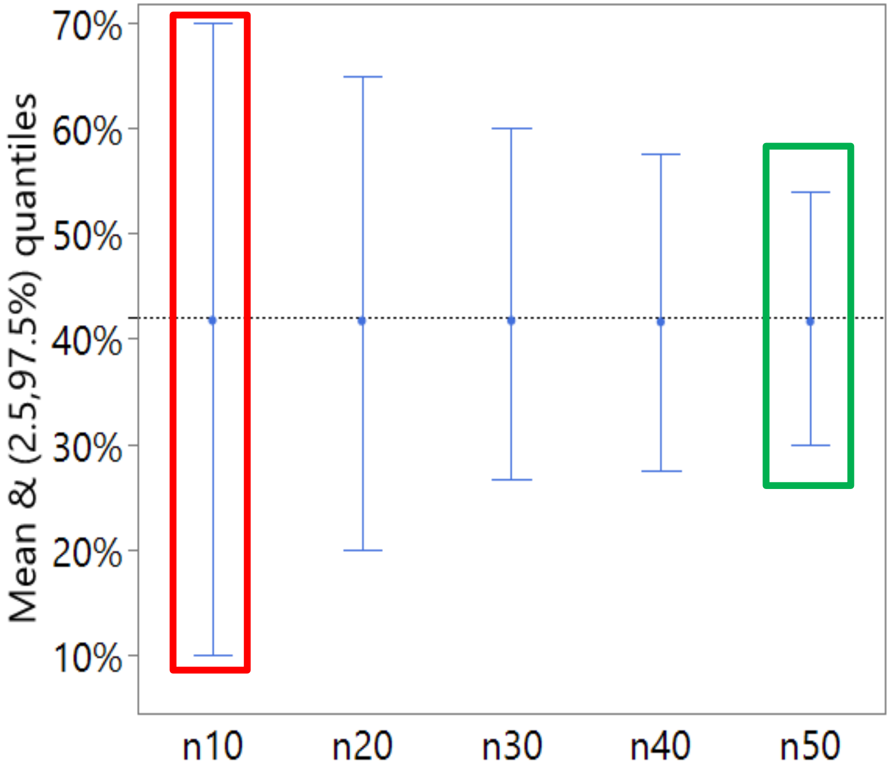
- Without a clearly defined hypothesis, sample size can be determined based on the precision of the estimates that can be obtained (confidence interval)

Observed response rate	95% CI n=10	95% CI n=50
10%	2%-40%	4%-21%
20%	6%-51%	11%-33%
30%	11%-60%	19%-44%
40%	17%-69%	28%-54%
60%	31%-83%	46%-72%

Case study 3: Design



Case study 3: Results



- Experiments with lower sample size were simulated by repeatedly taking a different subset of a large set of donors evaluated for ATR-107 (42%)

Observed response rate	n=10	n=50
20%	6%-51%	11%-33%

20% in sample is compatible with 42% in population

20% in sample is not compatible with 42% in population

Overview

Theory: what is important in determining sample size, and what is not?

Examples: sample size calculations for specific research questions

Resources: online freely available tools to perform sample size calculations

Resources

Manuscript in preparation:
"Statistical considerations on demonstrating unwanted immunogenicity"

- Sample size calculation
- Representativeness (HLA similarity)
- How to determine positivity per donor

https://shiny.sd-analytics.org/samplesize/

Conclusion

Clear definition of desired end conclusions

Calculations based on the binomial distribution:

- In collaboration with statistician
- With statistical software or online calculators

Sample size that allows to make the desired end conclusions

Questions?