One plate ahead of the unspecific binding: An innovative approach to solve obinutuzumab interference in a glofitamab-specific PK-Assay

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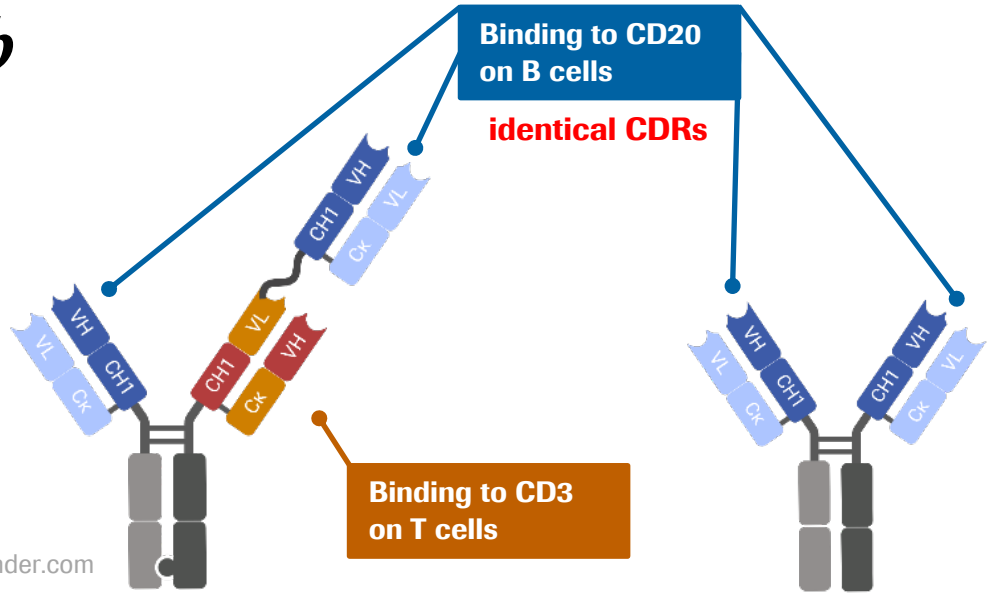
Glofitamab and Obinutuzumab



	Glofitamab	Obinutuzumab
Specificity 1	Human CD20	Human CD20
Specificity 2	Human CD3	
Dose	Starting with 5 µg flat	1000 mg flat, 7 days before dosing glofitamab (= pre-treatment)
Molar excess at cMax	1	Up to > 300000
Treatment Indication	Non-Hodgkin's lymphoma	

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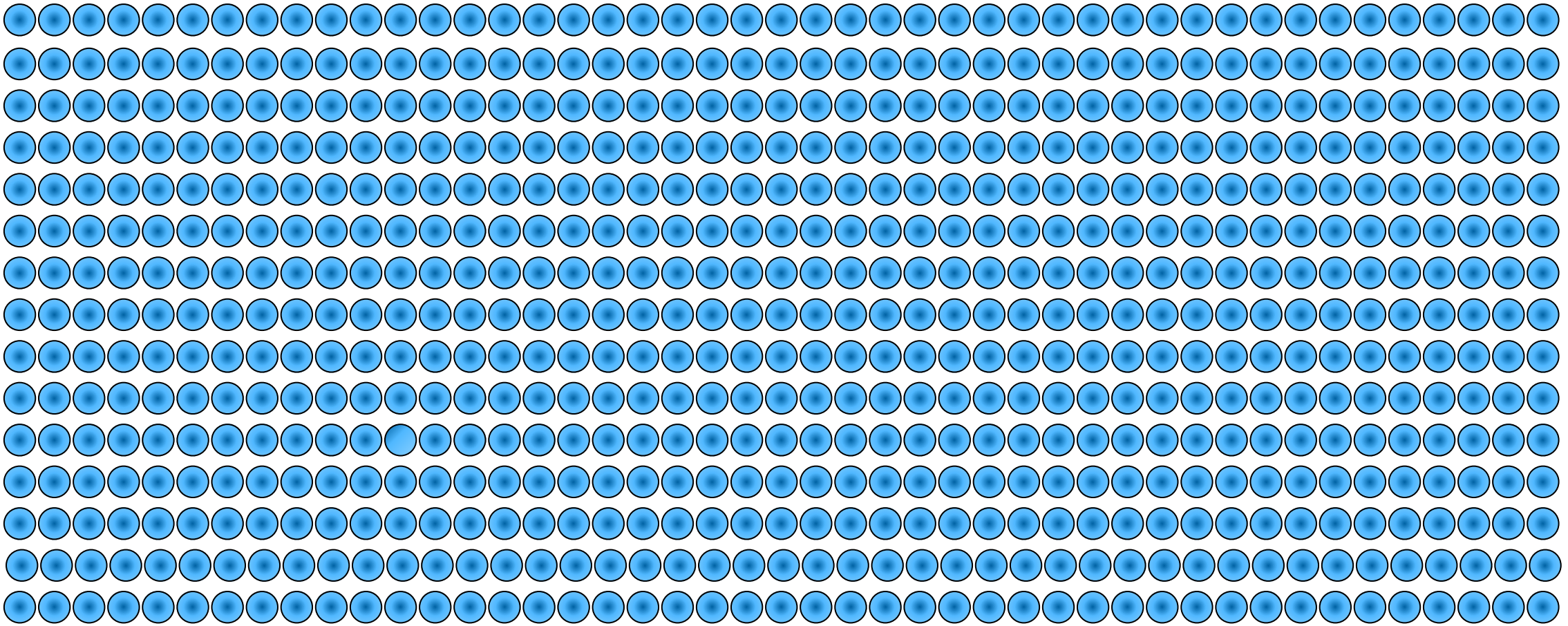
The Challenge: Spot the Difference



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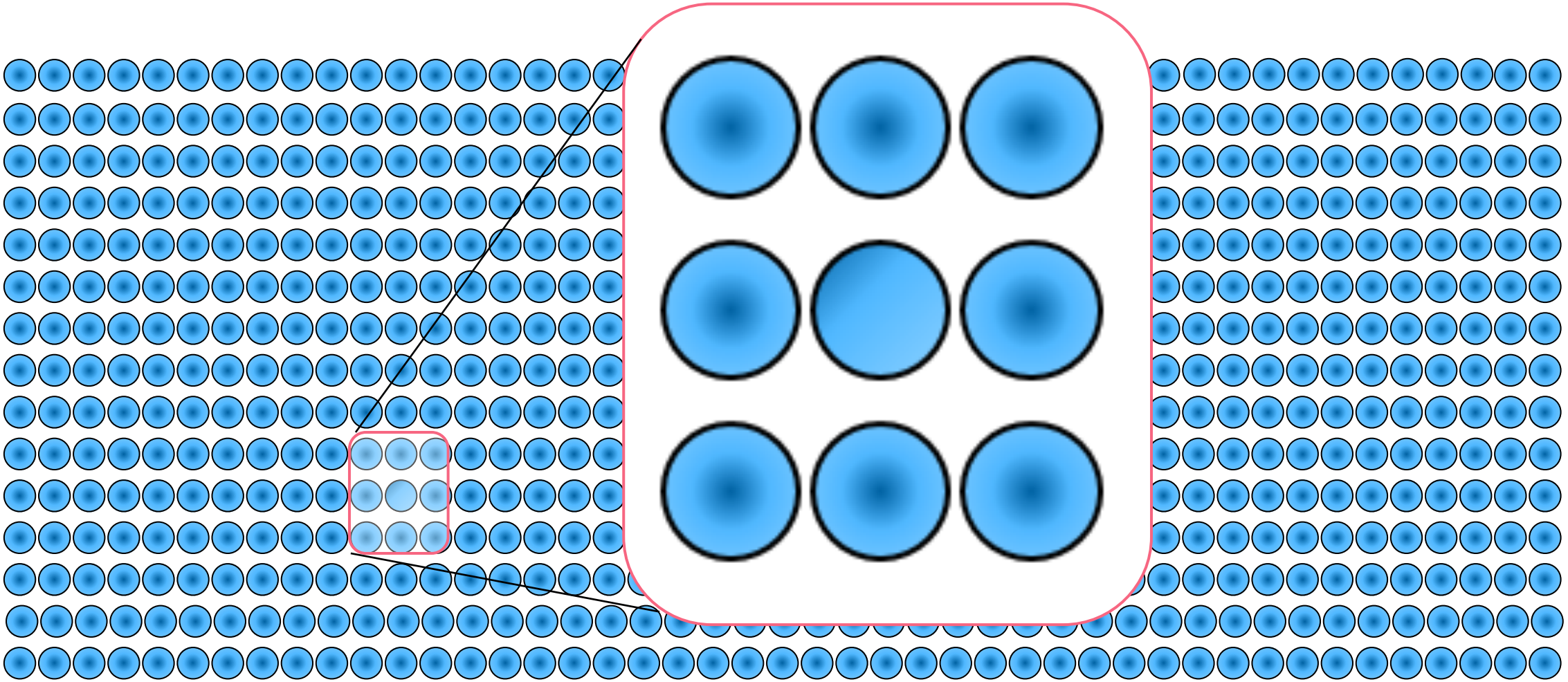

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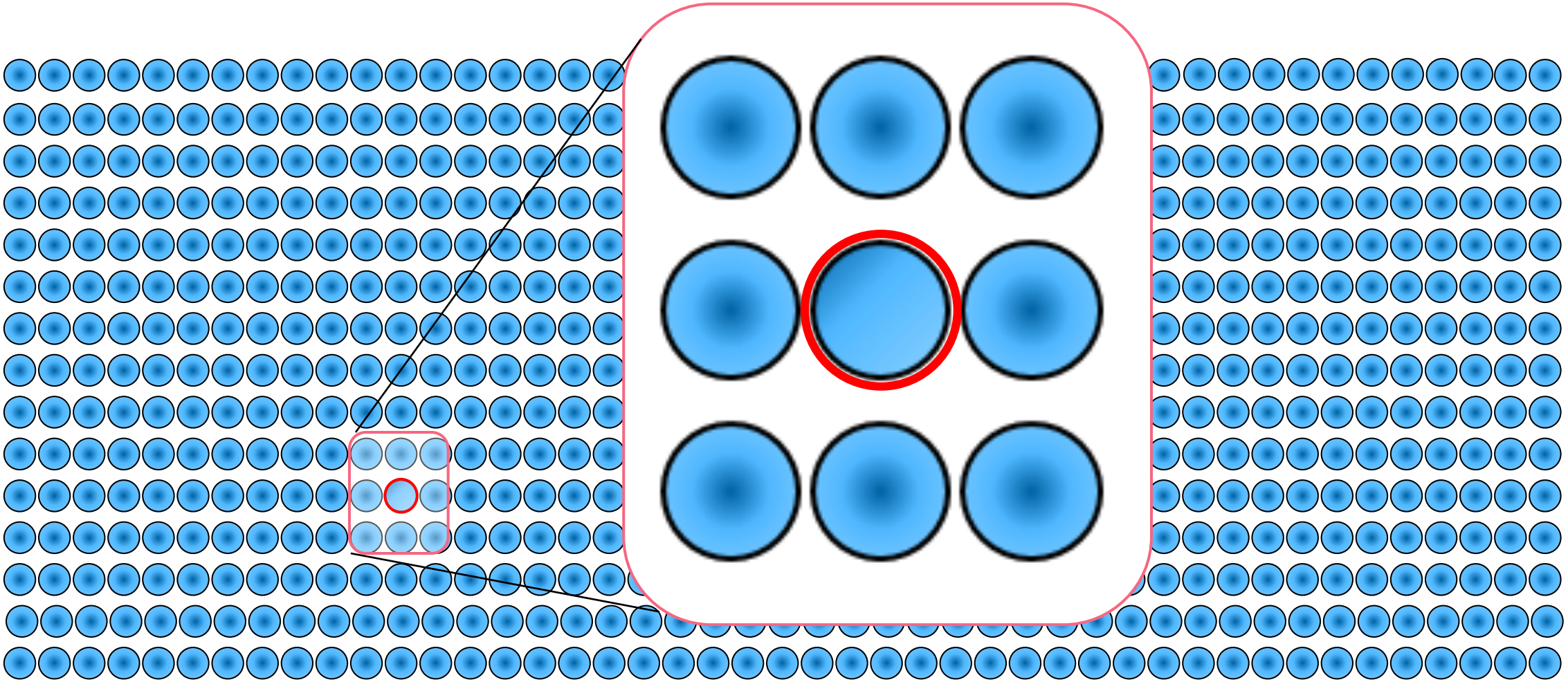
The Challenge: Spot the Difference



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Our Mission with regard to the clinical PK-Assay

Criteria for clinical PK Assay to support FiH trial of glofitamab administered after a fixed single-dose pre-treatment of obinutuzumab:

Develope and validate a method to quantify single digit ng/mL of glofitamab in the presence of up to 200 µg/mL obinutuzumab in serum of cancer patients

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Co-Medication of Biotherapeutics and the potential Interference on PK Evaluation

Co-medication and interference testing in bioanalysis: a European Bioanalysis Forum recommendation

Interference testing of co-medication in bioanalytical method validation has become an area of debate in view of the increased specificity offered by current state-of-the-art technology in both LC–MS/MS and ligand-binding assay platforms. In view of this, and considering the extensive experience within the European Bioanalysis Forum member companies, we evaluated the impact of co-medication on the performance of hundreds of bioanalytical methods with the aim of providing a science-based recommendation on how to evaluate and document potential interference from co-medication on the PK parameters in clinical studies in patients and volunteers.

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Keywords: co-medication • interference testing • method validation

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FDA guidance

During validation, the sponsor should confirm that the assay is free of potential interfering substances including endogenous matrix components, metabolites, anticipated concomitant medications, etc. If the study sample contains more than one analyte and the analytes are intended to be quantified by different methods, the sponsor should test each method for interference from the other analyte.

The sponsor should analyze blank samples of the appropriate biological matrix (e.g. plasma) from at least six (for CCs) or ten (for LBAs) individual sources. The sponsor should ensure that there are no matrix effects throughout the application of the method. Refer to Table 1 for details of selectivity and specificity requirements and acceptance criteria.

Bioanalytical Method Validation 7
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EMA guideline

It may also be necessary to investigate the extent of any interference caused by metabolites of the drug(s), interference from degradation products formed during sample preparation, and interference from possible co-administered medications. Co-medications normally used in the subject population studied which may potentially interfere should be taken into account at the stage of method validation, or on a study specific and compound specific base.

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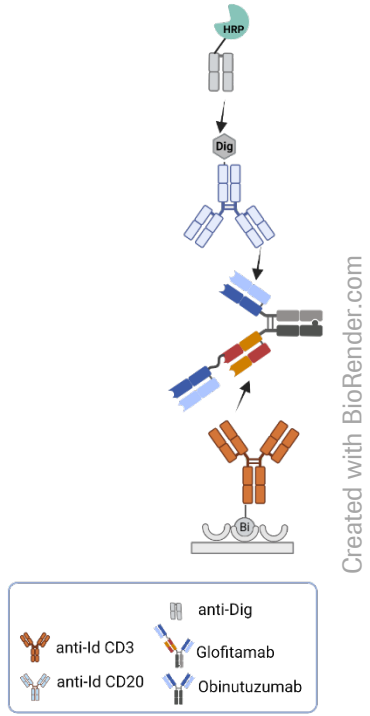
The Glofitamab-specific clinical PK-Assay: First Generation

Analysing glofitamab in human serum samples co-spiked with 200 µg/mL obinutuzumab

Assay Conditions: Sequential ELISA, Capture via anti-human CD3, Detection via anti-human CD20 domain, standard buffers

What we expected



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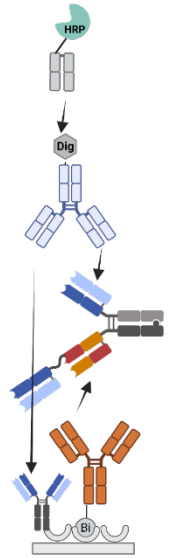
Sample	Nominal Glofitamab Concentration [ng/mL]	Actual Glofitamab Concentration [ng/mL]	Accuracy [%]
ULQC	100	142.47	**142**
HQC	75	125.36	**167**
MQC	15	69.10	**461**
LQC	3	61.12	**2037**
LLQC	1	59.00	**5900**
Blank	0	56.92	**N/A**

What we got



Detection of glofitamab at accuracies >120%

→ Hypothesis: Unspecific Obinutuzumab binding as the root-cause

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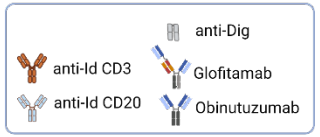
Confirming the unspecific binding of Obinutuzumab

Analysing human serum samples spiked only with obinutuzumab in two assay formats

Assay Conditions:

1. Sequential ELISA, Capture via anti-human CD3, Detection via anti-human CD20 domain, standard buffers
2. Sequential ELISA, ~~Capture via anti-human CD3~~, Detection via anti-human CD20 domain, standard buffers



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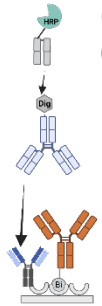
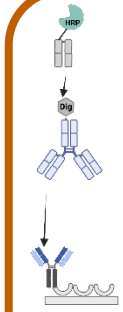


Capture via CD3 binder



No Capture via CD3 binder

Nominal Obinutuzumab Concentrations [ng/mL]	Nominal Glofitamab Concentrations [ng/mL]	Actual Glofitamab Concentations [ng/mL]	Actual Glofitamab Concentation [ng/mL]
50000	BLQ	238	**265**
5000	BLQ	72	**74**

Signal detection in the absence of anti-CD3 capture reagent confirms unspecific binding of obinutuzumab

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Optimization of the first generation Glofitamab-specific PK Assay

Options that were checked for their potential to reduce the unspecific binding of obinutuzumab:

- Medium instead of high binding streptavidin microtiter plates
- ELISA blocking reagents (e.g. Casein)
- Different coating conditions
- Washing buffer composition

None of the options alone resulted in acceptable assay performance

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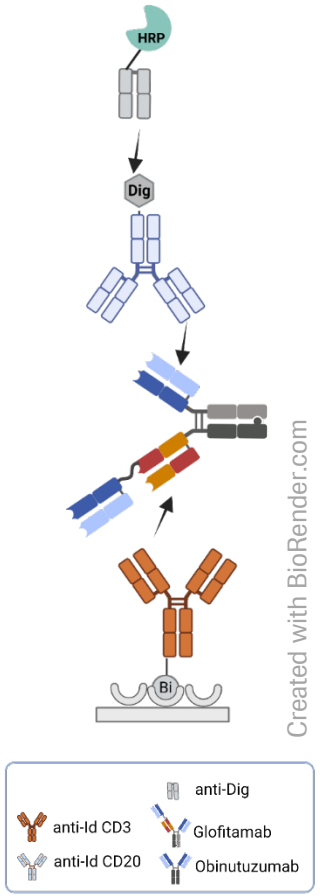
Performance of the optimized Glofitamab-specific PK Assay



Key Changes during Assay Optimization
- Microtiter Plates (high → medium binding capacity)
- Assay Buffer (PBS-T, BSA → Roche Universalbuffer)
- Washing Buffer (PBS-T → Washing Buffer at pH 5.5)

Sample	Nominal Concentration [ng/mL]	Actual Concentration [ng/mL]	Accuracy	Actual Concentration [ng/mL]	Accuracy	Actual Concentration [ng/mL]	Accuracy
		run 1		run 2		run 3	
ULQC	64	55.54	87	35.06	**55**	56.44	88
HQC	48	42.89	89	30.55	**64**	47.12	98
MQC	12	10.10	84	6.74	**56**	11.95	100
LQC	3	2.74	91	1.85	**62**	3.24	108
LLQC	1	1.05	105	0.81	81	1.70	**170**

Optimized glofitamab-specific PK Assay demonstrates reduced interference by obinutzumab

→ But assay is not performing robustly

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Key Considerations for a Two Plate Glofitamab-specific PK Assay

- Establish an ELISA using two microtiter plates where **the first plate acts as an absorber to remove** unspecifically bound **obinutuzumab**
- Find a way to **transfer glofitamab** from the first **to the the second microtiter plate** for final quantification

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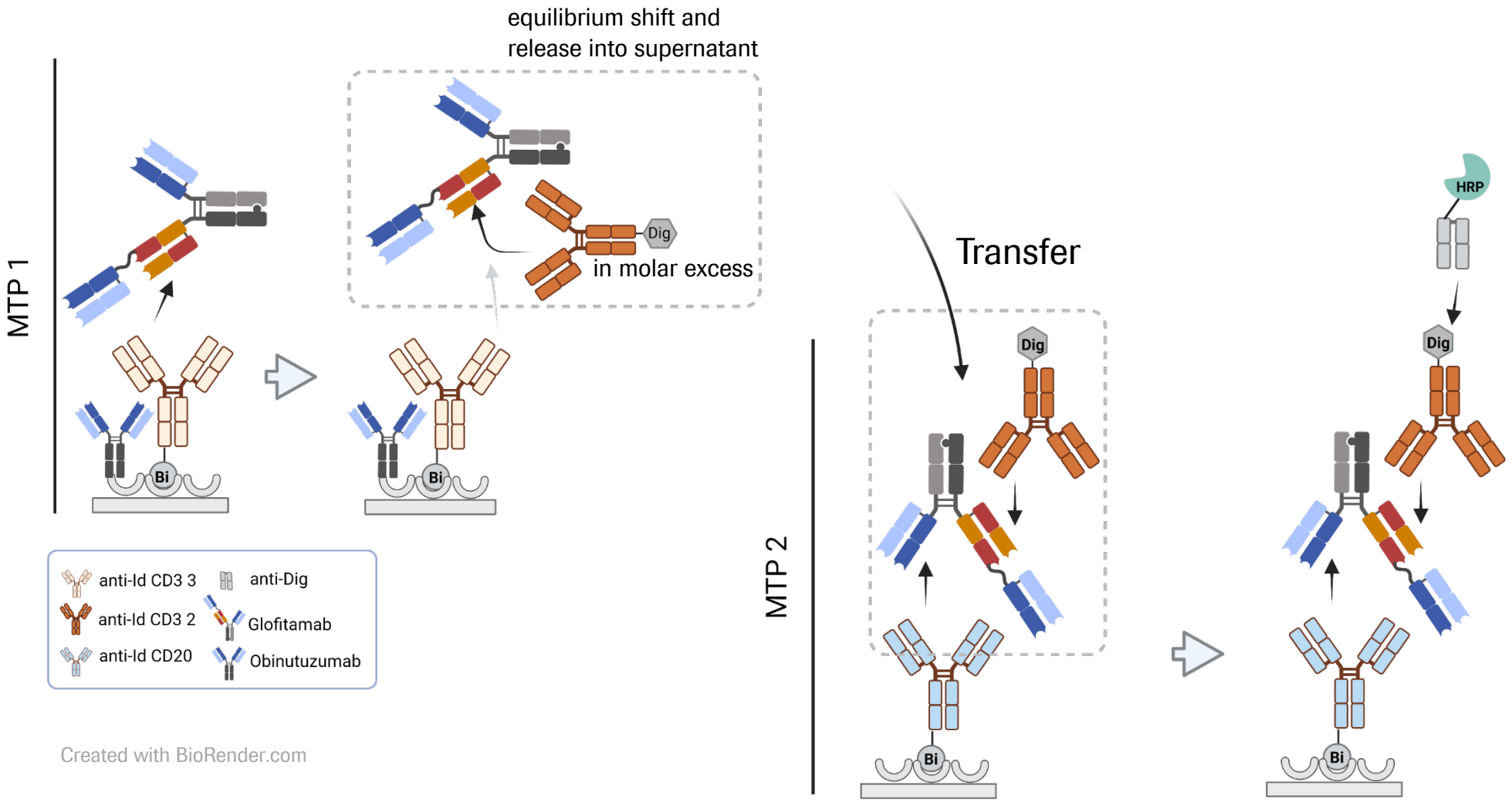
The Two Plate Glofitamab-specific clinical PK Assay



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How the right anti-idiotypic CD3 binder Antibodies were selected

Antibody Clone	k a (1/Ms)	k d (1/s)	t (1/2) [min]	KD nM
anti-Id CD3 1	1.96E+05	1.05E-04	110	0.54
anti-Id CD3 2	1.99E+05	1.11E-04	104	0.56
anti-Id CD3 3	1.66E+05	2.01E-04	55	1.21
Anti-Id CD3 4	2.55E+05	2.15E-03	5	8.43

Candidates for equilibrium shift

Candidates for capture on MTP1

Modeling informed selection of the capture antibody on MTP1

Capture	Yield after Capture on MTP1	Yield after Exchange	Max achievable Yield
anti-Id CD3 3	53%	32%	54%
anti-Id CD3 4	25%	22%	22%

based on the selected assay conditions (incl. a significant molar excess of the candidate used for the equilibrium shift)

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Performance of the Glofitamab –specific PK Assay in a GxP lab

- The method was **successfully validated in-house as well as at two independent GxP-regulated CRO laboratories** according to regulatory guidelines for pharmacokinetic assays
- Method **validation confirmed lack of interference** on the performance of the glofitamab-specific PK Assay **by obinutuzumab** of up to a serum concentration of 1 mg/mL
- In-study performance (cut-off July 2021): 261 total runs with **87.7% passing rate**

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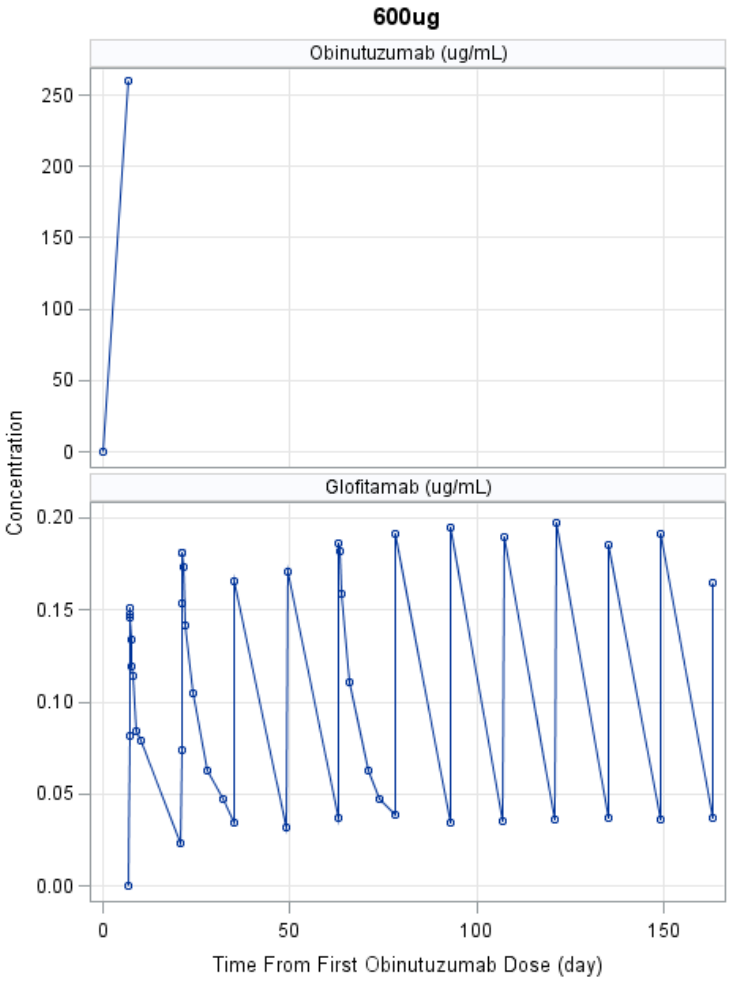
Application of the Glofitmab –specific PK Assay for routine analysis



Concentration profiles of Glofitamab are unaffected by Obinutuzumab

→ Data verify no interefence by obinutuzumab on the specific quantification of glofitamab in patient samples

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Conclusion

- **Combining biotherapeutics** with shared structures, that are relevant for pharmacologically activity, **can require very complex bioanalytical assay development** activities
- Using **two anti-idiotypic antibodies** with identical binding specificity to the CD3 binding domain of glofitamab but **which demonstrated different complex stability in combination with an applied molar excess to shift the equilibrium** offered the solution to develop the presented two plate glofitamab-specific PK assay
- Generation of **diverse bioanalytical reagents** and their **solid characterization** of the binding properties **are key**
- Don‘t shy away from **unorthodox assay procedures** – they may offer us the **opportunities to overcome the bioanalytical challenges of the future**

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Doing now what patients need next