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Development and Validation of a Trastuzumab/Pertuzumab Hybrid LC-MS Assay for Clinical Development

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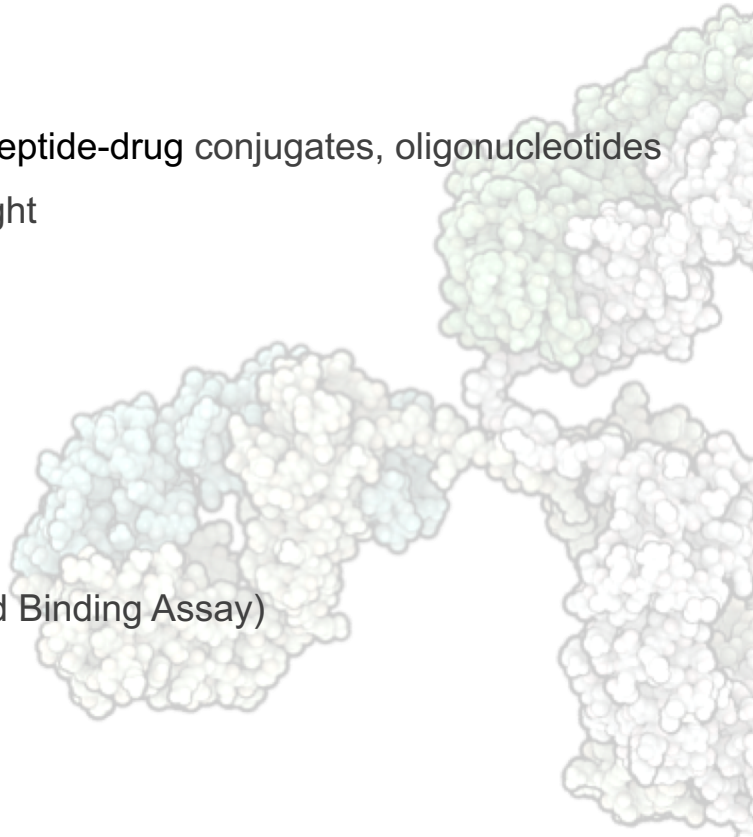
Ligand Binding or Mass Spectrometry?

KEY CRITERIA FOR DEFINING THE BIOANALYTICAL STRATEGY

- Proteins, therapeutic antibodies, multispecific, ADC's, peptides, peptide-drug conjugates, oligonucleotides
- Intrinsic molecular complexity/heterogeneity, large molecular weight

- Selectivity and sensitivity requirements
- Expected or demonstrated cross reactivity
- Stage of drug development (exploratory, preclinical, clinical)
- Regulatory aspects
- Availability of well-defined reagents (specific antibodies for Ligand Binding Assay)
- Timelines



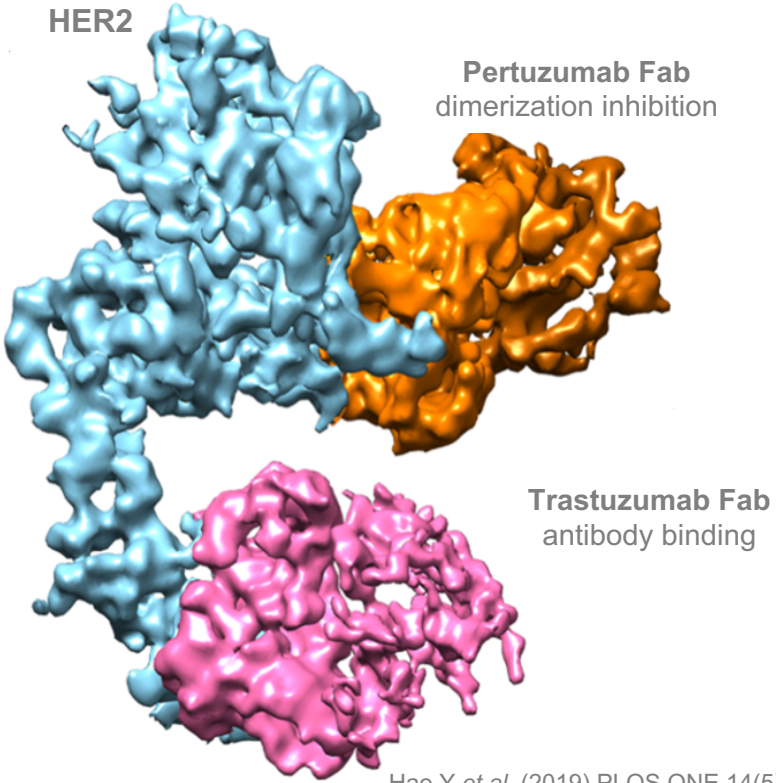
Pertuzumab/Trastuzumab

DUAL-AGENT HER2 BLOCKADE

- Human epidermal growth factor receptor 2 (HER2) is highly expressed in on the surface of various tumors
- Trastuzumab and Pertuzumab are therapeutic monoclonal antibodies that bind to different domains of HER2
- Complementary mechanism of action fully inhibits the receptor
- Used in combined cancer therapies

Bioanalytical Challenges

- Analytes cannot easily be distinguished and quantified in one ligand binding assay
- Low sample volume from patient studies

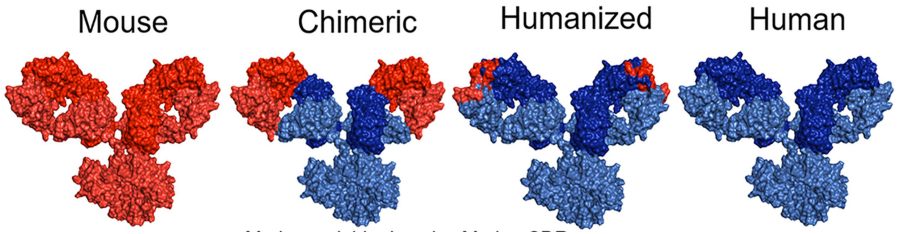


Hao Y *et al.* (2019) PLOS ONE 14(5)

Method Development

IN SILICO SEQUENCE ANALYSIS

- Surrogate peptide will determine selectivity and ability to multiplex
  - 6-25 amino acids
  - Hydrophobicity and length of the peptides (retention, ionization and fragmentation)
  - Cross-reactivity with endogenous proteins and co-medications



Adapted Lopes dos Santos (2018) Braz..J. Pharm. Sci.

N-mAb	*(IgG4)*	SDEQLKSGTASVVCLLNNFYPREAKVQWK**VDNALQSGNSQESVTEQDSK**DSTYSLSST
T-mAb	*(IgG1)*	SDEQLKSGTASVVCLLNNFYPREAKVQWK**VDNALQSGNSQESVTEQDSK**DSTYSLSST
P-mAb	*(IgG1)*	SDEQLKSGTASVVCLLNNFYPREAKVQWK**VDNALQSGNSQESVTEQDSK**DSTYSLSST
B-mAb	*(IgG1)*	SDEQLKSGTASVVCLLNNFYPREAKVQWK**VDNALQSGNSQESVTEQDSK**DSTYSLSST

generic

N-mAb	*(IgG4)*	KASGITFSNSGMHWVRQAPGK**GLEWVAVIWYDGSK**RYYADSVKGR**FTIS**RDNSKNTLF
T-mAb	*(IgG1)*	AASGFNIKDTYIHWVRQAPGKGLEWVAR**IYPNGYTR**YADSVKGR**FTISADTS**KNTAYL
P-mAb	*(IgG1)*	AASGFTFTDYTMDWVRQAPGK**GLEWVADVNPSGGSIYNQR**FKGR**FTLSVDR**SKNTLYL
B-mAb	*(IgG1)*	AASGYTFTNYGMNWVRQAPGK**GLEWVGWINTTGEPTYAADFK**RR**FTFSLDTS**KSTAYL

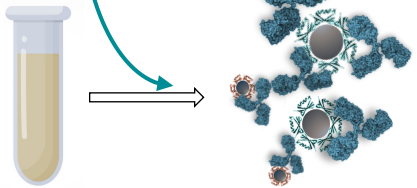
mAb-specific

Hybrid LC-LC/MS - General Experimental Setup

BOTTOM-UP QUANTITATIVE METHOD – PROTEOLYTIC PEPTIDE IS EQUIMOLAR TO PROTEIN CONCENTRATION

Internal standard
SIL or analogue

SIL extended peptide
Protein standard



Affinity Purification

magnetic beads
ready-to-use or customized

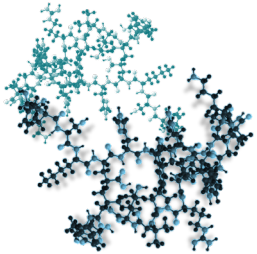
Protein A, L, G
Immuno-capture (Anti-Fc, Anti-ID, Anti-payload)
Functional pull-out (receptor, ligand, cofactor)

Sample treatment

Denaturation
Reduction
Alkylation

De-glycosylation

……



Proteolysis

Trypsin
LysC, GluC, AspN, …

SIL surrogate peptide

SPE

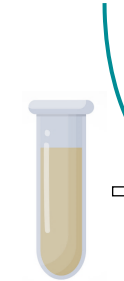
LC-MS/MS

Quantifier Surrogate peptide
(Qualifier peptides)

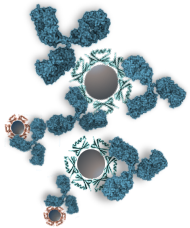
Hybrid LC-LC/MS - General Experimental Setup

BOTTOM-UP QUANTITATIVE METHOD – PROTEOLYTIC PEPTIDE IS EQUIMOLAR TO PROTEIN CONCENTRATION

SILu™Mab

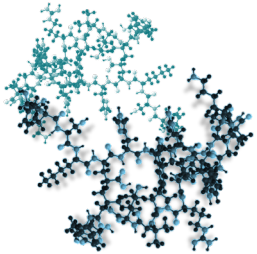


10 µl



Protein A magnetic beads

Reduction
Alkylation



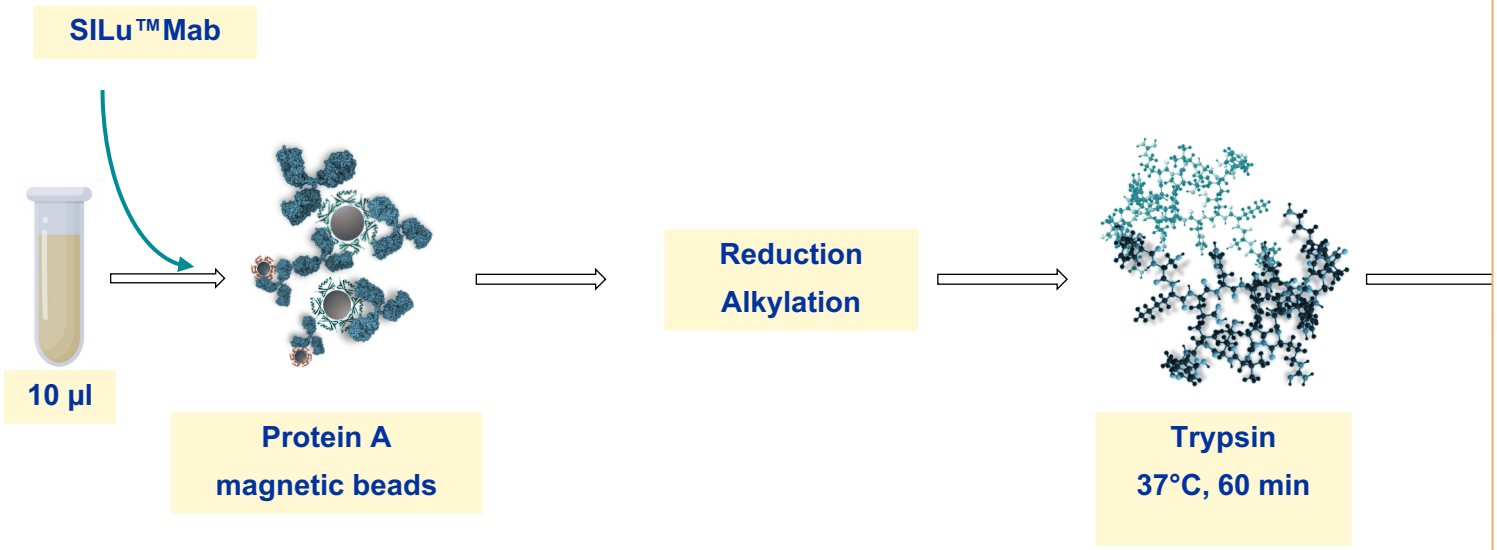
Trypsin
37°C, 60 min

Surrogate CDR peptides

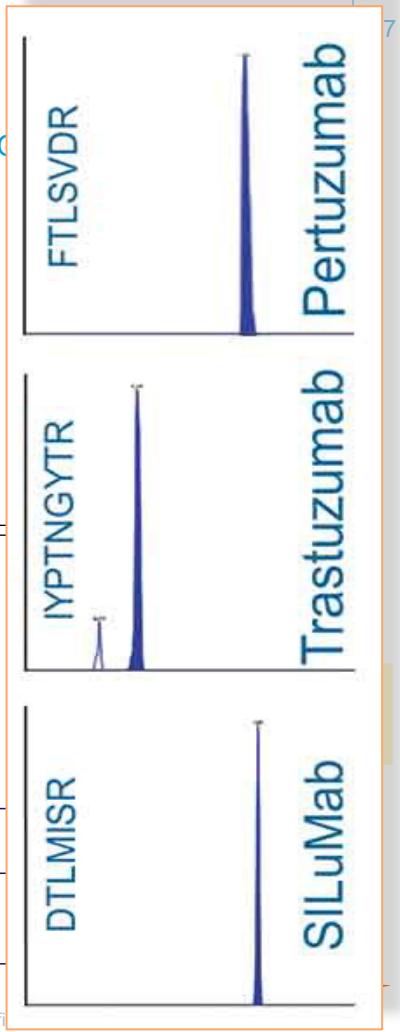
Analyte	Surrogate peptide	Q1 mass (amu)	Q3 mass (amu)	Time (min)
Trastuzumab	IYPTNGYTR	542.8	404.7	1.3
Pertuzumab	FTLSVDR	419.5	589.2	2.7
SILuMab	DTLMISR*($^{13}C_6{}^{15}N_4$)	423.2	516.3	2.2

Hybrid LC-LC/MS - General Experimental Setup

BOTTOM-UP QUANTITATIVE METHOD – PROTEOLYTIC PEPTIDE IS EQUIMOLAR TO PROTEIN C



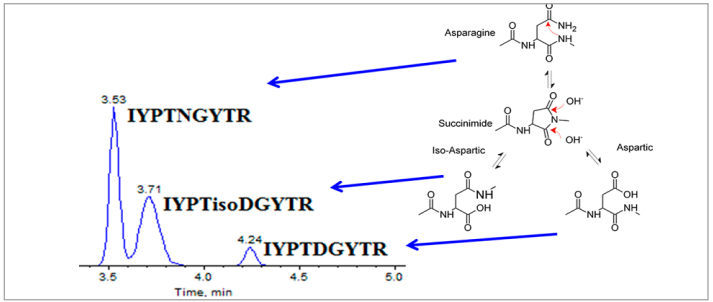
Analyte	Surrogate peptide
Trastuzumab	IYPTNGYTR
Pertuzumab	FTLSVDR
SILuMab	DTLMISR*($^{13}C_6{}^{15}N_4$)

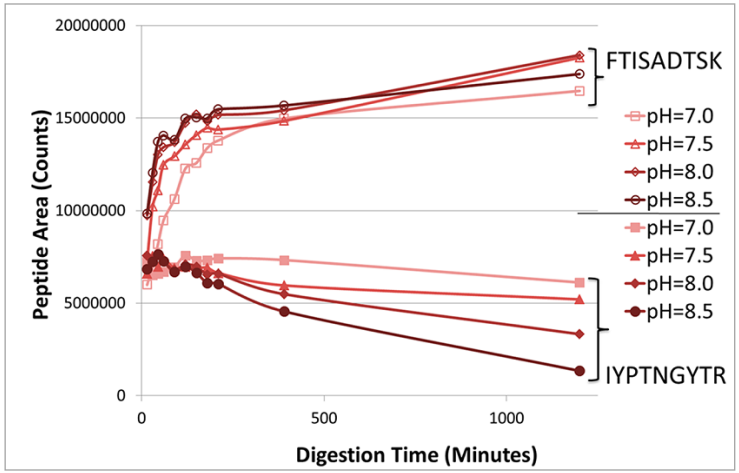


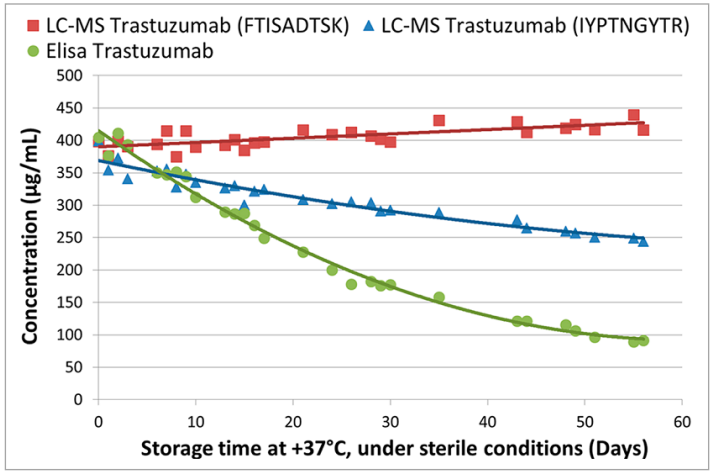
Deamidation of IYPTNGYTR Surrogate Peptide

NG motif in surrogate peptide indicates a susceptibility for deamidation

From IYPT**N**GYTR to IYPT**D**GYTR



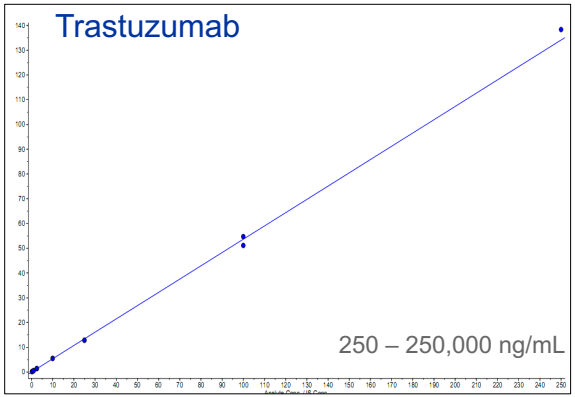




Bults P. *et al*. *Anal. Chem*. (2016) 88: 1871

Method Validated in Human Serum

Trastuzumab

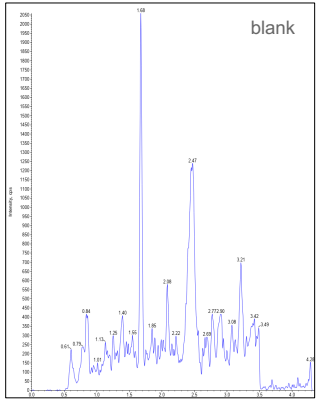


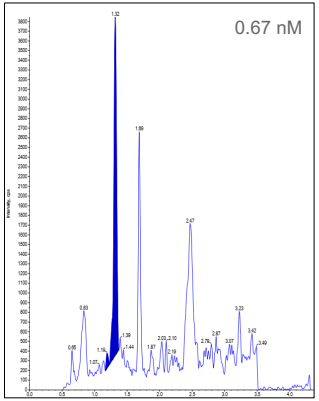
Precision (%CV)

	LLOQ	LQC	MQC	HQC
PA1	7.0	13.2	4.8	6.4
PA2	7.6	10.3	6.7	7.0
PA3	4.5	3.3	4.9	10.0

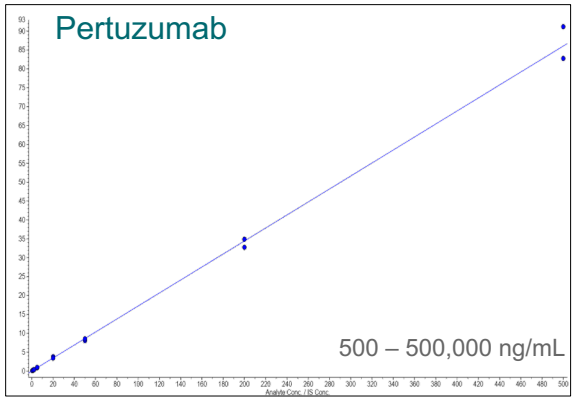
Accuracy (|%RE|)

	LLOQ	LQC	MQC	HQC
PA1	0.7	10.4	13.7	15.3
PA2	6.6	16.5	7.4	13.2
PA3	5.6	3.2	7.4	10.7





Pertuzumab



	LLOQ	LQC	MQC	HQC
PA1	8.5	8.2	4.0	4.2
PA2	18.9	5.8	14.8	5.2
PA3	8.6	4.0	5.7	9.8

Accuracy (|%RE|)

	LLOQ	LQC	MQC	HQC
PA1	0.7	10.4	13.7	15.3
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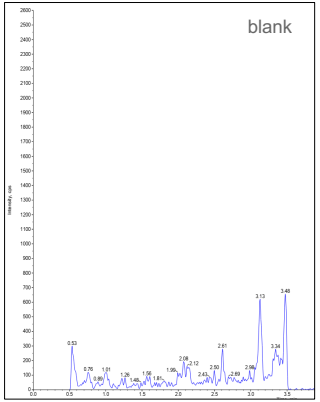
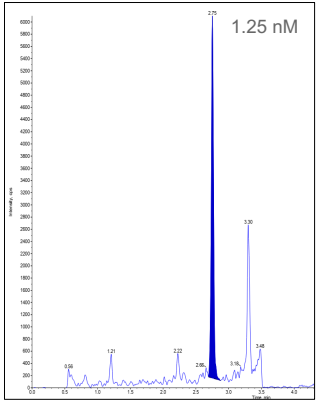
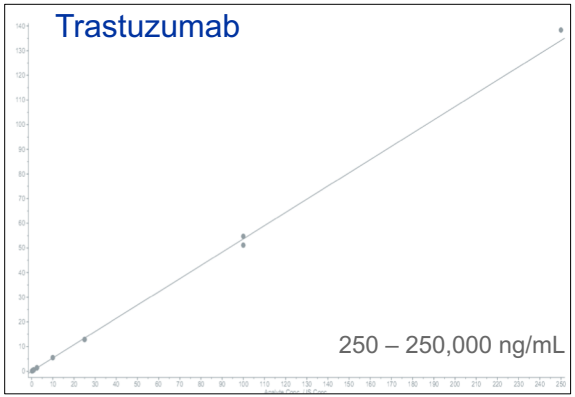




Schokker *et al*. (2020) *mAbs 12 (1), 1795492*

Method Validated in Human Serum

Trastuzumab



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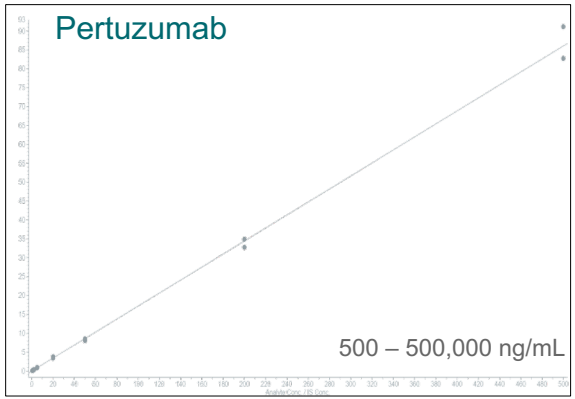
Accuracy (|%RE|)

	LLOQ	LQC	MQC	HQC
PA1	0.7	10.4	13.7	15.3
PA2	6.6	16.5	7.4	13.2
PA3	5.6	3.2	7.4	10.7

	LQC	MQC	HQC
Mean	0.717	19.7	202
SD	0.066	1.89	15
%RE	-4.4	-1.6	1.2
%CV	9.3	9.6	7.3

Pertuzumab



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PA2	18.9	5.8	14.8	5.2
PA3	8.6	4.0	5.7	9.8

Accuracy (|%RE|)

	LLOQ	LQC	MQC	HQC
PA1	0.7	10.4	13.7	15.3
PA2	6.6	16.5	7.4	13.2
PA3	5.6	3.2	7.4	10.7

	LQC	MQC	HQC
Mean	1.504	40.5	388
SD	0.112	2.58	23
%RE	0.3	1.3	-3.0
%CV	7.4	6.4	6.0

Schokker *et al.* (2020) *mAbs 12 (1), 1795492*

TRAP Study in EACs Patients

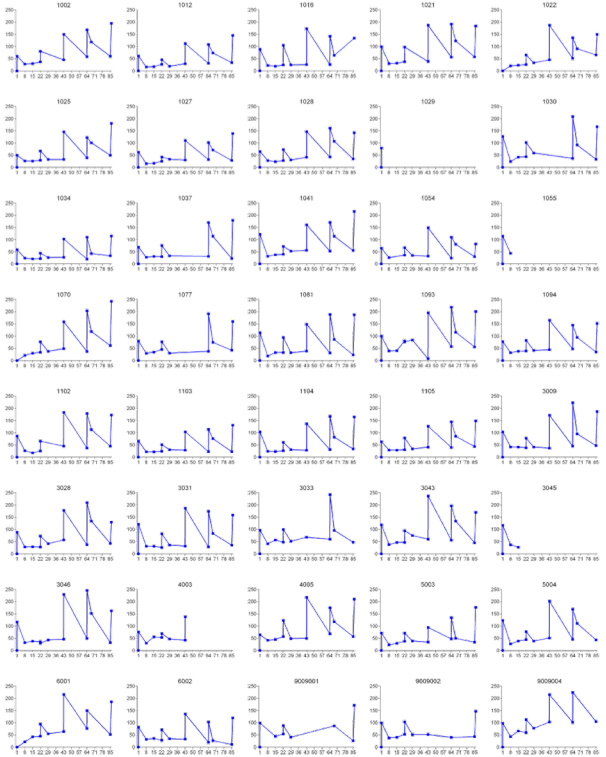
PHARMACOKINETICS OF TRASTUZUMAB AND PERTUZUMAB

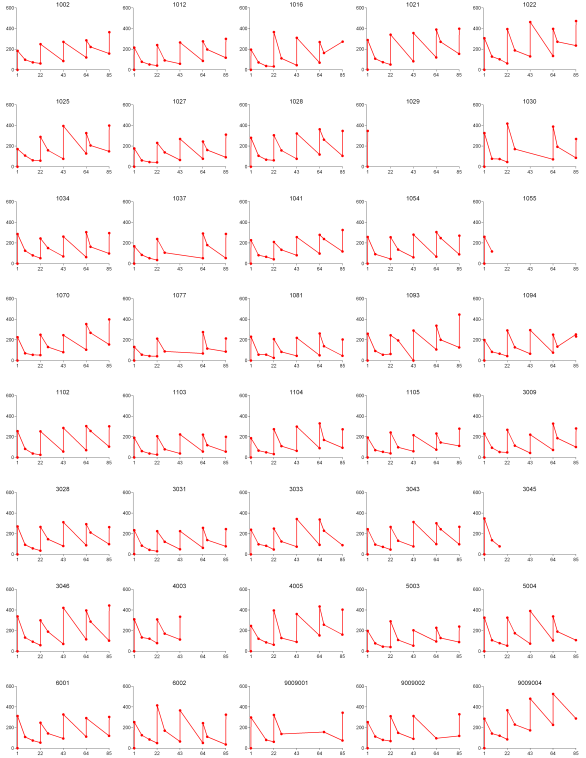
- 10–15% of breast cancer and esophagogastric cancer patients are HER2 positive, and HER2 can be used for targeted therapy in these patients
- 40 esophageal cancer patients (EACs) enrolled in seven centers in the Netherlands received chemoradiation in addition to:
  - Trastuzumab
    - 4 mg/kg on day 1
    - 2 mg/kg per week during weeks 2 to 6
    - 6 mg/kg per week during weeks 7, 10, and 13
  - Pertuzumab
    - 840 mg every 3 weeks
- PK and biomarkers to asses exposure–response relationship
- 57% subjects 3-year progression free
- Overall survival = 71% (higher for HER2 positive patients with a +3 overexpression)
- Pharmacokinetic analysis (670 study samples) did not correlate with survival or pathologic response

TRAP Study in EACs Patients

PHARMACOKINETICS OF TRASTUZUMAB AND PERTUZUMAB

Trastuzumab





Pertuzumab

Stroes C.I. *et al*. (2019) *J Clin Oncol*.

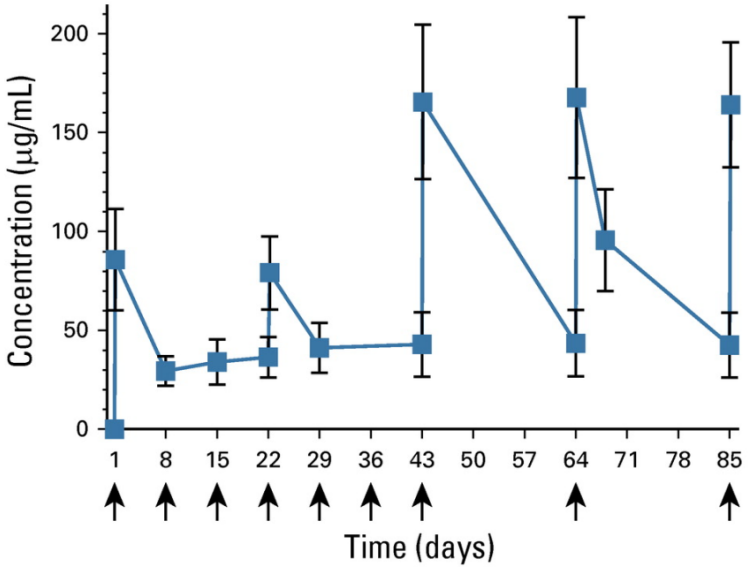
TRAP Study in EAC Patients

PHARMACOKINETICS OF TRASTUZUMAB AND PERTUZUMAB

Mean trough and peak concentrations in serum (mean C_{min} < 20 µg/mL)

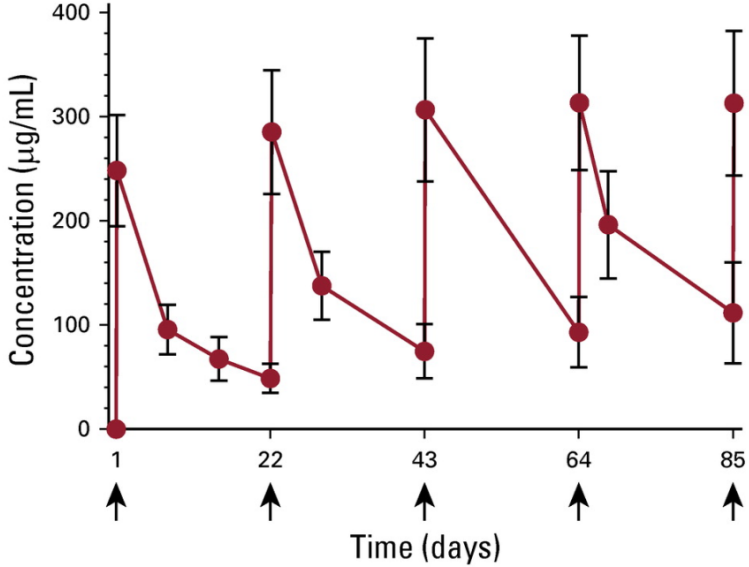
Trastuzumab

250 – 250,000 ng/mL



Pertuzumab

500 – 500,000 ng/mL



Schokker et al. (2020) mAbs 12 (1), 1795492; Stroes C.I. *et al*. (2019) *J Clin Oncol*.

Summary and Conclusions

- Increasing application of combined immuno-therapies → higher need for accurate multiplex PK assays
- Superior selectivity of hybrid LC-MS/MS is a valuable alternative to traditional ligand binding methods

- Accurate and selective for the simultaneous or individual determination of trastuzumab and pertuzumab
- Validated according to the current FDA/EMA guidelines for large molecule bioanalysis
- Robust and stable throughout a long period of time fit for monitoring patient studies
- Minimal sample volume
- Sufficient throughput (10 min injection-to-injection, >120 samples/day)
- Easily adaptable to multiplex quantifications of other mAb combinations in non-clinical and clinical studies

Schokker *et al*. (2020) *mAbs 12 (1), 1795492*

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- Martijn Hilhorst

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Thank You