



innovation for health & wellness

“trace & catch”

Instructions for Use

Trastuzumab

(Herclon[®], Herceptin[®]) ELISA

SHIKARI[®]

Q-TRAS

Enzyme immunoassay for the quantitative determination of Trastuzumab (Herceptin[®], Herclon[®]) in serum and plasma

REF TR-TRASv2



12 x8



2-8°C

Revision # 2.1 August 2017



Matriks Biotek[®] Laboratories
www.matriksbiotek.com

Contents	Page
Intended Use	3
Summary and Explanation.....	3
Test Principle	4
Warnings and Precautions.....	5
Storage and Stability of the Kit.....	6
Specimen Collection and Storage	6
Materials Supplied.....	7
Materials Required but not Supplied	8
Procedure Notes.....	8
Pre-Test Setup Instruction	9
Test Procedure.....	10
Quality Control	10
Calculation & Interpretation of Results	11
Assay Characteristics	12
Automation	12
References.....	13
Semi-Log Graph Paper	17
Semi-Log Graph Paper.....	18

	SHIKARI Q-TRAS
	Free Trastuzumab (Herclon [®] , Herceptin [®]) quantitative analyses
Required Volume (µl)	10
Total Time (min)	70
Sample	Serum, plasma
Sample Number	96
Detection Limit (ng/mL)	2
Spike Recovery (%)	Between 85-115
Shelf Life (year)	1

Intended Use

Enzyme immunoassay for the quantitative determination of **free Trastuzumab** (Herclon[®], Herceptin[®]) in serum and plasma. *Matriks Biotek[®] trastuzumab ELISA* has been developed for the quantitative analysis of free trastuzumab in serum and plasma samples at high specificity.

Summary and Explanation

The HER (or ErbB) family of transmembrane tyrosine kinase receptors is composed of four members, HER1 to HER4. HER2, a ligand-less Mr 185,000 receptor encoded by the neu proto-oncogene, is overexpressed in 25–30% of human breast cancer and has been associated with enhanced tumor aggressiveness and a high risk of relapse and death. Recent evidence indicates that HER2 amplifies the signal provided by other receptors of the ErbB family by heterodimerizing with them. The important biological role of HER2 in the signaling network that drives epithelial cell proliferation and transformation, together with its extracellular accessibility and its overexpression in some human tumors led to considering HER2 as an appropriate target for tumor-specific therapies.

The neu gene encodes a 185-kDa transmembrane glycoprotein, referred to as p185neu, HER2, or erbB-2, possessing intrinsic protein tyrosine kinase activity. The receptor consists of an extracellular domain, with four subdomains including two cysteine rich domains, a transmembrane domain, and an intracellular domain, consisting of a juxtamembrane region, a tyrosine kinase domain, and a carboxyl tail harboring autophosphorylation sites HER2 is homologous to, but distinct from, other members of the erbB family, which includes the epidermal growth factor receptor (EGFR or erbB-1), erbB-3, and erbB-4. The binding of cognate growth factors to these receptors regulates cell growth, proliferation, and differentiation through the activation of receptor tyrosine kinases, triggering an incompletely defined signal transduction cascade. Signal transduction by these receptors is believed to involve dimerization and oligomerization, both in the form of homo-oligomers and hetero-oligomers in various erbB receptor combinations.

Trastuzumab (Herclon[®], Herceptin[®]) is a recombinant DNA-derived humanized monoclonal antibody that selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). The antibody is an IgG1 kappa that contains human framework regions with the complementarity-determining regions of a murine anti-p185 HER2 antibody that binds to HER2. Trastuzumab is composed of 1,328 amino acids and has a molecular weight of 148 kDa.

Trastuzumab has antitumor activity against HER2-positive human breast tumor cells in laboratory models and is active for the treatment of women with HER2-overexpressing breast cancers. On the basis of trastuzumab clinical efficacy, this antibody was approved in 1998 for clinical use for HER2 overexpressing metastatic breast cancer. Trastuzumab seems to exert its antitumor effects by several mechanisms that are not yet completely understood. In HER2 overexpressing cells, trastuzumab markedly down-regulates HER2 expression by accelerating receptor endocytosis and degradation and inhibits cell cycle progression by inducing the formation of p27Kip1/Cdk2 complexes. Trastuzumab also induces antibody-dependent cell-mediated cytotoxicity against the HER2 expressing tumor cells in animal models. This process is regulated by antibody receptors FcγRIII and FcγRIIB on myeloid cells. Other additional mechanisms that have been proposed include suppression by trastuzumab of angiogenesis and metastasis.

The pharmacokinetics of trastuzumab according to the prescribing data; The pharmacokinetics of trastuzumab were studied in women with metastatic breast cancer. Short duration intravenous infusions of 10 to 500 mg trastuzumab once weekly demonstrated dose-dependent pharmacokinetics. Mean half-life increased and clearance decreased with increasing dose level. The half-life averaged 2 and 12 days at the 10 and 500 mg dose levels, respectively. The volume of distribution of trastuzumab was approximately that of serum volume (44 mL/kg). At the highest weekly dose studied (500 mg), mean peak serum concentrations were 377 mcg/mL. In studies using an initial dose of 4 mg/kg followed by a weekly dose of 2 mg/kg, a mean half-life of 6 days (range 1-32 days) was observed. Between weeks 16 and 32, trastuzumab serum concentrations reached a steady state with mean trough and peak concentrations of approximately 9 mcg/mL and 123 mcg/mL, respectively.

In a study of women receiving adjuvant therapy for breast cancer, a mean half-life of trastuzumab of 16 days (range: 11-23 days) was observed after an initial dose of 8 mg/kg followed by a dose of 6 mg/kg every three weeks. Between weeks 6 and 37, trastuzumab serum concentrations reached a steady-state with mean trough and peak concentrations of 63 mcg/mL and 216 mcg/mL, respectively.

In this context, identification of biomarkers for (non-)response and risk factors for adverse drug reactions relating to serum concentrations and therapeutic drug monitoring of trastuzumab might be very helpful.

Test Principle

Solid phase enzyme-linked immunosorbent assay (ELISA). Standards and samples (serum or plasma) are incubated in the microtitre plate coated with ligand antigen

(recombinant human HER2/ErbB2/CD340) for trastuzumab (Herclon®, Herceptin®). After incubation, the wells are washed. In the next step, a horse radish peroxidase (HRP) conjugated is added. Following incubation, wells are washed and the bound enzymatic activity is detected by addition of TMB chromogen-substrate. The colour developed is proportional to the amount of trastuzumab in the sample or standard. Results of samples are determined using the standard curve constructed.

Warnings and Precautions

1. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood. For further information (clinical background, test performance, automation protocols, alternative applications, literature, etc.) please refer to the local distributor.
3. In case of severe damage of the kit package please contact Matriks Biotek® or your supplier in written form, latest one week after receiving the kit. Do not use damaged components in test runs, but keep safe for complaint related issues.
4. Obey lot number and expiry date. Do not mix reagents of different lots.
5. Follow good laboratory practice and safety guidelines. Wear lab coats, disposable latex gloves and protective glasses where necessary.
6. Reagents of this kit containing hazardous material may cause eye and skin irritations. See MATERIALS SUPPLIED and labels for details.
7. Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guidelines or regulations.
8. Avoid contact with Stop solution. It may cause skin irritations and burns.
9. Some reagents contain sodium azide (NaN_3) as preservatives. In case of contact with eyes or skin, flush immediately with water. NaN_3 may react with lead and copper plumbing to form explosive metal azides. When disposing reagents, flush with large volume of water to avoid azide build-up.
10. All reagents of this test kit containing human serum or plasma have been tested and were found negative for HIV I/II, HBsAg and HCV by FDA approved procedures. However, a presence of these or other infectious agents cannot be excluded absolutely and therefore reagents should be treated as potential biohazards in use and for disposal.

Storage and Stability

The kit is shipped at ambient temperature and should be stored at 2-8°C. Keep away from heat or direct sun light. The storage and stability of specimen and prepared reagents is stated in the corresponding chapters.

Specimen Collection and Storage

Serum, Plasma (EDTA, Heparin)*

The usual precautions for venipuncture should be observed. It is important to preserve the chemical integrity of a blood specimen from the moment it is collected until it is assayed. Do not use grossly hemolytic, icteric or grossly lipemic specimens. Samples appearing turbid should be centrifuged before testing to remove any particulate material.

Storage:	2-8°C	-20°C	Keep away from heat or direct sun light Avoid repeated freeze-thaw cycles
Stability:	2 d	6 mon	

*Trastuzumab (Herclon®, Herceptin®) infusion camouflages/masks the presence of antibody to trastuzumab in serum/plasma samples. Therefore, blood sampling time is critical for detection of TRAS. The Matriks Biotek® Laboratories suggests to obtain blood sample just before the infusion of Trastuzumab (Herclon®, Herceptin®) or at least 2 weeks after the infusion of Trastuzumab (Herclon®, Herceptin®).

Materials Supplied

1 x 12 x 8	MTP	Microtiter Plate Break apart strips. Microtiter plate with 12 rows each of 8 wells precoated with recombinant human HER2/ErbB2/CD340.
7 x 0.3 mL	STND A-E HIGH CNTRL LOW CNTRL	Trastuzumab Standards A-E, Concentrate (10X), High Level Control, Low Level Control 3000; 1000; 333; 111; 0 nanogram/mL. Ready to use. Contains trastuzumab, human serum, stabilizers and <0.1% NaN ₃ .
1 x 30 mL	ASSAY BUF	Assay Buffer, Concentrate (5X) Blue colored. Ready to use. Contains proteins and <0.1% NaN ₃ .
1 x 12 mL	HRP CONJ	Horse radish peroxidase-Conjugated Probe Red colored. Ready to use. Contains HRP-conjugated preservatives and stabilizers.
1 x 12 mL	TMB SUBS	TMB Substrate Solution Ready to use. Contains TMB
1 x 12 mL	TMB STOP	TMB Stop Solution Ready to use. 1N HCl.
1 x 50 mL	WASHBUF CONC	Wash Buffer, Concentrate (20x) Contains Buffer with Tween 20.
2 x 1	ADH FILM	Adhesive Film For sealing of Microtiter Plate during incubation
2 x 1	SLGP	Semi-Log Graph Paper For constructing standard curve and calculation of results.

Materials Required But Not Supplied

1. Micropipettes (< 3% CV) and tips to deliver 5-1000 μ L.
2. Calibrated measures.
3. Tubes (1 mL) for sample dilution.
4. Wash bottle, automated or semi-automated microtiter plate washing system
5. Microtiter plate reader capable of reading absorbance at 450/650 nm.
6. Bidistilled or deionised water, paper towels, pipette tips and timer.

Procedure Notes

1. Any improper handling of samples or modification of the test procedure may influence the results. *The indicated pipetting volumes, incubation times, temperatures and pretreatment steps have to be performed strictly according to the instructions.* Use calibrated pipettes and devices only.
2. Once the test has been started, all steps should be completed without interruption. Make sure that required reagents, materials and devices are prepared ready at the appropriate time. Allow all reagents and specimens to reach room temperature (18-25 °C) and gently swirl each vial of liquid reagent and sample before use. Mix reagents without foaming.
3. Avoid contamination of reagents, pipettes and wells/tubes. Use new disposable plastic pipette tips for each reagent, standard or specimen. Do not interchange caps. Always cap not used vials. Do not reuse wells/tubes or reagents.
4. Use a pipetting scheme to verify an appropriate plate layout.
5. *Incubation time affects results. All wells should be handled in the same order and time sequences. It is recommended to use an 8-channel Micropipettor for pipetting of solutions in all wells.*

- Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Buffer, and that there are no residues in the wells.
- Humidity affects the coated wells/tubes. Do not open the pouch until it reaches room temperature. Unused wells/tubes should be returned immediately to the resealed pouch including the desiccant.

Pre-test Setup Instructions

Preparation of Components

Dilute/dissolve	Component	With	Diluent	Relation	Remarks	Storage	Stability
10 mL	Wash Buffer*	Up to 200 mL	bidist. Water	1:20	Warm up at 37°C to dissolve crystals. Mix vigorously.	2-8 °C	2 w
10 mL	Assay Buffer*	Up to 50 mL	bidist. Water	1:5		2-8 °C	2 w

*Prepare Wash Buffer and Assay Buffer before starting assay procedure.

Dilution of Standards and Samples (serum/plasma)*

Sample	To be diluted	With	Remarks
Standards	1:10	Assay Buffer	For dilution at 1:10; 20 µl Standard + 180 µl Assay Buffer
Serum/Plasma	1:1000	Assay Buffer	First; for dilution at 1:10; 10 µl Standard + 90 µl Assay Buffer Second; for dilution at 1:100; 5 µl Standard + 495 µl Assay Buffer

Patient samples with a concentration of trastuzumab above the measuring range are to be rated as > "Highest Standard (Standard A)". The result must not be extrapolated. The patient sample in question should be further diluted with Assay Buffer and retested.

Test Procedure

1	Dilute each of the standards and samples (serum/plasma) using Diluted Assay Buffer as described in "Dilution of Standards and Samples (serum/plasma)" section.
2	Pipette 100 µL of each Diluted Standards, High Level Control, Low Level Control and Diluted Samples into the respective wells of microtiter plate. Wells A1: Standard A B1: Standard B C1: Standard C D1: Standard D E1: Standard E F1: High Level Control G1: Low Level Control H1 and on: Sample (Serum / Plasma)
3	Cover the plate with adhesive film. Incubate 30 min at room temperature (18-25°C).
4	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
5	Pipette 100 µL of ready-to use HRP Conjugate into each well.
6	Cover the plate with adhesive film. Incubate 30 min at room temperature (18-25°C).
7	Remove adhesive film. Discard incubation solution. Wash plate 3 times each with 300 µL of diluted Wash Buffer . Remove excess solution by tapping the inverted plate on a paper towel.
8	Pipette 100 µL of TMB Substrate Solution into each well.
9	Incubate 10 min (without adhesive film) at room temperature (18-25°C) in the dark .
10	Stop the substrate reaction by adding 100 µL of Stop Solution into each well. Color changes from blue to yellow. Briefly mix contents by gently shaking the plate.
11	Measure optical density with a photometer at 450/650 nm within 15 min after pipetting of the Stop Solution.

Quality Control

The test results are only valid only if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable standards/laws. All standards must be found within the acceptable ranges as stated above and/or label. If the criteria are not met, the run is not valid and should be repeated. In case of any deviation the following technical issues should be proven: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, incubation conditions and washing methods.

Calculation & Interpretation of Results

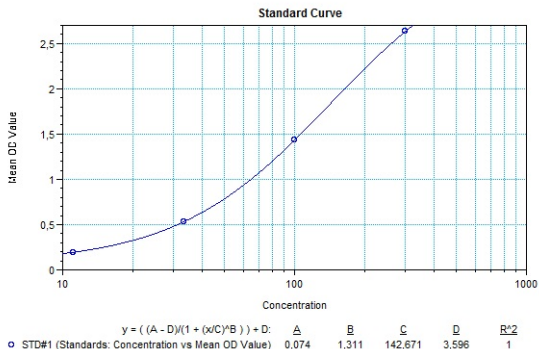
1. Using the diluted standards (300; 100; 33; 11; 0 ng/mL) disregarding zero standard, construct a standard curve by plotting the OD_{450/650} nm for each of 4 standards on the vertical (Y-axis) axis versus the corresponding trastuzumab concentration on the horizontal (X-axis) axis, thus creating a standard curve by 4 points obtained.
2. The concentration of the samples can be read directly from this standard curve. Using the absorbance value for each sample, determine the corresponding concentration of trastuzumab from the standard curve. Find the absorbance value on the Y-axis and extend a horizontal line to the curve. At the point of intersection, extend a vertical line to the X-axis and read the trastuzumab concentration for the unknown sample.
3. If computer data regression is going to be used, we recommend primarily "4 Parameter Logistic (4PL)" or secondly the "point-to-point calculation".
4. To obtain the exact values of the samples, the concentration determined from the standard-curve must be multiplied by the dilution factor (10x). Any sample reading greater than the highest standard should be further diluted appropriately with Assay Buffer and retested. Therefore, if the pre-diluted samples have been further diluted, the concentration determined from the standard curve must be multiplied by the further dilution factor.

E.g.; If the pre-diluted sample further diluted in a ratio of 1:10 then results should be multiplied by 100.

5. Automated method: Computer programs can also generally give a good fit.

Typical Calibration Curve

(Example. Do not use for calculation!)



Standard	Concentration (ng/mL)	Mean OD 450/650
A	300	2,631
B	100	1,432
C	33	0,530
D	11	0,194
E	0	0,032

Assay Characteristics

- Specificity:** Except for trastuzumab, there is no cross reaction with other therapeutic antibodies and native serum immunoglobins.
- Sensitivity:** Quantitative limit: 11 ng/mL
Detection limit: 2 ng/mL
- Precision Of Kit:**
Intra-assay CV: <15% for trastuzumab range 300-11 ng/mL.
Inter-assay CV: <15% for trastuzumab range 300-11 ng/mL.
- Recovery:** Recovery rate was found to be between 85-115% with native human serum spiked with Cmax (123µgr/mL) and Cmin (27mgr/mL) values of Trastuzumab.

Automation

Experiments have shown that the *Matriks Biotek*® SHIKARI® Trastuzumab ELISA is also suitable to run on an automated ELISA processor.

References

1. Molina MA, Codony-Servat J, Albanell J, Rojo F, Arribas J, Baselga J., Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. *Cancer Res.* 2001 Jun 15;61(12):4744-9.
2. Groenen, L. C., Nice, E. C., and Burgess, A. W. Structure-function relationships for the EGF/TGF- α family of mitogens. *Growth Factors*, 11: 235–257, 1994.
3. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* (Wash. DC), 235: 177–182, 1987.
4. Klapper, L. N., Kirschbaum, M. H., Sela, M., and Yarden, Y. Biochemical and clinical implications of the ErbB/HER signaling network of growth factor receptors. *Adv. Cancer Res.*, 77: 25–79, 2000.
5. Fendly B. M., Winget M., Hudziak R. M., Lipari M. T., Napier M. A., Ullrich A. Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor or HER2/neu gene product. *Cancer Res.*, 50:1550-1558, 1990.
6. Carter P., Presta L., Gorman C. M., Ridgway J. B., Henner D., Wong W. L., Rowland A. M., Kotts C., Carver M. E., Shepard H. M. Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc. Natl. Acad. Sci. USA*, 89:4285-4289, 1992.
7. Baselga J., Norton L., Albanell J., Kim Y. M., Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. *Cancer Res.*, 58:2825-2831, 1998.
8. *Slamon D., Leyland-Jones B., Shak S., Paton V., Bajamonde A., Fleming T., Eiermann W., Wolter J., Baselga J., Norton L. Addition of Herceptin (humanized anti-HER2 antibody) to first line chemotherapy for HER2 overexpressing metastatic breast cancer (HER2+/MBC) markedly increases anticancer activity: a randomized, multinational controlled Phase III trial (meeting abstract). Proc. Am. Soc. Clin. Oncol., 17:3771998.*
9. Pegram M. D., Lipton A., Hayes D. F., Weber B. L., Baselga J. M., Tripathy D., Baly D., Baughman S. A., Twaddell T., Glaspy J. A., Slamon D. J. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. *J. Clin. Oncol.*, 16:2659-2671, 1998.
10. Baselga J., Tripathy D., Mendelsohn J., Baughman S., Benz C. C., Dantis L., Sklarin N. T., Seidman A.D., Hudis C. A., Moore J., Rosen P. P., Twaddell T., Henderson I. C., Norton L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *J. Clin. Oncol.*, 14:737-744, 1996.

11. Sliwkowski M. X., Lofgren J. A., Lewis G. D., Hotaling T. E., Fendly B. M., Fox J. Nonclinical studies addressing the mechanism of action of Trastuzumab (Herceptin). *Semin. Oncol.*, 26 (Suppl. 12):60-70, 1999.
12. Sarup J. C., Johnson R. M. K. L. K., Fendly B. M., Lipari M. T., Napier M. A., Ullrich A., Shepard H.M. Characterization of an anti-p185HER2 monoclonal antibody that stimulates receptor function and inhibits tumor cell growth. *Growth Reg.*, 1:72-82, 1991.
13. Lane H. A., Beuvink I., Motoyama A. B., Daly J. M., Neve R. M., Hynes N. E. ErbB2 potentiates breast tumor proliferation through modulation of p27Kip1/Cdk2 complex formation: receptor overexpression does not determine growth dependency. *Mol. Cell Biol.*, 20:3210-3223, 2000.
14. Clynes R. A., Towers T. L., Presta L. G., Ravetch J. V. Inhibitory Fc receptors modulate in vivo cytotoxicity against tumor targets. *Nat. Med.*, 6:443-446, 2000.
15. Petit A. M., Rak J., Hung M. C., Rockwell P., Goldstein N., Fendly B., Kerbel R. S. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases downregulate vascular endothelial growth factor production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors. *Am. J. Pathol.*, 151:1523-1530, 1997.
16. Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J., Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts., *Cancer Res.* 1998 Jul 1;58(13):2825-31.
17. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D, Baly D, Baughman SA, Twaddell T, Glaspy JA, Slamon DJ., Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neuoverexpressing metastatic breast cancer refractory to chemotherapy treatment., *J Clin Oncol.* 1998 Aug;16(8):2659-71
18. Wong WM., Drug update: trastuzumab: anti-HER2 antibody for treatment of metastatic breast cancer., *Cancer Pract.* 1999 Jan-Feb;7(1):48-50.
19. Pegram MD, Pauletti G, Slamon DJ. ,HER-2/neu as a predictive marker of response to breast cancer therapy., *Breast Cancer Res Treat.* 1998;52(1-3):65-77.
20. Hanna W, Kahn HJ, Trudeau M., Evaluation of HER-2/neu (erbB-2) status in breast cancer: from bench to bedside., *Mod Pathol.* 1999 Aug;12(8):827-34
21. Beuzeboc P, Scholl S, Garau XS, Vincent-Salomon A, Cremoux PD, Couturier J, Palangié T, Pouillart P., Herceptin, a monoclonal humanized antibody anti-HER2: a major therapeutic progress in breast cancers overexpressing this oncogene?., *Bull Cancer.* 1999 Jun;86(6):544-9
22. Pegram M, Hsu S, Lewis G, Pietras R, Beryt M, Sliwkowski M, Coombs D, Baly D, Kabbinnar F, Slamon D., Inhibitory effects of combinations of HER-2/neu antibody and **chemotherapeutic agents used for treatment of human breast cancers.**, *Oncogene.* 1999 Apr 1;18(13):2241-51

23. Goldenberg MM., Trastuzumab, a recombinant DNA-derived humanized monoclonal antibody, a novel agent for the treatment of metastatic breast cancer., *Clin Ther.* 1999 Feb;21(2):309-18
24. Brenner TL, Adams VR., First MAb approved for treatment of metastatic breast cancer., *J Am Pharm Assoc (Wash).* 1999 Mar-Apr;39(2):236-8
25. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L, Sklarin NT, Seidman AD, Hudis CA, Moore J, Rosen PP, Twaddell T, Henderson IC, Norton L., Phase II study of weekly intravenous trastuzumab (Herceptin) in patients with HER2/neu-overexpressing metastatic breast cancer., *Semin Oncol.* 1999 Aug;26(4 Suppl 12):78-83.
26. Shak S., Overview of the trastuzumab (Herceptin) anti-HER2 monoclonal antibody clinical program in HER2-overexpressing metastatic breast cancer. Herceptin Multinational Investigator Study Group., *Semin Oncol.* 1999 Aug;26(4 Suppl 12):71-7.
27. Schaller G, Bangemann N, Becker C, Bühler H, Opri F, Weitzel HK., Therapy of metastatic breast cancer with humanized antibodies against the HER2 receptor protein., *J Cancer Res Clin Oncol.* 1999 Aug-Sep;125(8-9):520-4
28. Albanell J, Baselga J., The ErbB receptors as targets for breast cancer therapy., *J Mammary Gland Biol Neoplasia.* 1999 Oct;4(4):337-51.
29. Kunisue H, Kurebayashi J, Otsuki T, Tang CK, Kurosumi M, Yamamoto S, Tanaka K, Doihara H, Shimizu N, Sonoo H., Anti-HER2 antibody enhances the growth inhibitory effect of anti-oestrogen on breast cancer cells expressing both oestrogen receptors and HER2., *Br J Cancer.* 2000 Jan;82(1):46-51.
30. Tokuda Y, Watanabe T, Omuro Y, Ando M, Katsumata N, Okumura A, Ohta M, Fujii H, Sasaki Y, Niwa T, Tajima T., Dose escalation and pharmacokinetic study of a humanized anti-HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer., *Br J Cancer.* 1999 Dec;81(8):1419-25.
31. Dillman RO., Infusion reactions associated with the therapeutic use of monoclonal antibodies in the treatment of malignancy., *Cancer Metastasis Rev.* 1999;18(4):465-71.
32. Dillman RO., Perceptions of Herceptin: a monoclonal antibody for the treatment of breast cancer., *Cancer Biother Radiopharm.* 1999 Feb;14(1):5-10.
33. Pestalozzi BC, Brignoli S., Trastuzumab in CSF., *J Clin Oncol.* 2000 Jun;18(11):2349-51
34. Pegram MD, Konecny G, Slamon DJ., The molecular and cellular biology of HER2/neu gene amplification/overexpression and the clinical development of herceptin (trastuzumab) therapy for breast cancer., *Cancer Treat Res.* 2000;103:57-75.
35. Stebbing J, Copson E, O'Reilly S., Herceptin (trastuzumab) in advanced breast cancer., *Cancer Treat Rev.* 2000 Aug;26(4):287-90.
36. Feldman AM, Lorell BH, Reis SE., Trastuzumab in the treatment of metastatic breast cancer : anticancer therapy versus cardiotoxicity., *Circulation.* 2000 Jul 18;102(3):272-4.
37. Sakamoto G, Mitsuyama S., New molecule-targeting therapy with herceptin (trastuzumab), an anti-HER2 (c-erbB-2) monoclonal antibody., *Breast Cancer.* 2000;7(4):350-7.
38. Pittsley K., Trastuzumab., *Clin J Oncol Nurs.* 2000 Sep-Oct;4(5):235-6.

39. Jamieson D, Cresti N, Verrill MW, Boddy AV., Development and validation of cell-based ELISA for the quantification of trastuzumab in human plasma., *J Immunol Methods*. 2009 Jun 30;345(1-2):106-11. doi: 10.1016/j.jim.2009.04.006.
40. Petersen ER, Sørensen PD, Jakobsen EH, Madsen JS, Brandslund I., Serum HER-2 predicts response and resistance to trastuzumab treatment in breast cancer., *Clin Chem Lab Med*. 2013 Feb 18:1-10. doi: 10.1515/cclm-2012-0558.
41. Leyland-Jones B, Colomer R, Trudeau ME, Wardley A, Latreille J, Cameron D, Cubedo R, Al-Sakaff N, Feyereislova A, Catalani O, Fukushima Y, Brewster M, Cortés J., Intensive loading dose of trastuzumab achieves higher-than-steady-state serum concentrations and is well tolerated., *J Clin Oncol*. 2010 Feb 20;28(6):960-6. doi: 10.1200/JCO.2009.23.1910.
42. Preithner S, Elm S, Lippold S, Locher M, Wolf A, da Silva AJ, Baeuerle PA, Prang NS., High concentrations of therapeutic IgG1 antibodies are needed to compensate for inhibition of antibody-dependent cellular cytotoxicity by excess endogenous immunoglobulin G., *Mol Immunol*. 2006 Mar;43(8):1183-93.
43. Baselga J, Carbonell X, Castañeda-Soto NJ, Clemens M, Green M, Harvey V, Morales S, Barton C, Ghahramani P., Phase II study of efficacy, safety, and pharmacokinetics of trastuzumab monotherapy administered on a 3-weekly schedule., *J Clin Oncol*. 2005 Apr 1;23(10):2162-71.
44. Leyland-Jones B, Gelmon K, Ayoub JP, Arnold A, Verma S, Dias R, Ghahramani P., Pharmacokinetics, safety, and efficacy of trastuzumab administered every three weeks in combination with paclitaxel., *J Clin Oncol*. 2003 Nov 1;21(21):3965-71.
45. Leyland-Jones B., Dose scheduling--Herceptin., *Oncology*. 2001;61 Suppl 2:31-6. Review.
46. Tokuda Y, Watanabe T, Omuro Y, Ando M, Katsumata N, Okumura A, Ohta M, Fujii H, Sasaki Y, Niwa T, Tajima T., Dose escalation and pharmacokinetic study of a humanized anti-HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer., *Br J Cancer*. 1999 Dec;81(8):1419-25.
47. Wong JY, Raubitschek A, Yamauchi D, Williams LE, Wu AM, Yazaki P, Shively JE, Colcher D, Somlo G., A pretherapy biodistribution and dosimetry study of indium-111-radiolabeled trastuzumab in patients with human epidermal growth factor receptor 2-overexpressing breast cancer., *Cancer Biother Radiopharm*. 2010 Aug;25(4):387-94. doi: 10.1089/cbr.2010.0783.
48. Alvarez-Rueda N, Ladjemi MZ, Béhar G, Cognac S, Pugnière M, Roquet F, Bascoul-Mollevi C, Baty D, Pèlerin A, Navarro-Teulon I., A llama single domain anti-idiotypic antibody mimicking HER2 as a vaccine: Immunogenicity and efficacy., *Vaccine*. 2009 Jul 30;27(35):4826-33. doi: 10.1016/j.vaccine.2009.05.067.
49. Magdelaine-Beuzelin C, Kaas Q, Wehbi V, Ohresser M, Jefferis R, Lefranc MP, Watier H., Structure-function relationships of the variable domains of monoclonal antibodies approved for cancer treatment., *Crit Rev Oncol Hematol*. 2007 Dec;64(3):210-25.
50. Taylor C, Hershman D, Shah N, Suci-Foca N, Petrylak DP, Taub R, Vahdat L, Cheng B, Pegram M, Knutson KL, Clynes R., Augmented HER-2 specific immunity during treatment with trastuzumab and chemotherapy., *Clin Cancer Res*. 2007 Sep 1;13(17):5133-43..

