Application of intrathecal trastuzumab (Herceptin™) for treatment of meningeal carcinomatosis in HER2-overexpressing metastatic breast cancer

H.J. STEMMLER[1*], M. SCHMITT[2*], N. HARBECK[2], A. WILLEMS[2], H. BERNHARD[3], D. LÄSSIG[1], S. SCHOENBERG[4] and V. HEINEMANN[1]

[1]Medical Department III, Ludwig-Maximilians-University of Munich, Klinikum Grosshadern; [2]Department of Obstetrics and Gynaecology, and [3]Medical Department III, Technical University of Munich, Klinikum rechts der Isar; [4]Department of Radiology, Ludwig-Maximilians-University of Munich, Klinikum Grosshadern, Germany

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Abstract. Leptomeningeal carcinomatosis represents a rare manifestation of metastatic breast cancer (MBC). A 39-year-old female presenting with HER2-overexpressing MBC and suffering from meningeal carcinomatosis was treated with the humanized antibody trastuzumab directed to HER2 by intrathecal administration. The patient was diagnosed with HER2-overexpressing stage III breast cancer in December 2003. In August 2004, the patient developed a singular intracerebral metastasis which was resected by neurosurgery followed by whole-brain radiotherapy. Since MRI and cerebrospinal fluid (CSF) analyses indicated meningeal carcinomatosis, the patient was commenced on trastuzumab (6 mg/kg q3w) and capecitabine (2.500 mg/m^2 d1-14, q3w). Prompted by clinical deterioration, 5 repeated doses of intrathecal methotrexate (15 mg/dose) were administered, yet without clinical improvement. There is initial evidence that trastuzumab does not reach an adequate concentration in CSF after intravenous application. Nevertheless, infiltration of trastuzumab into CSF is facilitated under conditions of an impaired blood-brain barrier, as it is known for meningeal carcinomatosis. For patients with leptomeningeal disease, intrathecal application of trastuzumab may provide an interesting therapeutical approach for patients with HER2 overexpressing metastatic breast cancer. Therefore, an Ommaya reservoir for intrathecal treatment with trastuzumab was placed surgically and intrathecal therapy was begun with escalating doses of trastuzumab (5-20 mg), which proved to be effective and well tolerated by the patient. Within 2 weeks after treatment, the patients' condition improved significantly and cell counts in CSF obtained from the Ommaya reservoir remained low for 11 months after first diagnosis of meningeal carcinomatosis when clinical symptoms and MRI indicated progression of meningeal and cerebral disease.

Correspondence to: Dr Hans-Joachim Stemmler, Department of Internal Medicine III, University of Munich - Klinikum Grosshadern, Marchioninistrasse 15, D-81377 Munich, Germany
E-mail: joachim.stemmler@med.uni-muenchen.de

*Contributed equally

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Introduction

Women with metastasized breast cancer and HER2 (human epidermal growth factor receptor type 2) overexpressing tumors are candidates for treatment with the HER2-directed humanized monoclonal antibody trastuzumab (Herceptin™). The oncoprotein HER2 (also known as neu or c-erbB-2), is a transmembrane growth factor receptor belonging to the epidermal growth factor receptor family which is over-expressed in approximately 25-30% of all cases of human breast carcinoma. Compared to chemotherapy alone, the addition of trastuzumab improves the patient's progression-free survival (median time to progression of 7.4 vs. 4.6 months; $p<0.001$), the response rate (50 vs. 32%; $p<0.001$) and the time of overall survival (median time of survival of 25.1 vs. 20.3 months; $p=0.046$) (1,2).

Numerous investigators reported an increased rate of brain metastases (BM) in patients receiving trastuzumab-based treatment for HER2-positive MBC as compared to patients without HER2-overexpression (25-48 vs. 6-16%) (3-11) (Chock JY *et al*, Proc Am Soc Clin Oncol 21: abs. 218, 2002) (Weitzen R *et al*, Proc Am Soc Clin Oncol 21: abs. 1936, 2002). Leptomeningeal carcinomatosis represents a rare manifestation of MBC (3.5%); whereas the incidence of secondary meningeal seeding in patients suffering from parenchymal brain involvement is estimated as ≤6% (11-13).

There is some evidence that trastuzumab is unable to penetrate the brain or infiltrate into the cerebrospinal-fluid (CSF) which is explained by the blood-brain barrier (BBB) which permits central nervous system penetration to molecules with molecular weights not exceeding 200 Da (14,15). Increase in the permeability of the BBB for larger macro-molecules has been described for patients undergoing whole-brain radiotherapy (WBRT) for treatment of malignant brain

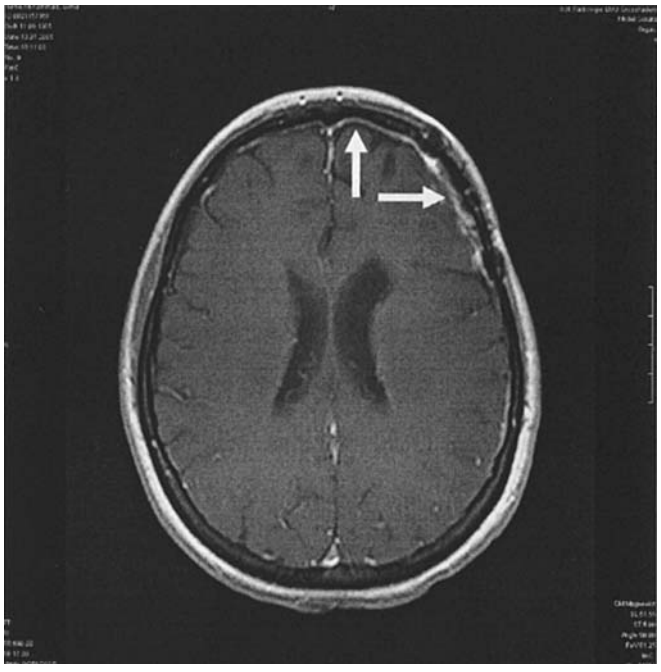

Figure 1. MRI post contrast scan: meningeal enhancement leading to the diagnosis of meningeal carcinomatosis (January 2005). Arrows indicate meningeal enhancement.

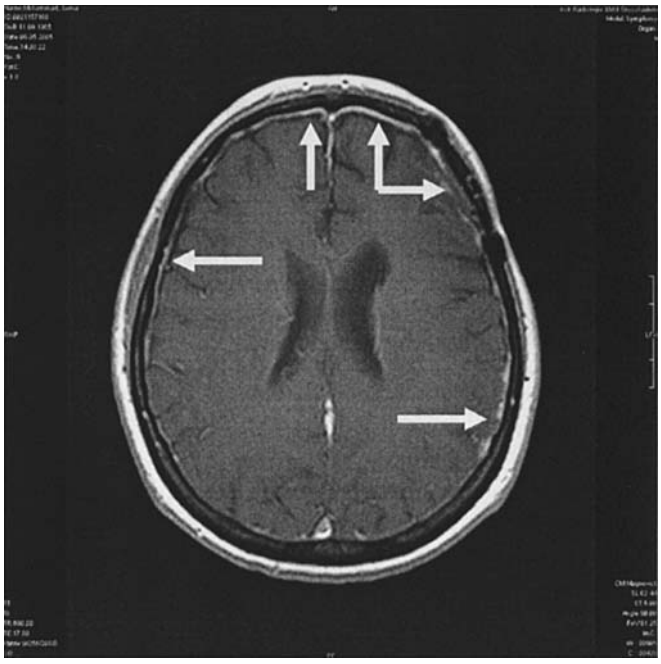

Figure 2. MRI post contrast scan: progressive meningeal enhancement (June 2005). Arrows indicate progressive meningeal enhancement.

tumors (16). Moreover, trastuzumab in CSF was determined in a single patient but was found to be 300-fold lower as compared to the corresponding serum level (14,17). An impairment of the BBB allowing the antibody to reach effective concentrations in CSF can be suspected in a case reported by Baculi *et al*, who reported on a breast cancer patient suffering from meningeal carcinomatosis who responded to treatment with trastuzumab (18). Furthermore, successful treatment of meningeal carcinomatosis by intrathecal trastuzumab via an Ommaya reservoir was reported by Laufman *et al* (19).

The present case report gives details regarding the clinical course of a breast cancer patient who received 2nd-line intrathecal trastuzumab via a surgically placed Ommaya reservoir for meningeal carcinomatosis in HER2-over-expressing MBC. Particulars of the functionally reactive levels of trastuzumab in serum and CSF, determined quantitatively by a novel-type ELISA, are also given.

Materials and methods

A newly designed ELISA detecting quantitatively and with high reactivity functional trastuzumab in body fluids makes use of the interaction of trastuzumab with the extracellular domain (ECD) of HER2 and detection of this complex by an antibody to human IgG. For this, a 96-well microtiter plate (Maxisorp™, Nunc, Wiesbaden, Germany) was coated with recombinant extracellular domain (ECD) of HER2. To construct a standard curve, serum from healthy female donors were spiked with different concentrations of trastuzumab (0-200 ng/ml) and then added to the ECD-coated wells of the microtiter plate. Likewise, sera or CSF from breast cancer patients were applied. As the detecting antibody, alkaline phosphatase-conjugated mouse antibody to human-IgG (Dianova™, Hamburg, Germany) was employed. The amount of reactive trastuzumab was determined by addition of p-nitrophenyl phosphate and the absorption of the color developed measured at 405 nm in a multiwell-ELISA reader (SLT-Spectra II™, SLT Instruments, Germany). The assay sensitivity ranges from 10 to 100 ng/ml of trastuzumab.

Case report

A 39-year-old female was diagnosed with left-sided breast cancer in December 2003. After mastectomy and axillary dissection (pT2, $pN2_{(7/16)}$, G3, ER and PgR negative, HER2 3+ according to Dako-HercepTest™) the patient received 6 cycles of adjuvant $FE_{100}C$ (5-fluorouracil, epirubicin, cyclophosphamide) followed by irradiation of the chest wall (50 Gy). In August 2004, the patient developed clinically symptomatic CNS involvement with headaches and dizziness. MRI detected a singular intracerebral metastasis which was resected by neurosurgery and then the patient was treated by whole-brain radiotherapy (WBRT). Close to completion of WBRT, the patient was re-submitted to our ward presenting with myoclonic seizure. MRI indicated leptomeningeal enhancement leading to the diagnosis of meningeal carcinomatosis (Fig. 1). At day 24 after WBRT, CSF obtained by lumbal puncture was examined which showed an increased cell count with an elevated level of protein (101 mg/dl) but was devoid of tumor cells. Microbiological results were negative. The patient was then commenced on trastuzumab, according to standard regimen with a loading dose of 4 mg/kg followed by weekly doses of 2 mg/kg.

Since at day 35 after WBRT-repeated lumbal punctures revealed the presence of single tumor cells in CSF, the 5-fluorouracil pro-drug capecitabine (Xeloda™, 1,250 mg/m^2 twice daily orally, d1-14, q3w) was added to trastuzumab.

Table I. Levels of reactive trastuzumab in serum and CSF.

Treatment	Days after WBRT	Days on trastuzumab	CSF cytology (x/3 cells)	Trastuzumab levels in CSF (ng/ml)	Trastuzumab levels in serum (ng/ml)
	24	0	9	0	0
	59	35	77	258	16,304
	140	116	52	-	-
15 mg MTX i.th. lumbal	154	130	60	-	-
15 mg MTX i.th. lumbal	158	134	19	-	-
15 mg MTX i.th. lumbal	161	137	11	-	-
15 mg MTX i.th. lumbal	164	140	12	356	22,885
15 mg MTX i.th. lumbal	168	144	40	-	-
Ommaya reservoir placed	180	156	20	-	-
Prior to T 5 mg Ommaya	186	162	1	76	11,675
Prior to T 10 mg Ommaya	192	168	-	195	18,676
Prior to T 15 mg Ommaya	196	172	-	3,460	82,303
Prior to T 20 mg Ommaya	200	176	-	1,446	78,129
	206	182	1	-	-
	215	191	1	-	-
T 20 mg Ommaya	221	197	-	-	-
	223	199	1	1,466	79,883
	231	207	1	231	87,581
	334	310	0	-	-

WBRT, whole brain radiotherapy; T, trastuzumab; MTX; methotrexate; i.th., intrathecal therapy by lumbal puncture; Ommaya reservoir, intrathecal therapy by Ommaya reservoir.

Still, shortly after treatment, the patient's general condition deteriorated with symptoms such as dizziness, hearing loss, and headache. Then, repeated doses of intrathecal methotrexate (15 mg total dose by lumbal puncture) were given. Despite decreasing cell counts and lower protein levels in CSF, the patient's condition deteriorated and therefore an Ommaya reservoir was placed into the brain surgically in order to allow intrathecal treatment. CSF samples taken from the Ommaya reservoir showed no increased cell counts.

Owing to the unchanged clinical presentation and an updated MRI indicating progressive leptomeningeal disease, intrathecal therapy with escalating doses of trastuzumab was begun (Fig. 2). The patient received 4 dose levels (by steps of 5 mg each), beginning with 5 mg administered via the Ommaya reservoir on day 162 after WBRT, followed by application of trastuzumab on days 168, 172, and 176. Since determination of reactive trastuzumab in serum and CSF persistently showed substantially lower trastuzumab levels in CSF compared to serum, an additional dose of 20 mg trastuzumab was given intrathecally three weeks later on day 197 (Table I). All intrathecal doses of trastuzumab were tolerated well by the patient. Within the next 2 weeks, the patient's condition improved significantly and tumor cell counts in CSF obtained from the Ommaya reservoir remained low for 11 months after first diagnosis of meningeal carcinomatosis. Treatment with trastuzumab and capecitabine was changed to trastuzumab and temozolomide in October 2005, when clinical symptoms such as dizziness, nausea, left-sided amaurosis, and hearing-loss, as well as imaging studies (MRI) indicated progression of meningeal and cerebral disease.

Discussion

Trastuzumab (Herceptin) is highly effective in the treatment of HER2-overexpressing patients with MBC. While trastuzumab improves the overall survival, an unexpectedly high incidence of cerebral metastases (BM) has been observed in MBC patients receiving and responding to trastuzumab. The biological explanation for this high incidence of BM may be an increased affinity of HER2 overexpressing breast cancer metastases for the CNS. Alternatively, it may be argued that trastuzumab prolongs survival to such an extent that BM, which is known to be a late event in the course of metastatic disease, becomes apparent (3-11). Moreover, the intact blood-brain-barrier may prevent trastuzumab reaching an adequate concentration in the cerebrospinal fluid (CSF), thus making BM common in patients with trastuzumab-sensitive MBC (8-10,14,17).

Besides the frequent diagnosis of BM, leptomeningeal carcinomatosis represents an uncommon manifestation of MBC (incidence of 3.5%) (11-13). Treatment options for meningeal carcinomatosis are limited since only a few chemotherapeutic

agents do penetrate the BBB or are administered intrathecally. Such agents include methotrexate, thiotepa, and standard or liposome-bound cytosine arabinoside (20,21). In addition, there are a few reports indicating that the 5-fluorouracil pro-drug capecitabine is efficient in patients with meningeal carcinomatosis derived from solid tumors (20). If intrathecal therapy is chosen, a safer and more efficient delivery of trastuzumab may be achieved by treating the patient using an Ommaya reservoir, compared to standard intrathecal treatment via repeated lumbal punctures (22,23).

Little is known about the biodistribution and pharmacokinetics of trastuzumab in CSF compared to that in the circulating blood compartment. At present, there is only a single report in the literature regarding this topic showing that in a patient screened, the trastuzumab concentration was 300-fold lower in CSF than in serum (14,17). Whether trastuzumab can infiltrate the brain tissue or diffuse into CSF in patients with impaired BBB has not been evaluated so far. Yet, there is some evidence that permeability of the BBB is increased in cancer patients who received radiotherapy for malignant brain tumors (16,18).

HER2 overexpressing intra-cerebral MBC in an experimental athymic rat model can be targeted by direct intracerebral microinfusion of trastuzumab (24). The rats, treated by intracerebral microinfusion exhibited significantly improved survival compared to untreated rats. Intracerebral microinfusion may thus be an effective way to treat BM, despite the fact that this approach does require a high degree of technical skills. Concerning the human situation, Laufman *et al* reported on a patient with meningeal carcinomatosis who was successfully treated by intrathecal infusion of trastuzumab using an Ommaya reservoir (19).

To allow the assessment of the functional reactive status of trastuzumab present in blood or CSF, we established a novel enzymometric test format which makes use of the specific interaction of trastuzumab with the extracellular domain (ECD) of HER2. Using this assay, we determined the functional levels of trastuzumab in serum and CSF of an MBC patient presenting with clinically progressive meningeal carcinomatosis. Following repetitive yet clinically ineffective intrathecal administrations of methotrexate and intermittent systemic corticosteroid treatment, finally an Ommaya reservoir was surgically placed in order to allow inthrathecal treatment of the patient with trastuzumab. The patient in our study was treated with trastuzumab according to the schedule of Laufman *et al* (19) applying increasing concentrations of trastuzumab at four-day intervals. With this type of treatment, the patient's condition improved significantly and no obvious side-effects were observed.

Before placement of the Ommaya reservoir, CSF levels of trastuzumab were determined and compared to serum levels. Although the patient had been on trastuzumab therapy for a relatively short period of time (35 days), the functional serum trastuzumab level was 16,304 ng/ml and thereby 63 times higher than the trastuzumab concentration of 258 ng/ml in CSF. One could argue that trastuzumab may need more time to achieve a steady-state in CSF, however, on day 164 after WBRT and after 140 days on trastuzumab, reactive trastuzumab levels in CSF remained at a low level (356 ng/ml) whereas the serum level stayed high (22,885 ng/ml) at a ratio of 1:62. In this respect, we would like to note though that CSF levels achieved by intrathecal administration of trastuzumab were significantly higher (peak value of 3,460 ng/ml) than CSF levels determined prior to intrathecal use of trastuzumab (Table I).

In conclusion, there is initial evidence that penetration of trastuzumab into CSF may be facilitated under conditions of an impaired BBB, as is known for meningeal carcinomatosis. We demonstrate further that high levels of trastuzumab can also be achieved by direct application of trastuzumab into the brain using an Ommaya reservoir. Given the increasing use of trastuzumab to treat HER2-overexpressing breast cancer patients in the metastatic and the adjuvant setting, more patients are expected to benefit from this therapy and may experience a considerable survival advantage. Nevertheless, these patients treated with trastuzumab are at risk to develop progressive disease to the CNS during or after this treatment. For these patients, individualized trastuzumab-based therapy regimens may be developed as we are now able to monitor effective reactive concentrations of trastuzumab in serum and CSF by a novel bioassay for functional trastuzumab, as presented herein.

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