Intrathecal trastuzumab (Herceptin) and methotrexate for meningeal carcinomatosis in HER2-overexpressing metastatic breast cancer: a case report

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Leptomeningeal carcinomatosis represents a rare manifestation of metastatic breast cancer (MBC). We herewith report on a patient suffering from HER2 overexpressing MBC who received intrathecal methotrexate and trastuzumab for meningeal carcinomatosis. A 48-year-old woman was diagnosed with breast cancer in December 2002. Following surgery, six cycles of adjuvant $FE_{100}C$ plus irradiation and, subsequently for 1 year, trastuzumab were given. As a result of disseminated metastatic spread in October 2005, the patient received whole-brain radiotherapy for symptomatic central nervous system involvement, and was put on several trastuzumab-based combination regimens (capecitabine, vinorelbine, paclitaxel). In June 2006, the patient developed clinical signs of terminal cone involvement with overflow incontinence and paraparesis of the legs. Immediate radiation led to partial relief from clinical symptoms. Subsequently, the patient was put on the tyrosine kinase inhibitor lapatinib and capecitabine (August to October 2007), but on November 6th the patient suffered again from overflow incontinence and weakness of the legs. Failing to respond to lapatinib, the patient received gemcitabine/cisplatin and, additionally, was recommenced on intravenous trastuzumab. Owing to progressive leptomeningeal disease, the patient received repeated doses of intrathecal methotrexate and trastuzumab. Within 2 weeks and four intrathecal treatments, cerebrospinal fluid cytology showed the absence of tumor cells. Moreover, a striking clinical improvement with resolution of the paraparesis of the legs and overflow incontinence was observed. This case report gives details regarding the clinical course of a breast cancer patient who received intrathecal trastuzumab and methotrexate via lumbar puncture for meningeal carcinomatosis of HER2-overexpressing MBC. *Anti-Cancer Drugs* 19:832–836

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Introduction

Numerous investigators reported an increased rate of central nervous system (CNS) metastases in patients receiving trastuzumab-based regimens for HER2-overexpressing metastatic breast cancer (MBC) as compared with patients without HER2-overexpression (25–34 vs. 6–16%) [1–10]. A retrospective analysis of 3871 breast cancer patients with known HER2 status indicated that the cumulative 10-year risk of brain metastasis was significantly higher in patients with tumors overexpressing HER2 (6.8 vs. 3.5%, $P < 0.01$) [11]. In contrast to frequent parenchymal CNS involvement, leptomeningeal carcinomatosis represents a rare but often disastrous manifestation of MBC (3.5%) [9,12,13].

Little is known so far about the pharmacokinetics and state of activity of trastuzumab in the CNS. Owing to the blood–brain barrier (BBB), which restricts CNS penetration to molecules with molecular weights exceeding 200 Da, trastuzumab may not reach sufficient levels in the cerebrospinal fluid (CSF) [14,15]. When determined in a few cases, trastuzumab levels in CSF were found consistently to be 300-fold to 400-fold lower as compared with the corresponding serum level [14,15].

Yet, an increase in the permeability of the BBB for larger macromolecules including trastuzumab has been described for patients undergoing whole-brain radiotherapy (WBRT) and for patients with an impairment of the BBB owing to other causes (e.g. leptomeningeal carcinomatosis) [15,16]. Baculi *et al.* [17] reported on a breast cancer patient suffering from meningeal carcinomatosis who responded to trastuzumab-based treatment. Restrictively, the response was transient and limited to radiological findings. Moreover, the groups of Laufman, Stemmler, and Platini *et al.* reported on the successful

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treatment of meningeal carcinomatosis by intrathecal trastuzumab [18–20].

This case report gives details on treatment schedule, regimen, time points as well as the clinical outcome of a breast cancer patient who received combined intrathecal immunotherapy consisting of trastuzumab and methotrexate for meningeal carcinomatosis of HER2-overexpressing MBC.

Methods

Determination of functionally active trastuzumab by ELISA

Quantification of trastuzumab in blood samples and CSF makes use of the interaction of trastuzumab with the extracellular domain 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 of HER2. Serum and CSF from the herewith-reported breast cancer patient, taken at four different times, were applied (27 November 2007, 30 November 2007, 6 December 2007, 13 December 2007; Table 1). As the detecting antibody, alkaline phosphatase-conjugated mouse antibody to human-IgG (Dianova, Hamburg, Germany) was used. 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, Crailsheim, Germany).

Preparation and intrathecal application of trastuzumab

Trastuzumab at an absolute dose of 20 mg was injected via standard lumbar puncture. For that, lyophylisated trastuzumab (150 mg) was reconstituted with 7.2 ml of sterile water resulting in a solution, which contains 21 mg/ml of trastuzumab (pH 6.0). For each intrathecal use, 0.95 ml (= 20 mg) of this solution was injected. No other co-medication was given.

Sequence of intrathecal treatment

Before the application of methotrexate, three CSF samples (1 ml each) were taken for further analyses. First, methotrexate (12-mg absolute dose) was given, followed immediately by intrathecal trastuzumab at an absolute dose of 20 mg.

Case report

A 48-year-old woman was diagnosed with right-sided breast cancer in December 2002. Following breast sparing surgery and axillary lymph-node dissection [pT1c, $pN1_{(2/12)}$, cM0, G3, ER, and PgR negative (IHC 0/12 each), HER2 3 + according to DAKO-HercepTest, Copenhagen, Denmark], the patient received six cycles of adjuvant $FE_{100}C$ (5-fluorouracil, epirubicin, cyclophosphamide), irradiation, and, subsequently, trastuzumab for 1 year.

In October 2005, the patient developed clinical symptoms of CNS involvement including vision disorders, headaches, and dizziness. Contrast-enhanced MRI detected multiple brain metastases, which were successfully treated by WBRT. Simultaneous metastases were diagnosed in the liver, lung, and the musculoskeletal system. In addition to continuous application of bisphosphonates, the patient was put on trastuzumab combined capecitabine (October 2005 to April 2006), vinorelbine (May to October 2006), and paclitaxel (November 2006 to June 2007) administered in succession.

In June 2006, the patient developed clinical signs of terminal cone involvement with the occurrence of overflow incontinence and paraparesis of the legs. Immediate radiation was started resulting in partial relief of the clinical symptoms and then, the patient was administered lapatinib and capecitabine (August to October 2007).

On 6 November, the patient reported again with clinical symptoms of terminal cone involvement with overflow incontinence and weakness of the lower extremities. Contrast-enhanced MRI of the spine showed focal leptomeningeal nodules with contrast material enhancement establishing the diagnosis of meningeal carcinomatosis (Fig. 1). CSF obtained by lumbar puncture was examined, which showed an increased cell count with an

Table 1 Clinical course of meningeal involvement, treatment, and levels of reactive trastuzumab in serum and CSF

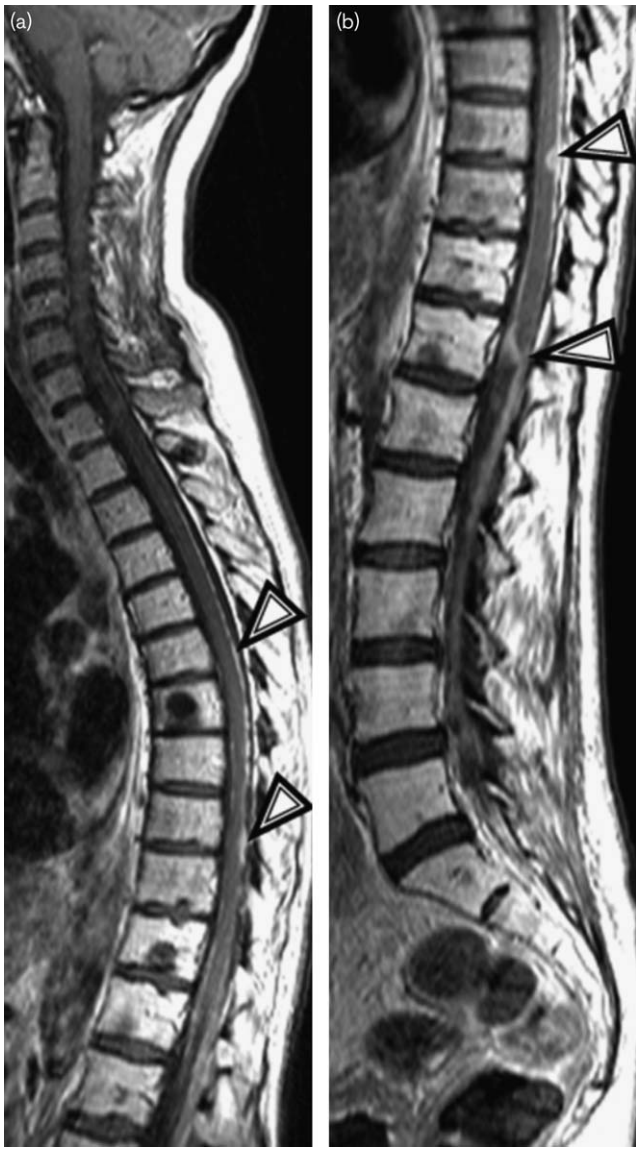
Date	i. th. treatment by lumbar puncture (absolute doses mg i. th.)	Days after WBRT	Days on i.v. TRAS[b]	Tumor cell count in CSF (%)	TRAS levels CSF (ng/ml)	TRAS levels SER (ng/ml)
21 October 2005	–	Before WBRT	–	0	–	–
6 November 2007	–	730	–	0	–	–
23 November 2007	12-mg MTX/20-mg TRAS i.th.	747	7	12	–	–
27 November 2007	12-mg MTX/20-mg TRAS i.th.	751	11	4	211	34 274
30 November 2007	12-mg MTX/20-mg TRAS i.th.	754	14	2	6425[a]	–
6 December 2007	12-mg MTX/20-mg TRAS i.th.	760	20	0	446	–
13 December 2007	–	767	27	0	204	–

CSF, cerebrospinal fluid; i. th., intrathecal therapy by lumbar puncture; i.v., intravenous; MTX, methotrexate; SER, serum; TRAS, trastuzumab; WBRT, whole-brain radiotherapy.

[a]Sanguineous and xanthochrome CSF (1304/3 erythrocytes).

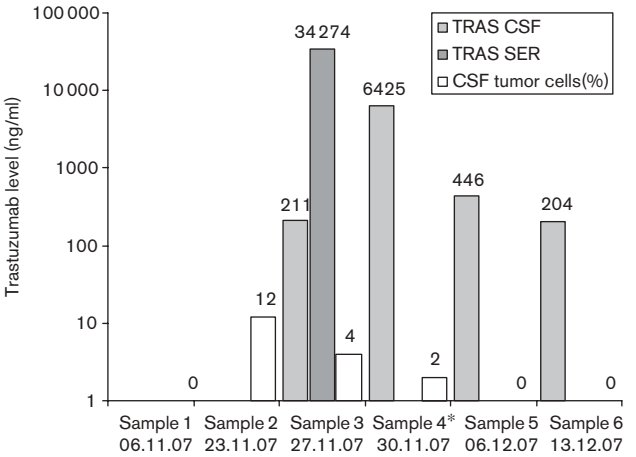
[b]Re-exposition to trastuzumab (first dose 4 mg/kg body weight, 2 mg/kg BW q1w) after failure of lapatinib.

Fig. 1



T1-weighted MRI post-Gd diethylene triamine pentaacetic acid contrast scan of the cervical, thoracic (a), and lumbar (b) spine: increased meningeal contrast material enhancement and meningeal nodular thickening (arrows) led to the diagnosis of meningeal carcinomatosis. Arrows indicate meningeal enhancement.

Fig. 2



Levels of reactive trastuzumab in serum and CSF. CSF, cerebrospinal fluid; SER, serum; TRAS, trastuzumab. *Sanguineous and xanthochrome CSF (1304/3 erythrocytes).

elevated level of protein (101 mg/dl), but was devoid of tumor cells. Microbiological results were negative (sample 1, 6 November 2007). Failing to respond to lapatinib, the patient received a combination chemotherapy, which consisted of gemcitabine and cisplatin, and, additionally, was recommenced on trastuzumab, according to standard regimen with a loading dose of 4 mg/kg followed by weekly doses of 2 mg/kg. Nevertheless, clinical symptoms of meningeal carcinomatosis deteriorated and the patient developed overflow incontinence and incomplete paraparesis of the lower extremities. Repeated lumbar punctures confirmed meningeal carcinomatosis with 12% tumor cells in CSF (sample 2, 23 November 2007). Owing to the progressive clinical presentation and a follow-up MRI indicating progressive leptomeningeal disease, the patient received intrathecal methotrexate (12-mg absolute dose) and trastuzumab (20-mg absolute dose) by lumbar puncture, which was tolerated well. Repeated doses of intrathecal methotrexate and trastuzumab were given at 3–6 days intervals (sample 3, 27 November 2007; sample 4, 30 November 2007; sample 5, 6 December 2007). As demonstrated in Table 1, the tumor cell count in CSF decreased within 2 weeks and moreover, the patient's general condition improved significantly (Eastern Cooperative Oncology Group 3 to 1) and she was able to walk again without assistance.

Trastuzumab levels in CSF were determined at each lumbar puncture. Trastuzumab was found to be 78-fold to 168-fold lower in the CSF as compared with serum (Fig. 2). A final lumbar puncture confirmed remission of meningeal carcinomatosis with the absence of tumor cells in CSF (sample 6, 13 December 2007) (Table 1).

Unfortunately, close to successful treatment of meningeal carcinomatosis, the patient's general condition deteriorated with rapid general progression of visceral metastases in lung and liver and the patient died on 1 January 2008.

Discussion

Trastuzumab (Herceptin) is highly effective in the treatment of HER2 overexpressing MBC. Nonetheless, numerous previous investigators have reported an unexpectedly high incidence of clinically overt CNS

metastasis ranging from 25 to 34% of patients having received trastuzumab-based regimens for HER2 overexpressing MBC [1–10]. Plausible explanations for this observation are as follows. (i) HER2 overexpressing breast cancer cells have an increased affinity to the CNS. (ii) Trastuzumab-based therapy prolongs survival to such an extent that CNS metastasis, which is known to be a late event in the course of metastatic disease, becomes apparent. The apparent change in the pattern of metastasis in HER2 positive breast cancer may thus be (partially) interpreted as a consequence of prolonged survival owing to successful trastuzumab treatment. (iii) On the other hand, the intact BBB is crossed only by small lipid-soluble molecules: chemotherapy agents such as anthracyclines, vinca alkaloids, or the taxanes are poorly taken up into the brain. Specifically, monoclonal antibodies such as trastuzumab are too large (148 kDa) to pass the BBB [21].

In contrast to the relatively frequent diagnosis of parenchymal CNS metastases, leptomeningeal carcinomatosis represents a rare but often disastrous manifestation of MBC (incidence of 3.5%) [9,12,13]. Alternative treatment options for progressive CNS metastases and meningeal carcinomatosis, which failed to respond to surgery and/or radiotherapy, are limited as only a few agents do pass the BBB. Treatment options are therefore considered as follows.

(1) *Loco-regional chemotherapy* with agents which are administered intrathecally including methotrexate, thiotepa, and standard or liposome-bound cytosine arabinoside [12].
(2) *Systemic chemotherapy* with selected agents such as capecitabine, 5-fluorouracil, platinum analogues, temozolomide, methotrexate, topotecan, or bendamustine, however, are chosen because of their known activity in the brain [4].
(3) *Manipulation of the permeability of the BBB*. The intact BBB limits CNS penetration to molecules with molecular weights up to 200 Da [21]. Yet, changes in the permeability of BBB were observed in experimental animals as well as in clinical studies evaluating patients who received WBRT for several malignant brain tumors [16]. Even in patients with CNS metastases of HER2 overexpressing MBC, it was shown that penetration of trastuzumab into the CSF may be facilitated during WBRT [15].
(4) *Small molecules.* Lapatinib, which is a 'small molecule' (< 1 kDa), acts as a dual inhibitor of EGFR1- (HER2) and EGFR2- (HER2) tyrosine kinases [22–24]. Preclinical and clinical studies demonstrated that breast cancer cell lines, which were resistant to trastuzumab, remained sensitive toward lapatinib. Furthermore, it was shown in a mouse model that lapatinib could effectively inhibit brain metastasis [25]. These preclinical data were matched for humans by the results of the EGF100151 trial reported by Geyer *et al.* [26]. A recent update of this randomized phase III trial indicated that a combined administration of capecitabine with lapatinib was effective in reducing CNS metastasis as a first site of disease recurrence (2 vs. 6%, $P = 0.045$) [27]. Based on the preclinical and clinical evidence, the efficacy of single-agent lapatinib was then evaluated in the EGF105084 phase II trial in which patients received single-agent lapatinib at a dose of 750 mg twice daily [28]. The trial included 241 HER-positve MBC patients who had been exposed to trastuzumab and had received prior radiotherapy of the CNS. A $\geq 50\%$ volumetric reduction of CNS lesions was observed in 19/241 (7%) of patients, whereas a $\geq 20\%$ reduction was reported in 19% of patients. These data indicate that lapatinib exerts consistent but modest activity as a single agent for the treatment of brain metastasis in HER2-positive MBC patients.
(5) *Loco-regional therapy with trastuzumab.* Little is known so far about the biodistribution and pharmacokinetics of trastuzumab in CSF compared with that in the circulating blood compartment. At present, there are few reports in the literature regarding this topic showing that in patients screened for trastuzumab, the concentration was 300-fold to 400-fold lower in CSF than in serum [14,15]. Furthermore, there is some evidence that accessibility for trastuzumab to the brain may be facilitated in patients who received WBRT for treatment of metastases in the CNS or whose BBB was impaired by meningeal carcinomatosis [15,20].

Intrathecal administration of trastuzumab is a more individual approach of treatment. Yet, only a few reports of patients with meningeal carcinomatosis are available who were successfully treated by intrathecal infusion of single-agent trastuzumab [18–20].

The patient reported here had failed to respond to radiotherapy, various trastuzumab-based systemic chemotherapy regimens therapy, and to treatment with lapatinib. As an alternative approach, the patient finally received intrathecal methotrexate and trastuzumab for rapidly progressive clinical symptoms of terminal cone involvement with cytological confirmed meningeal carcinomatosis. Intrathecal methotrexate/trastuzumab led to notable relief from clinical symptoms including the nearly complete resolution of the paraparesis of the legs and the disappearance of the overflow incontinence without any side effects being observed.

We demonstrate that high levels of trastuzumab can be achieved by direct application of trastuzumab into the

CSF by lumbar puncture. 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 of developing progressive disease in the CNS during or after this treatment. Progression in the CNS after surgery, radiotherapy, and systemic therapy, including the tyrosine kinases inhibitors, remains a disastrous situation. For these patients, individualized trastuzumab-based therapy regimens including the intrathecal approach may now be developed. The possibility of assessing the functional, reactive status of trastuzumab present in blood or CSF with a biocatch ELISA may serve as a helpful tool in monitoring the intrathecally administered dose of trastuzumab. A phase-I trial, which investigates the optimal dose in this highly investigational approach, has been conducted.

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