

Brain Metastases in Breast Cancer – an *In Vitro* Study to Evaluate New Systemic Chemotherapeutic Options

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Abstract. *Background:* Fifteen-30% of breast cancer patients develop central nervous system (CNS) metastases. The most potent drugs for the treatment of breast cancer like taxanes, anthracyclines and trastuzumab have limited efficacy for brain metastases. No standardized therapy has yet been established for this condition. Drugs with proven efficacy in the CNS and which are commonly used for primary brain tumors were applied. We evaluated the capacity of these drugs to inhibit breast tumor cell growth in vitro. *Materials and Methods:* Twelve primary cell cultures of pulmonary/pleural metastases of breast cancer and 3 commercially available cell lines were used for non-radioactive cytotoxicity assays to evaluate the efficacy of 3 different concentrations of Topotecan, Cisplatin, Nimustine, Vincristine, Irinotecan, Caelyx[®] (pegylated liposomal Doxorubicin) and Etoposide. *Results:* Topotecan, Cisplatin, Caelyx[®] and Vincristine showed significantly higher cytostatic activity in vitro than Irinotecan, Etoposide and Nimustine. With regard to the median cytotoxicity, the order of drugs in our assays was Topotecan, Cisplatin, Vincristine, Caelyx[®], Irinotecan, Etoposide and Nimustine. Nimustine showed almost no efficacy against breast cancer cells. *Conclusion:* Topotecan, Cisplatin, Vincristine and Caelyx[®] seem to be suitable candidates for further clinical evaluation. The data and the "liposomal packaging" suggest that Caelyx[®] might be effective in the CNS. Since pulmonary metastases are often associated with brain metastases, evaluating primary cell cultures from malignant pleural effusions could be a valuable approach for the testing of new cytostatic drugs for brain metastases.

Brain metastases (BM) from systemic primary cancers are the most common cause of neoplastic disease of the central nervous system (CNS). They outnumber primary intracranial

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neoplasms by at least 10 to 1 (13, 36). After lung cancer, breast cancer is the second most common cause of brain metastases. Overall 15-30% of all metastasized breast cancers lead to CNS metastases (14, 44). Breast cancer, which is characterized by early and frequent metastases, is the most frequent cancer in women with approximately 500,000 cases each year worldwide.

Nowadays, systemic treatment of breast cancer is beneficial for survival in the adjuvant and metastatic setting. Both, the 20-year follow-up of the Bonadonna study and a meta-analysis of the early breast cancer triallists' Collaborative Group (1998) demonstrated the benefits of chemotherapy (CT) concerning disease-free and overall survival (6, 42). However, the incidence of diagnosed brain metastases is increasing (7). This is partly due to the use of modern imaging techniques like MRI, PET and SPECT. Moreover, longer survival of patients with metastasized breast cancer leads to an increase of metastases in uncommon locations such as the brain, skin and vagina. The frequency of the different metastatic locations in breast cancer is 20-30% CNS, 55% lung and pleura, 35% liver, 35% chest wall and 77% bone metastases.

The most potent drugs used in modern breast cancer therapy are anthracyclines, taxanes and trastuzumab (24). In many cases, good control of disease spread to lungs, liver, or bones can be achieved. Unfortunately, none of these drugs reach the central nervous system in efficacious concentrations. Hence, there are several reports of patients showing a good response of their visceral metastases who suffer from newly occurring or progressing CNS metastases (16, 11, 24, 25, 34, 17). A recent retrospective review of 122 women with metastatic breast cancer treated with trastuzumab found that one-third developed CNS metastases in a median time of 6 months after starting trastuzumab therapy. Remarkably, at the time brain metastases (BM) were identified, in half of the patients other systemic disease was either stable or responding to therapy (4).

For the management of BM corticosteroids, radiotherapy and surgical therapy have an established place (36). Surgical resection is preferable in cases of single accessible BM and

no evidence of progressive extracranial disease. Surgery should be followed by radiotherapy (36, 42). All other patients should be offered radiotherapy. Systemic therapy for BM of breast cancer is a palliative approach aiming to control disease and neurologic dysfunction. Therefore, only single substances expected to be less toxic than combinations were tested. Patients with BM that have progressive extracranial metastatic disease or relapse after radiotherapy are candidates for chemotherapy (29). Contraindications are acute danger of cranial herniation, severe neurological dysfunction or poor general condition.

A frequently anticipated obstacle for chemotherapy of metastatic CNS disease is the blood-brain barrier (BBB), which acts as a barrier for most hydrophilic and large lipophilic substances in normal brain, due largely to the tight junctions between brain capillary endothelial cells. Other mechanisms contributing to form the BBB are high electric resistance, organic anion transporters, transmembrane efflux mechanism, e.g. P-glycoprotein and multidrug-resistance associated proteins (MRP). Remarkably, the BBB seems to be more permeable in the situation of BM (7, 20, 21, 33). This also seems to be the case in primary brain tumors like glioma, where formation of pathological tumor vessels inhibits the forming of a functioning BBB (38).

The lung is the most frequently involved distant metastatic site associated with breast cancer (39, 9, 44). In a series of patients investigated by Saito and coworkers (39), 27 patients with node-negative breast cancer developed BM, whereas 26 had developed pulmonary or pleural metastases before CNS spread. Based on this observation, we used primary cell cultures from pleural effusions to test drugs with proven efficiency in the treatment of primary CNS tumors.

Currently, there is not enough clinical evidence to recommend a specific drug or drug regimen for BM of breast cancer. In order to offer these patients the most efficacious chemotherapeutic options, we tested drugs with efficacy in the CNS as proven by their established use in the treatment of primary central nervous tumors (18, 27, 37). In the present study, we investigated primary cell cultures of metastasized breast cancer with cytotoxicity assays, aiming to discover the most promising candidates for future clinical testing in patients suffering from BM.

Materials and Methods

Patients. The median age of patients was 50 years (range 34-75 years) at the time of occurrence of malignant pleural effusion. Receptor status and grading of the tumors is shown in Table I. All patients were pretreated with standard adjuvant chemotherapy. In most cases, additional second- and third-line chemotherapy had been necessary in the course of the disease. Hormonal treatment was administered in case of positive hormone receptor status. At the time of this writing, four patients have died. One had developed multiple BM and died from progress of systemic/pulmonary disease.

Table I. Characteristics of breast cancer patients from which primary cell cultures were gained.

Patient	Age	Histology	Grading	Receptor
1	35	ductal invasive	III	neg
2	37	ductal invasive	II	neg
3	39	ductal invasive	II	ER+, PR+
4	47	ductal invasive	III	neg
5	47	lobular invasive	III	ER+, PR+
6	48	ductal invasive	II	neg
7	51	ductal invasive	II	ER+, PR+
8	63	ductal invasive	II	ER+, PR+
9	64	ductal invasive	III	ER+, PR+
10	70	lobular invasive	III	ER+, PR+
11	71	ductal invasive	III	ER+, PR+
12	75	ductulolobular	III	ER+, PR-

Chemotherapeutic agents. The following substances were used in the study: Nimustine (ACNU®; Baxter Oncology), Vincristine (Vincritinsulfat®; Hexal), Cisplatin (Platinex®; Bristol Myers Squibb), Topotecan (Hycamtin®; Glaxo Smith Kline Beecham) and Etoposide (Eto-Gry®; Grypharma). Pegylated liposomal Doxorubicin (Caelyx®; Essex Pharma) was also tested.

Three different concentrations of all drugs were used in the experiments. The institutional pharmacy prepared and dissolved the agents for our tissue culture experiments in the same way as they are prepared and transported for patient use. Drugs were compared in their efficacy with respect to the doses administered in humans. The assumed doses for one cycle were: Topotecan 7.5 mg/m², Cisplatin 100 mg/m², Nimustine 100 mg/m², Caelyx® 20 mg/m², Etoposide 700 mg/m², Irinotecan 300 mg/m² and Vincristine 1.4 mg/m². These doses were considered for the preparation of the stock solutions for cytotoxicity assays.

Primary tumor cell culture and commercial cell lines. Primary tumor cell lines from 17 patients with metastasizing breast cancer were isolated and cultivated from pleural effusions. In 12 of these patients, we were able to gain a suitable primary cell culture, which enabled us to perform cytotoxicity experiments. Tumor cells were grown in several different media to establish a primary cell line and the culture from the medium in which the cells grew best was selected. Experiments were performed in RPMI1640 medium (Biochrom, Berlin, Germany).

Effusions (20 - 50 ml) were centrifuged, cell pellets washed twice in phosphate-buffered saline (PBS, Biochrom) and then cultivated in HBCA-medium supplemented with 10% fetal bovine serum (FBS, PAA Laboratories, Cölbe, Germany) and gentamycin at 1 x 10⁶ cells/ml in a plastic cell culture flask, in a humidified incubator under 5% CO₂ atmosphere. Fibroblasts were deleted by trypsin treatment every other day and the remaining tumor cell monolayer was cultivated until homogeneous morphology of the cells (passage 3-4). If tumor cells had divided adequately, leukocytes and fibroblasts were absent after a few passages.

In order to establish the experimental setting, we used commercially available cell lines (MCF7, MDA-MB, BT20) to test different concentrations and incubation times. These cell lines were

Table II. Comparison of median cytotoxicities exhibited by the tested chemotherapeutics (Mann-Whitney U-test).

	Cisplatin	Topotecan	Caelyx®	Vincristine	Irinotecan	Etoposid	Nimustine
Cisplatin	*	n. s.	n. s.	n. s.	$p < 0.05$	$p < 0.05$	$p < 0.01$
Topotecan	n. s.	*	n. s.	n. s.	$p < 0.01$	$p < 0.01$	$p < 0.01$
Caelyx®	n. s.	n. s.	*	n. s.	$p < 0.05$	$p < 0.05$	$p < 0.01$
Vincristine	n. s.	n. s.	n. s.	*	n. s.	$p < 0.05$	$p < 0.01$
Irinotecan	n. s.	n. s.	$p < 0.05$	n. s.	*	n. s.	$p < 0.01$
Etoposide	$p < 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.05$	n. s.	*	$p < 0.01$
Nimustine	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	$p < 0.01$	*

not significant = n.s.

obtained from Cell Line Services (Heidelberg, Germany) and cultivated in RPMI1640 medium (Biochrom) supplemented with 10% FBS and gentamycin (R10).

Cytotoxicity assay and photometric evaluation. To quantify the cytotoxicity of drugs, viability of cells was measured with a non-radioactive cell counting assay (Cell Counting Kit-8 Alexis® Biochemicals, Grünberg, Germany), which allows a sensitive colorimetric determination of viable cells in cell proliferation and cytotoxicity assays. A tetrazolium salt (WST-8; 2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monodium salt) is reduced by dehydrogenases in cells to give a yellow-colored product (formazan), which can be analyzed *via* absorbance at 460 nm in an ELISA plate reader. The amount of formazan dye generated is directly proportional to the number of living cells. We seeded approximately 20,000 cells in a volume of 95 µl R10 medium per well in a 96-well cluster plate. Quintuplicate wells were set for each drug at each concentration.

Drugs in various concentrations were added in a volume of 5 µl. Dilutions of the drugs were done in conventional NaCl 0.9%-solution. For establishing the experimental system, MCF7, MDA-MB and BT20 cells were initially seeded on 4 plates each and identical settings of drugs were added. For each cell line, one of the plates was then analyzed with the cell counting kit after the specified incubation time of 12, 24, 48 and 72 hours, respectively. Best signal/noise ratios were obtained with the plates incubated for 48-hours. Therefore, we decided to perform all the following trials with a 48-hour incubation time. Several dilutions were tested (1:20, 1:100, 1:500). After 48 hours, 10 ml of WST-8 were added, and after one-hour incubation, the plates were evaluated with the plate reader. Controls were run with PBS, media and NaCl.

Statistical evaluation of results. The median numbers of viable cells in percent were determined using the non-parametric Mann-Whitney U-test (significance set at $p < 0.05$) using Statistica version 6 (Statsoft).

Results

Pre-testing. Testing with the commercially available breast cancer cell lines MCF-7, MDA-MB and BT20 revealed that the results gained with the cell counting assay were reproducible (data not shown). These cell lines have a rather high proliferation activity. Smaller differences in the capacity of drugs to inhibit cell growth were easier to discover with

these cells. We tested different incubation times (12, 24, 48 and 72 hours) and dilutions of chemotherapeutics to establish the experimental setting.

Testing. We aimed to imitate the *in vivo* situation in our experimental *in vitro* setting. Therefore, we used primary cell cultures of 12 patients with as few passages (3-5) *in vitro* as possible. We tested seven different chemotherapeutic agents (Table II) at three different concentrations with regard to their ability to inhibit growth of breast cancer cells. The determined differences in the number of viable cells after exposure to the cytostatic compounds was smaller in primary cell lines compared to commercial cell lines. The results are illustrated in Figure 1.

A dilution of 1:500 of the chemotherapeutic agents proved to be too high and did not result in reproducible rates of cytotoxicity. Nimustine, Etoposide and Irinotecan were less efficacious than the other agents tested and did not exhibit sufficient inhibition of cell growth if diluted 1:100 to allow a reliable comparison of the whole group of drugs. Therefore, drugs were compared in terms of their median cytotoxicities after 48 hours at a dilution of 1:20. We administered a dilution of 1:20 resulting in concentrations for Topotecan of 3.75×10^{-3} mg/ml, Cisplatin 0.05 mg/ml, Nimustine 0.05 mg/ml, Caelyx® 0.01 mg/ml, Etoposide 0.35 mg/ml, Irinotecan 0.15 mg/ml and Vincristine 7×10^{-4} mg/ml. This dilution yielded the most reproducible results in the cytotoxicity assays.

Topotecan proved to be the most potent drug based on the median cytotoxicity evaluated for all drugs in our cell culture assays. It was significantly more potent than Irinotecan, Etoposide and Nimustine. Through all experiments performed, Cisplatin was always among the most potent agents. Cisplatin showed a slightly higher median cytotoxicity than Vincristine and Caelyx®, but was not statistically significantly more efficacious than the other two. The anthracycline Caelyx® also showed a considerable inhibition of tumor cell growth. Vincristine, Topotecan, Cisplatin, and pegylated liposomal Doxorubicin form a group of four drugs that are significantly more toxic to breast cancer cells than the rest of the agents tested. Irinotecan and

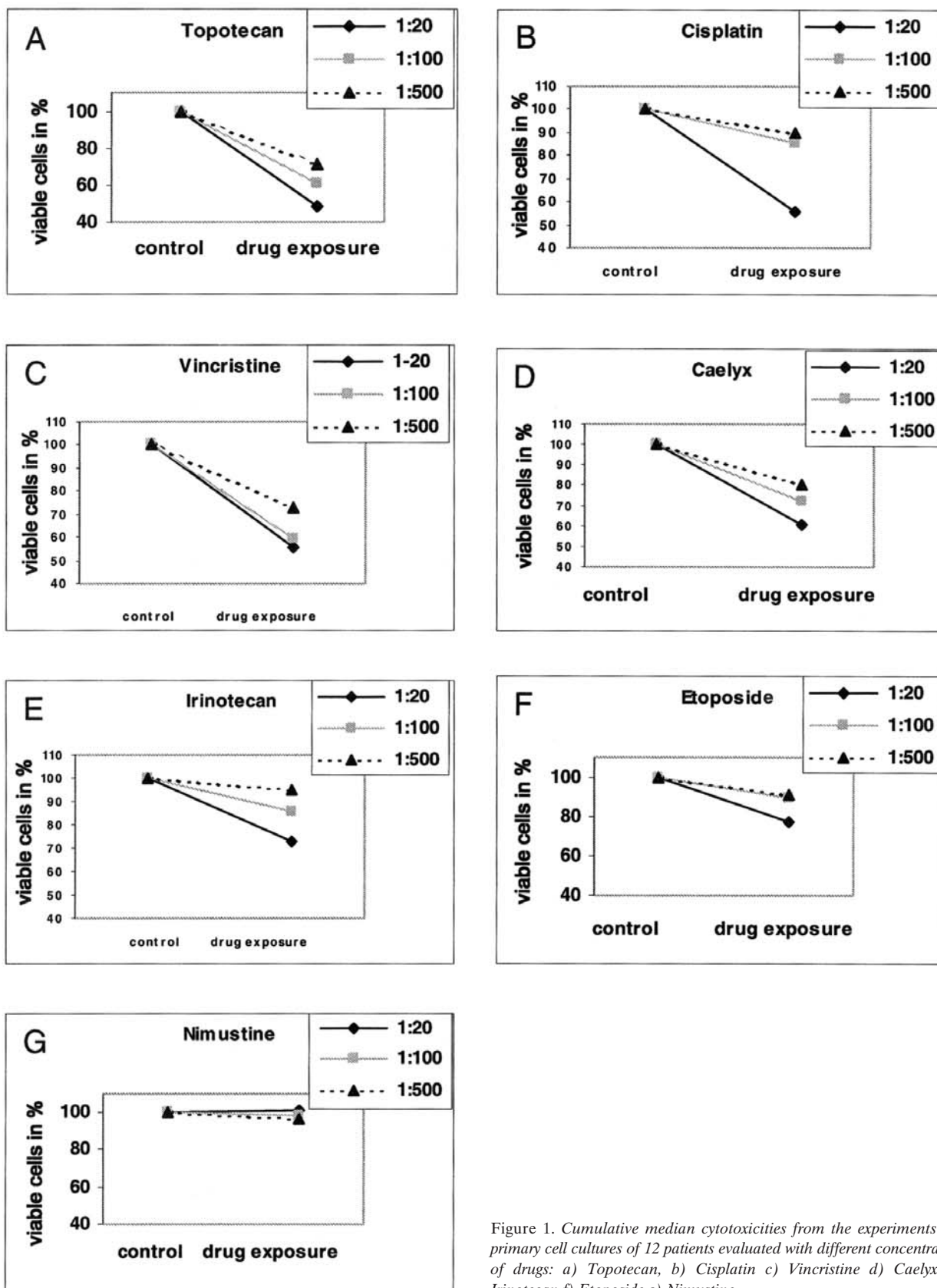


Figure 1. Cumulative median cytotoxicities from the experiments with primary cell cultures of 12 patients evaluated with different concentrations of drugs: a) Topotecan, b) Cisplatin c) Vincristine d) Caelyx® e) Irinotecan f) Etoposide g) Nimustine.

Etoposide showed comparable rates of growth inhibition, but were significantly less cytotoxic ($p < 0.05$) than the drugs mentioned above. The data are shown in Table II. Nimustine turned out to be significantly weaker than all the other substances tested ($p < 0.01$). Surprisingly, in a few primary cell lines cells incubated with Nimustine, tumor cells grew better than the control (Figure 1G).

The variation of the proliferation activity between the different primary cell lines was substantial. When the same drug was tested in different cell lines, consequently the standard deviation of the median cytotoxicity of a specific drug was enlarged.

We also compared the capacities of agents with regard to the age of patients. The median age of all patients was 49 years and was used as a cut off to split the collective into two subgroups. There was a tendency, for tumors from younger patients to be more resistant to cytostatic agents than malignancies of elderly women, but the difference did not prove to be statistically significant. The lack of statistical significance could be attributed to the relatively small numbers of subjects.

Discussion

In the present study, we performed an *in vitro* evaluation of chemotherapeutics in order to search for potent therapeutic options for brain metastases (BM) from breast cancer. Patients that suffer from this condition are, in general, relatively young and have mostly hormone receptor-negative and more aggressive undifferentiated G2 and G3 tumors (10, 40, 44). These young patients are frequently in good general condition and demand valuable treatment. It is noteworthy that patients with BM from breast cancer are a diverse group characterized by a large subset of patients surviving only a few months, but also a remarkable number of patients that survive for more than a year, with an unsatisfying median overall survival of 6 months (28).

Hall *et al.* showed, in an retrospective analysis of patients with brain metastases from different tumors, that among other therapeutic approaches, chemotherapy was a beneficial factor for prolonged survival (23). One of the crucial factors for a successful systemic treatment of BM is that the BBB becomes permeable in the situation of metastatic brain disease. Some authors (16) claim that most substances with good penetration of the BBB have limited activity against breast cancer. In contrast, Landonio *et al.* stated, that chemotherapy has proven efficacy in patients with BM from several different primaries, of which breast cancer is just one. Surprisingly, the observed responses to chemotherapy almost resemble those in other metastatic sites (27). Effective treatment of the extracranial disease is often possible. Consequently, as opposed to patients with BM from other solid tumors who generally die of extensive

systemic disease, at least half of the patients with BM from breast cancer die of their neurological disease (14, 22).

In our *in vitro* study, we applied drugs that have proven efficacy in the CNS and are used for primary brain tumors (18, 27, 37). We evaluated the capacity of these drugs to inhibit the growth of breast cancer cells. Topotecan belongs to a class of agents that has shown substantial promise in preclinical studies (37). It is a camptothecin derivative which inhibits topoisomerase I. Topotecan is one of the chemotherapeutic agents with the highest cerebrospinal fluid levels after intravenous administration, reaching 30-40% of plasma concentrations (5). In spite of encouraging *in vitro* findings, so far there is no clinical evidence of good response rates *in vivo*. There might be some inhibitory mechanism against, or efflux transport of, Topotecan that only takes effect *in vivo*. In one study performed by Levine *et al.* only 4 out of 53 Topotecan-treated patients showed an objective response (30). In general, clinical evidence regarding the systemic treatment of BM is limited (16). In spite of its superiority in *in vitro* assays, Topotecan might not be the first choice for the situation *in vivo*. Other compounds with satisfying results in *in vitro* assays, like platinum analogs, have clinically shown efficacy against breast cancer in the past. Cisplatin is very active as first-line chemotherapy of metastasized breast cancer, with response rates of 50% (31). Naturally, sensitivity to drugs varies between tumor cells, but Cisplatin showed comparable efficacy to Topotecan and might be a valuable alternative option, especially in cases where Topotecan is not tolerated or leads to progressive disease.

Interest in platinum compounds in the treatment of breast cancer has been reawakened, because preclinical studies indicated a possible synergism between trastuzumab and platinum compounds in human breast cancer cell lines overexpressing Her2/neu (12, 31). In early trials, platinum-taxane-trastuzumab combinations have shown promising clinical activity (12). Our data justify the evaluation of platinum analogs clinically in BM of breast cancer. This adds another oncological disease setting in which the very widely used platinum analogs are applicable.

Caelyx[®] showed good cytotoxicity in our experiments. It has no established role for the treatment of CNS neoplastic disease yet, but its "lipophilic packaging" suggests that it enters the CNS. It was seen in one clinical study that the accumulation of radiolabelled liposomal Doxorubicin in glioblastoma tissue and BM from breast cancer was 10 times higher than in the surrounding normal brain. Objective responses were seen in all 3 breast cancer patients treated with liposomal Doxorubicin (26). The primary breast cancer cultures we exposed to Caelyx[®] reflected the expected cytotoxicity of an anthracycline, but more evidence is needed that it reaches the metastatically affected CNS in efficacious concentrations *in vivo*.

Vincristine showed good efficacy in inhibiting growth of breast cancer cells in our *in vitro* model. It has to be mentioned

that Vincristine generally exhibits good cytotoxicity in tissue culture experiments. Nevertheless, our results warrant clinical testing in breast cancer patients. Proteins like P-glycoprotein and multidrug resistance-associated protein (MRP) maintain the BBB by transmembrane efflux mechanisms that are believed to transport vinca alkaloids out of the CNS (3). To some minor extent, these mechanisms could take effect even if the BBB is disrupted by metastases, weakening the effect of Vincristine on metastatic tumor growth *in vivo*.

Etoposide is a topoisomerase II inhibitor used for high-grade gliomas. Our results revealed only a minimal efficacy of Etoposide against breast cancer cells and, even for the treatment of primary brain tumors like gliomas, the data in the literature is ambiguous (43).

Nitrosoureas were among the earliest agents to have demonstrable clinical efficacy in brain tumors (45). They are widely used as compounds of drug regimens. We tested the nitrosourea Nimustine with regard to its ability to inhibit breast cancer cell growth. It did not exhibit considerable cytotoxicity in our *in vitro* assays. Nevertheless, the results have to be interpreted with caution. It could very well be that it has minor efficacy as monotherapy, but is a valuable compound if combined with other substances, as demonstrated for the combination Nimustine and Irinotecan (18). Furthermore, its comparably weak performance in *in vitro* assays has frequently been observed and, therefore, a final judgment on Nimustine is difficult. The conclusion from our results is that there is no *in vitro* basis that would warrant clinical evaluation of the drug as monotherapy for BM from breast cancer.

Irinotecan (CP-11) belongs to the family of topoisomerase I inhibitors similar to Topotecan. As pointed out for Nimustine, as part of a drug regimen it might have considerable value but, considering our results, Irinotecan, Etoposide and Nimustine are not the first choice for a clinical evaluation in breast cancer patients with BM.

Final evaluation of all these drugs was not done in commercially available cell lines. It is characteristic of such cell lines that they divide rather rapidly, but they seem to lose a lot of their *in vivo* properties during several years of *in vitro* culturing and obtain new ones while adapting to culture conditions. Some of those properties lost and/or gained might play an important role for the cell's susceptibility to cancer drugs. Bahr *et al.* showed that culturing of glioma cell lines can lead to a multidrug resistance (MDR)-phenotype that primary glioma tumor cells do not show (2). The authors hereby exemplified a possible pitfall of *in vitro* testing. This illustrates how crucial it is to mimic the situation *in vivo* as far as possible, which we attempted with the use of primary cell lines and only 3-5 passages *in vitro*.

To the best of our knowledge, this is the first report using primary cell lines derived from pleural effusions to test chemotherapeutics which potentially can be used for the therapy of BM in breast cancer patients. The data presented

here might help to increase the treatment options. This will enable us to take age, performance status, tumor biology and prior therapy into account when deciding about treatment of metastatic CNS disease.

The approach of testing potential therapies with primary tissue cultures with the non-radioactive cell counting kit appears to be a valid option for preclinical testing of new chemotherapeutics for the treatment of breast cancer. Therefore, this experimental approach could be applied for promising substances, such as Capecitabine (41), Thiotepa and Temozolamide (1), to enlarge the therapeutic spectrum for metastatic CNS disease. Clinical trials with large patient populations are needed to identify the subgroup of patients that would benefit the most from chemotherapy for brain metastases.

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References

- 1 Abrey LE, Olson JD, Raizer JJ, Mack M, Rodavitch A, Boutros DY and Malkin MG: A phase II trial of temozolomide for patients with recurrent or progressive brain metastases. *J Neurooncol* 53(3): 259-265, 2001.
- 2 Bahr O, Rieger J, Duffner F, Meyermann R, Weller M and Wick W: P-glycoprotein and multidrug resistance-associated protein mediate specific patterns of multidrug resistance in malignant glioma cell lines, but not in primary glioma cells. *Brain Pathol* 13(4): 482-494, 2003.
- 3 Bart J, Groen JM, Hendrikse NH, van der Graaf WTA, Vaalburg W and de Vries EGE: The blood-brain barrier and oncology: new insights into function and modulation. *Cancer Treat Rev* 26: 449-462, 2000.
- 4 Bendell JC, Domchek SM, Burstein HJ, Harris L, Younger J, Kuter I, Bunnell C, Rue M, Gelman R and Winer E: Central nervous system metastases in women who receive trastuzumab-based therapy for metastatic breast carcinoma. *Cancer* 97: 2972-2977, 2003.
- 5 Blaney SM, Cole DE, Balis FM, Godwin K and Poplack DG: Plasma and cerebrospinal fluid pharmacokinetic study of topotecan in nonhuman primates. *Cancer Res* 53: 725-727, 1993.
- 6 Bonadonna G, Valagussa P, Moliterni A, Zambetti M and Brambilla C: Adjuvant cyclophosphamide, methotrexate, and fluorouracil in node-positive breast cancer: the results of 20 years of follow-up: *N Engl J Med* 332(14): 901-906, 1995.
- 7 Boogerd W: Central nervous system metastasis in breast cancer. *Radiother Oncol* 40: 5-22, 1996.
- 8 Brandes AA, Palmisano V and Monfardini S: Medulloblastoma in adults: clinical characteristics and treatment. *Cancer Treat Rev* 25(1): 3-12, 1999.
- 9 Clamon G and Doebbeling B: Meningeal carcinomatosis from breast cancer: spinal cord vs. brain involvement. *Breast Cancer Res Treat* 9: 213-217, 1987.

- 10 Clark GM, Sledge GW Jr, Osborne CK and McGuire WL: Survival from first recurrence: relative importance of prognostic factors in 1,015 breast cancer patients. *J Clin Oncol* 5(1): 55-61, 1987.
- 11 Crivellari D, Pagani O, Veronesi A, Lombardi D, Nole F, Thurlimann B, Hess D, Corner M, Bauer J, Martinelli G, Graffeo R, Sessa C and Goldhirsch A: High incidence of central nervous system involvement in patients with metastatic or locally advanced breast cancer treated with epirubicin and docetaxel. *Ann Oncol* 12: 353-356, 2001.
- 12 Crown J and Pegram M: Platinum-taxane combinations in metastatic breast cancer: an evolving role in the era of molecularly targeted therapy. *Breast Cancer Res Treat* 79(Suppl 1): 11-18, 2003.
- 13 DeAngelis LM: Current diagnosis and treatment of leptomeningeal metastasis. *J Neurooncol* 38: 245-252, 1998.
- 14 DiStefano A, Yong Yap Y, Hortobagyi GN and Blumenschein GR: The natural history of breast cancer patients with brain metastases. *Cancer* 44: 1913-1918, 1979.
- 15 Early breast cancer triallists' Collaborative Group: Poly-chemotherapy for early breast cancer: an overview of the randomized trials. *Lancet* 352: 930-942, 1998.
- 16 Fenner MH and Possinger K: Chemotherapy for breast cancer brain metastases. *Onkologie* 25: 474-479, 2002.
- 17 Freilich RJ, Seidmann AD and DeAngelis LM: Central nervous system progression of metastatic breast cancer in patients treated with paclitaxel. *Cancer* 76: 232-236, 1995.
- 18 Friedman HS, Keir ST and Houghton PJ: The emerging role of irinotecan (CPT-11) in the treatment of malignant glioma in brain tumors. *Cancer* 97(9 Suppl): 2359-2362, 2003.
- 19 Fuchs IB, Loebecke M, Buhler H, Stoltenburg-Didinger G, Heine B, Lichtenegger W and Schaller G: HER2 in brain metastases: issues of concordance, survival and treatment. *J Clin Oncol* 20: 4130-4133, 2002.
- 20 Greig NH: Brain tumors and the blood tumor barrier. *In: Implications of the Blood-Brain Barrier and its Manipulation*, Plenum Press, Vol. 2, pp. 77-106, 1989.
- 21 Greig NH: Optimizing drug delivery to brain tumours. *Cancer Treat Rev* 14: 1-28, 1987.
- 22 Hagemeister FB, Buzdar AU, Luna MA and Blumenschein GR: Causes of death in breast cancer: a clinicopathologic study. *Cancer* 46: 162-167, 1980.
- 23 Hall WA, Djalilian HR, Nussbaum ES and Cho KH: Long-term survival with metastatic cancer to the brain. *Med Oncol* 17: 279-286, 2000.
- 24 Kirsch DG and Hochberg FH: Targeting HER2 in brain metastases from breast cancer *Clin Cancer Res* 9: 5435-5436, 2003.
- 25 Kosmas C, Malamos NA, Tsavaris N and Antonopoulos M: Chemotherapy-induced complete remission of choroidal metastases and subsequent isolated leptomeningeal carcinomatosis in advanced breast cancer: a case report and literature review. *J Neurooncol* 47: 161-165, 2000.
- 26 Koukourakis MI, Koukouraki S, Fezoulidis I, Kelekis N, Kyrias G, Archimandritis S and Karkavitsas N: High intratumoral accumulation of stealth liposomal doxorubicin (Caelyx) in glioblastomas and in metastatic brain tumours. *Br J Cancer* 83: 1281-1286, 2000.
- 27 Landonio G, Sartore-Bianchi A, Gianetta L, Renga M, Riva M and Sienna S: Controversies in the management of brain metastases: the role of chemotherapy. *Forum* 11(1): 59-74, 2001.
- 28 Lentzsch S, Reichardt P, Weber F, Budach V and Dörken B: Brain metastases in breast cancer: prognostic factors and management. *Eur J Cancer* 35(4): 580-585, 1999.
- 29 Lesser GJ: Chemotherapy of cerebral metastases from solid tumors. *Neurosurg Clin N Am* 7(3): 527-536, 1996.
- 30 Levine EG, Cirrincione CT, Szatrowski TP, Canellos G, Norton L and Henderson IC: Phase II trial of topotecan in advanced breast cancer: a Cancer and Leukemia Group B study. *Am J Clin Oncol* 22(3): 218-222, 1999.
- 31 Martin M: Platinum compounds in the treatment of advanced breast cancer. *Clin Breast Cancer* 2: 190-209, 2001.
- 32 Massot-Punyet R, Almajano J and Camacho JM: Cerebral metastasis. *Rev Neurol* 31(12): 1242-1247, 2000.
- 33 Morrow CP and Curtin JP: *In: Synopsis of Gynecologic Oncology* 5th edition. Churchill Livingstone pp. 343, 1998
- 34 Oberhoff C, Kieback DG, Wurstlein R, Deertz H, Sehoul J, van Soest C, Hilfrich J, Mesroglu M, von Minckwitz G, Staab HJ and Schindler AE: Topotecan chemotherapy in patients with breast cancer and brain metastases: results of a pilot study. *Onkologie* 24: 256-260, 2001.
- 35 Patchell RA, Tibbs PA, Walsh JW, Dempsey RJ, Maruyama Y, Kryscio RJ, Markesbery WR, Macdonald JS and Young B: A randomized trial of surgery in the treatment of single metastases to the brain. *N Engl J Med* 322: 494-500, 1990.
- 36 Patchell RA: The management of brain metastases. *Cancer Treat Rev* 29: 533-540, 2003.
- 37 Pollack IF, Boyett JM and Finlay JL: Chemotherapy for high-grade gliomas of childhood. *Child's Nerv Syst* 15: 529-544, 1999.
- 38 Rieger J, Roth W, Glaser T, Winter S, Rieger L, Dichgans J and Weller M: Glioblastoma multiforme: mechanisms of resistance to chemotherapy. *Neurol Psych Brain Res* 7: 37-46, 1999.
- 39 Saito M, Yoshimoto M, Akiyama F, Sakamoto G, Kasumi F and Kaminishi M: Characteristics of brain metastases from node negative breast cancer. *Breast* 12 (Suppl 1): 28-29, 2003.
- 40 Schouten LJ, Rutten J, Huveneers HA and Twijnstra A: Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma. *Cancer* 94(10): 2698-2705, 2002.
- 41 Siegelmann-Danieli N, Stein M and Bar-Ziv J: Complete response of brain metastases originating in breast cancer to capecitabine therapy. *Isr Med Assoc J* 5(11): 833-834, 2003.
- 42 Singletary SE, Walsh G, Vauthey JN, Curley S, Sawaya R, Weber KL, Meric F and Horobagyi GN: A role for curative surgery in the treatment of selected patients with metastatic breast cancer. *Oncologist* 8(3): 241-251, 2003.
- 43 Tirelli U, D'Incalci M, Cancetta *et al*: Etoposide (VP-16-213) in malignant brain tumors; a phase II study. *J Clin Oncol* 2: 432-437, 1984.
- 44 Tsukada Y, Fouad A, Pickren JW and Lane WW: Central nervous system metastasis from breast carcinoma. Autopsy study. *Cancer* 52(12): 2349-2354, 1983.
- 45 Walker MD, Alexander E Jr, Hunt WE, MacCarty CS, Mahaley MS Jr, Mealey J Jr, Norrell HA, Owens G, Ransohoff J, Wilson CB, Gehan EA and Strike TA: Evaluation of BCNU and/or radiotherapy in the treatment of anaplastic gliomas. A cooperative clinical trial. *J Neurosurg* 49(3): 333-343, 1978.

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