MA 0918

Full Title:
Intra-hepatic chemotherapy with oxaliplatin every 2 weeks combined with systemic capecitabine and in patients with HER2-positive tumors additionally with trastuzumab (Herceptin®) in patients with non-resectable liver metastases from breast cancer.

A Phase II study of patients with limited extrahepatic disease.

Brief title:

Intra-hepatic Chemotherapy in Patients With Liver Metastases From Breast Cancer and Limited Extrahepatic Disease

EudraCT: 2009-014821-17

ClinicalTrials.gov: NCT01387295

Protocol version 8 dated Nov, 8th 2012

Protocol Committee and Clinical Investigators:

Consultant, Dr. med. Dorte Nielsen, Department of Oncology [1)]

Consultant Benny Vittrup Jensen, Department of Oncology [1)]

Senior Project Nurse Birgitte Christiansen[1)]

Consultant Bjørn Skjoldby, Surgical Section, Gastrounit [1)]

Consultant Henrik Nørgaard, Department of Radiology [1)]

Consultant, Professor, Dr. med. Julia S. Johansen, Department of Oncology1[1)]

Head of the Biobank, PhD. Estrid Høgdall, Department of Pathology[1)]

Consultant Eva Balslev, Department of Pathology[1)]

Consultant, PhD, Ann Knoop, Department of Oncology [2)]

Professor, Consultant, PhD, Per Pfeiffer, Department of Oncology [2)]

[1)] Herlev Hospital, 2730 Herlev, Denmark.

[2)] Odense University Hospital, 5000 Odense, Denmark.

Coordinating Investigator:

Consultant Dorte Nielsen, Department of Oncology, Herlev Hospital

Sponsor:

Consultant Dorte Nielsen, Department of Oncology, Herlev Hospital

"Trial Office"

Clinical Research Unit

Department of Oncology

Herlev Hospital

Monitor:

The University of Copenhagen's GCP unit is the coordinating GCP unit. Monitoring at the Department of Oncology, Aarhus and Odense will take place at the local GCP units.

Signature page

The study will be conducted in accordance with ICH-GCP (E6). All personnel responsible for the design and conduct of the study have undergone the GCP training.

The signature below approves the Protocol and its Annexes and is intended to ensure that the trial is conducted in accordance with the Protocol.

Having read and understood the requirements and obligations contained in the Protocol, I agree to conduct the clinical trial in accordance with national laws and international guidelines for good clinical practice.

Sponsor

Dorte Nielsen, Consultant, Dr. med.
Department of Oncology
Herlev Hospital
Herlev Ringvej 75
2730 Herlev
Email: dornie01@heh.regionh.dk
Tel: 44884000 82344----------

Date Signature

Principal investigator

Dorte Nielsen, Consultant, Dr. med.
Department of Oncology
Herlev Hospital
Herlev Ringvej 75
2730 Herlev
Email: dornie01@heh.regionh.dk
Tel: 44884000 82344----------

Date Signature

Investigator

xxxxxxxxxxx
Department of Oncology
xxxxxxxxxx
xxxxxxxxxxxxx
xxxxxxxxxxxx
Tel. xxxxxxxxx----------

Date Signature

1 Protocol synopsis

Title	Intrahepatic chemotherapy with oxaliplatin every 2 weeks combined with systemic capecitabine and in patients with HER2-positive tumors, in addition, trastuzumab (Herceptin®) in patients with non-resectable liver metastases from breast cancer
Studio design	Phase II study
Primary objective	Tumor response in the liver according to RECIST criteria version 1.1
Secondary objectives	a) Progression free survival (Intrahepatic) b) Progression free survival (Extrahepatic) c) Number of patients who can have the tumor burden reduced so much that they become suitable for local treatment (radiofrequency ablation (RF)) d) Survival (e) Toxicity
Paraclinical endpoints	Correlation between known markers in tissues (hormone receptor status, HER2 and topo IIα) and project biomarkers in blood and tissues in the form of gene profiles (e.g. mRNA and microRNA, SNP array profiles), proteins (e.g. p53, P1NP, P3NP, TIMP-1, IL-6, YKL-40, EGFR and VEGF), as well as metabolites and clinical parameters (response, time to progression, survival)
Patient population	Histologically or cytologically proven breast adenocarcinoma Liver metastases where local treatment (surgery/RF) is not indicated Patients with bone metastases or lymphnode metastases, without progression in the extrahepatic sites within 6 months and whose tumor burden is predominantly hepatic (as assessed by PET-CT scan) Measurable disease according to RECIST criteria version 1.1 No progression on treatment with capecitabine Age 18 years or older Performance status <2 Life expectancy over 3 months. Previous treatment with taxane either adjuvant or for metastatic disease. If treatment has been given for metastatic disease, treatment should have continued to the maximum response or cessation due to toxicity

Number of patients	Up to 50 evaluable patients receiving a permanent catheter and up to 50 evaluable patients undergoing transarterial chemoembolism with Degradable Starch Microsperes (DSM-TACE) recruited over 3 years period. Initiated October 2009. Last patient included October 2012.
Participating centers	Herlev Hospital, Odense University Hospital Other departments in Denmark may refer to the participating centres for consideration to be treated within the trial.
Treatment:	Oxaliplatin 85 mg/m2 (max. 2 m^2) intrahepatically over 1/2 hour every 2 weeks Concomitant systemic treatment: capecitabine 1300 mg/m^2 divided into two daily doses continuously (max, 2 m^2). Patients with HER-2 positive tumours: Trastuzumab (Herceptin®) 8mg/kg day 1 followed by 6 mg/kg every 3 weeks. Due to anatomical conditions, in 1/2 of the patients it is not possible to apply a permanent catheter, in these patients DSM-TACE is performed, where chemotherapeutic followed by EmboCeptS is injicated in A.hepatica. Treatment given: Oxaliplatin intrahepatic with 70-85 mg/m^2 over 10-30 minutes followed by 0-7 ½ ml EmboCeptS (until flow stop) every 4 weeks. Oxaliplatin intravenously with 85 mg/m^2 over 1/2 hour every 4 weeks (alternating) Oral capecitabine 1300 mg/m^2 daily divided into 2 doses continuously. In HER2-positive tumour, in combination with trastuzumab (Herceptin ®) 8 mg/kg day 1 followed by 6 mg/kg every 3 weeks For 4 patients, pharmacokinetics are carried out, taking 2 ml of blood on time 0, 15, 30, 60 min. and 2, 2½, 3, 4 and 6 hours for determination of oxaliplatin concentration.
Local treatment **Treatment after intrahepatic therapy**	At each evaluation every 2 months, it is assessed whether patients are suitable for radiofrequency (RF) - ablation. At investigators discretion: Capecitabine 2000 mg/m2 for 14 days every 3 weeks and if applicable with trastuzumab (Herceptin®) 6 mg/kg every 3 weeks in patients with HER-2 positive tumours. Treatment is given for progression and/or unacceptable toxicity.

EVALUATION **Baseline** **Before each series** **After every 4th series in connection with treatment then every 3 months**	Clinical evaluation, CT scan (possibly PET). If there are extrahepatic focuses in the bones, supplemented by MRI scans/x-ray (depending on the assessment at baseline). Hematological / biochemical status (hemoglobin, leucocyte count, neutrophil count, platelet count, sodium, potassium, creatinine, ASAT, LDH, alkaline phosphatase, billirubin), Project blood samples for the determination of biomarkers as above indicated in blood and described in Appendix 2. Tissue sample from the primary tumor is stored, and liver metastasis and possibly other metastases A biobank of blood and tissue from the patients is established. Hemoglobin, leucocyte count, neutrophil count, platelet count, sodium, potassium, creatinine, ASAT, LDH, alkaline phosphatase and bilirubin. Evaluation of side effects before each series. Tumor evaluation after every 4th series (every 7 weeks) according to RECIST criteria version 1.1 in during intrahepatic treatment thereafter every 3 months. At the time of each evaluation, project blood samples are taken to determine biomarkers as indicated in blood and described in Appendix 2. If biopsy is taken, tissue is stored from metastases. Disse samples are stored in the biobank.
Duration of treatment	Until disease progression Until unacceptable toxicity Until the patient wants the treatment discontinued A maximum of 12 series of intrahepatic treatment is given

1. Introduction

1.1 Background

Breast cancer is the most common cancer in women. The frequency has increased steadily in the last 50 years. In Europe, one in ten women will get breast cancer during their lives. Despite advances in diagnosis and treatment, breast cancer is the second leading cause of death in women. In the EU, around 179,000 new patients are diagnosed each year (28% of all cancers in women), of which around 74,000 patients die (15% of all deaths are related to cancer). The median survival rate for patients with metastasizing breast cancer (MBC) is 17-20 months, and the 5-year survival rate is about 15%. Patients in which the disease is confined to the bones may experience a more indolent course of the disease and have a greater chance of survival over a 5-year period (30-40%). Patients with visceral disease, especially liver metastases, have very poor prognosis and a median survival of 8-10 months. First-line treatment of metastasizing disease includes endocrine therapy or chemotherapy for hormone receptor positive tumor depending on localization of metastases, and chemotherapy for hormone receptor negative or endocrine non-responding disease. Patients who have received adjuvant chemotherapy generally respond worse to first-line treatment in advanced disease, and the response to chemotherapy continues to decline with second and third-line therapy, both in terms of response rate and time to disease progression.

1.2 Intrahepatic treatment

Several Phase III multicenter trials have been published regarding hepatic arterial chemotherapy in patients with liver metastases from colorectal cancer. These articles describe higher response rates with intraarterial than with intravenous therapy. However, no difference in median survival has been described, which to some extent may be due to a high complication rate of intraarterial therapy. In contrast, Kemeny et al. (1999) showed a low complication rate with promising median survival compared to established chemotherapy regimens. Besides, Ricke et al. (2004) shown very low complication rate by the use of "a minimally invasive hepatic arterial port" established via arteria subclavia or on the thigh. In a subsequent report, Vogl et al. (2007) describe the efficacy of intraarterial chemotherapy with a combination of gemcitabine and mitomycin C in 12 patients with colorectal cancer and 12 patients with breast cancer. They observed 5 partial response

(PR) and 3 cases of stable disease (SD) in patients with colorectal cancer, one case with complete response (CR), 4 PR, and 6 SD in patients with breast cancer. The median survival was 10 months for patients with colorectal cancer and 11 months for patients with breast cancer. The authors concluded that treatment was safe with acceptable tumor control. Recently, four studies with promising survival data in colorectal cancer have been published. The studies suggest that combined locoregional and systemic chemotherapy may be a step towards improved survival in the treatment of metastatic colorectal cancer.
If a patient with breast cancer develops liver metastases, all future treatment is considered palliative. Local tumor control in the form of hepatic resection and "radiofrequency" (RF) remains controversial treatment options, and there is no general consensus on whether patients benefit from these procedures. However, several authors have reported median survival of more than 30 months after such treatment approaches. These have been small patient populations that were probably highly selected. Hepatic intraarterial chemotherapy has so far only been very sparsely studied for the treatment of liver metastases in breast cancer. Thus, in most cases, only "case stories" (often in the media) have been reported. Only one Phase I/II trial has been reported in which a combination of adriamycin and 5-fluorouracil was used (first and second line treatment, a total of 28 patients (26 evaluable), 11 also had extrahepatic disease). The overall response rate was 63% with a remarkably long median survival of 25 months, in contrast to recent studies that reported a median survival of around 12 months. However, in some studies, there seems to be a trend towards increased survival for patients who have undergone regional therapy. To our knowledge, only 12 cases have been published where patients have been treated with both intra- and extrahepatic disease (there are no independent reports for these patients)..
Patients with bone and lymphatic metastases from breast cancer often have an indolent course, while patients with lever metastases have an exceedingly poor prognosis. Some of the latter patients could potentially benefit from intrahepatic treatment.
In addition to the above, a retrospective report of 217 patients with liver metastases from c. mammae has been published as an abstract (it is not clear whether the patients had extrahepatic disease). Patients received mitomycin C alone, gemcitabine alone, or a combination of the two drugs, as well as embolization with lipiodol and starch microspheres. PR in the liver was achieved in 13%, SD in 50% and progressive disease in 36%. One-year survival was 68% and 2-year survival 43%. Today, the treatment is offered in Germany, and many patients have a great desire to be able to receive the treatment in

Denmark. The protocol has been approved by the National Committee for Experimental Cancer Treatment.

2 Chemotherapy

2.1 Capecitabine (Xeloda®)

Capecitabine is administered as a non-cytotoxic, systemic pro-drug of 5'-deoxy-5-fluorouridine. After administration, it is largely absorbed unchanged from the gastrointestinal tract and is sequentially converted to the cytotoxic 5-fluorouracil (5-FU) via a series of metabolic steps. The final reaction in this signaling pathway is catalyzed by thymidine-phosphorylase (TP). 5-FU is preferably generated at the tumor site via utilization of the higher concentrations of TP found in tumor tissue compared to normal tissue.
In MBC, capecitabine has been evaluated in clinical trials in combination with docetaxel and as monotherapy. Capecitabine in combination with docetaxel is indicated for the treatment of patients with MBC after lack of efficacy with anthracyclins. Capecitabine monotherapy is indicated for the treatment of patients with MBC who are resistant to both paclitaxel and a chemotherapy regimen containing anthracycline or who are resistant to paclitaxel and where further treatment with anthracycline is not indicated.
Capecitabine has been studied in patients with liver metastases with mild to moderate liver dysfunction. There was no difference in the pharmacological parameters of the main metabolites from capecitabine in this group compared to patients with normal liver function.
The dose-limiting side effects are especially diarrhea and hand and foot syndrome.

2.2 Oxaliplatin (Eloxatin®)

Oxaliplatin is a 3rd generation platinum derivative with mechanism of action such as cisplatin. Oxaliplatin forms cross-links between base pairs in DNA, which inhibits replication and transcription and results in cell death. After a 2-hour infusion, 15% of the given platinum is present in the systemic circulation while the remaining 85% is rapidly distributed in the tissues. The bulk of platinum is eliminated in the urine especially within the first 48 hours. After 5 days, 54% is excreted in the urine and 3% in the faeces. Oxaliplatin has an adverse reaction profile that is different from cisplatin in that no reports of renal toxicity, ototoxicity, hair loss or severe haematological toxicity have been reported. The dose-limiting side effect is a cumulative peripheral sensory polyneuropathy (pSPN), which is aggravated in low temperatures.

In several *in vivo* tumor models, the combination of oxaliplatin and 5-FU causes synergistic antiproliferative effect.
Oxaliplatin was initially administered as an intravenous infusion over 2 hours. Studies have shown that the substance can be given over 1/2 hour with less local toxicity and unchanged systemic toxicity.
Platinum derivatives have only been used to a limited extent by MBC. Previous studies have shown significant efficacy of cisplatin in combination with epirubicin and studies suggest that the platinums should be used in patients who have BRCA gene mutation.
Oxaliplatin has only been used to a small extent but is considered one of the new promising substances.

2.3 Oxaliplatin intrahpatic

A Phase I study of intrahepatic infusion of oxaliplatin over 4 hours in combination with folic acid (200 mg/m^2 over 1 h) and 5-FU (600 mg/m2 over 2 hours) was conducted in patients with colorectal cancer. Patients were treated every 3 weeks with increasing doses of oxaliplatin. 21 patients were evaluated, and all patients were evaluable. Dose-limiting toxicity (DLT) was observed in oxaliplatin 150 mg/m^2/3rd week and consisted of leukopenia, obliteration of A. hepatica and acute pancreatitis. Toxicity consisted mostly of vomiting (16/21), anemia (16/21), upper abdominal pain (15/21), sensory neuropathia (10/21), diarrhea (9/21), and trombocytopenia (9/21). Ten of 18 evaluable patients (56%) achieved a complete or partial response. The authors recommended doses in a Phase II study for intrahepatic oxaliplatin 125 mg/m^2 every 3 weeks.

2.4 Oxaliplatin intrahepatic in combination with capecitabine

The Department of Oncology, Herlev Hospital has considerable experience with intrahepatic infusion of oxaliplatin in combination with capecitabine for patients with colorectal cancer. The following regimen was used: oxaliplatin 100 mg/m^2 intrahepatically over 2 hours day 1 every 2 weeks combined with Xeloda® up to 3500 mg/m2 daily divided into 2 doses for one week every 2 weeks. At present, 42 evalauble patients have been treated in a Phase II protocol. In connection with the treatments, the following side effects have been seen: hand-foot syndrome, diarrhea, thrombus formation at catheter tip (rarely), convulsive abdominal pain in connection with and after infusion of oxaliplatin, ulcers (rarely), fatigue, peripheral neuropathy, nausea, anorexia and vomiting. In all cases, the

side effects have been manageable. No patients have died as a result of treatment. The side effects have ledt to that almost all patients have received a reduced dose. We have therefore chosen to reduce the dose of oxaliplatin (85 mg/m2) and administer capecitabine continuously in a reduced dose (1300 mg/m2 daily divided into 2 doses) instead of over 1 week (total dose of capecitabine is reduced). Continuous treatment with capecitabine is used in patients with ventricular cancer and the treatment is well tolerated.

2.5 Trastuzumab

Trastuzumab is a recombinant DNA-derived humanized IgG monoclonal antibody that selectively binds to the human epidermal growth factor receptor 2 protein (HER2). Trastuzumab used in combination with chemotherapy shows efficacy on all endpoints in a randomized study of 469 patients with MBC with overexpression of HER2 who had not previously been treated with chemotherapy for metastatic disease. Overall, the group of patients receiving combination therapy (trastuzumab and chemotherapy) achieved better response rates (50% versus 32%), longer time to disease progression (7.4 months versus 4.6 months) and extended survival (25.1 months versus 20.3 months) compared to the group of patients treated with chemotherapy only. Den median survival was extended almost 5 months, despite the fact that approximately 70% of patients receiving chemotherapy only as first line therapy received trastuzumab upon progression. Trastuzumab added to the two chemotherapy regimens did not give rise to any more serious adverse events except for cardiac dysfunction, which was specifically increased in the group receiving both anthracyklin and trastuzumab.

The most common side effects of trastuzumab are infusion-related symptoms such as fever and chills, usually after the first infusion of trastuzumab. Other side effects associated with the infusion may include nausea, vomiting, pain, headache, hypotension, rash, and asthenia. Symptoms rarely appear with subsequent trastuzumab infusions. Serious side effects of trastuzumab are rare, but may include ARDS and heart failure, such as dyspnea, increasing cough, peripheral edema or reduced left ventricle ejection fraction. Therefore, patients with severe dyspnea at rest due to advanced malignancy or patients requiring supplemental oxygen therapy should not be treated with trastuzumab. In addition, an increased incidence of anemia and leukopenia has been observed after administration of trastuzumab in combination with chemotherapy. Allergic reactions and hypersensitivity that occur during the first infusion of trastuzumab have rarely been reported. Today, the drug is routinely used in all patients with HER2 positive tumor.

2.5.1 Overview of clinical experience with continuation of trastuzumab after progression in metastatic breast cancer

More recently, a prospective, randomized Phase III trial has been closed prematurely following the recruitment of 156 of planned 482 patients. The trial was conducted by German Breast Group to evaluate the safety and efficacy of treatment with trastuzumab after progression. The primary purpose of this trial was to compare capecitabine alone or in combination with trastuzumab in patients with HER2-positive MBC and progression after prior treatment with trastuzumab (alone or in combination with chemotherapy drugs other than capecitabine). The majority of patients had received trastuzumab with chemotherapy as a 1st line treatment. An initial analysis indicates that continued treatment with trastuzumab after progression in addition to capecitabine had fewer cases of tumor progression (48 *versus* 53, hazard ratio 0.71 in favor of the trastuzumab arm) and fewer deaths (26 *versus* 31), and that continued treatment with trastuzumab combined with capecitabine achieved a response rate of 49% *versus* 25% achieved with capecitabine alone. The incidence of severe toxicities, including cardiac toxicities, did not differ significantly in the two arms. However, the final analysis has not yet been published. Several retrospective trials have also been conducted with continuation of treatment with trastuzumab after progression. The authors consistently conclude that data clearly support the clinical benefit of continued treatment with trastuzumab.

2.5.2 Overview of clinical experience with combination therapy with trastuzumab and capecitabine

A recently published open-label Phase II trial of capecitabine plus trastuzumab recruited 27 patients with MBC with overexpression of HER2 who had previously been treated with anthracylines and/or taxanes. Patients received 2500 mg/m^2 of capecitabine for 14 days, followed by a 7-day break combined with trastuzumab 2 mg/kg weekly. Twelve patients (45%) achieved objective response, including CR in 4 patients (15%) and PR in 8 patients (35%). A further 9 (33%) of patients achieved stabilisation of the disease. Median survival (OS) was 28 months, time to progression (PFS) 6.7 months. The safety profile of the combination was favorable and predictable with a low incidence of grade 3 or 4 adverse events. The most common adverse events were pain (estimated primarily related to the disease), hand-foot syndrome (grade 1-2 in 17 patients, grade 3 in 4 patients), and gastrointestinal side effects (including grade 1-2 diarrhea in 13 patients). Severe

myelosuppression was rare (3 patients) and severe alopecia did not occur. One patient experienced trastuzumab-induced heart failure.
Another trial investigated the efficacy of capecitabine combined with trastuzumab in breast cancer patients. Patients had previously received treatment with either anthracycycline and docetaxel or vinorelbine, and all patients had previously been treated with trastuzumab for metastatic disease. 40 patients were recruited. The median time to progression was 8 months, and the OS was 24 months. No significant difference was found between 2nd line treatment and treatment thereafter. The CR rate was 2.5% and the PR rate was 17.5%. The disease was stable in fifty percent of patients for at least 6 months, resulting in a clinical benefit of 70%. Treatment-related adverse events of grade 3/4 were diarrhea (5%) and hand-foot syndrome (15%). In addition, three patients (7.5%) developed brain metastases during treatment.
For HER2 positive MBC, there is further evidence that capecitabine in combination with lapatinib is an effective treatment. Elevated liver enzymes are seen relatively frequently when treated with lapatinib, which is why the pharmaceutical company (GSK) advises against lapatinib being given in combination with intrahepatic therapy. Results regarding continued treatment with trastuzumab are comparable to results regarding lapatinib, which is why it is considered justifiable that patients are offered this treatment.

2.5.3 Administration of the drug

Trastuzumab is administered according to the department's standard guidelines. In very rare cases, patients have experienced infusion symptoms or pulmonary symptoms occurring more than six hours after the start of the infusion. Patients are warned of the possibility of this and informed that the doctor should be contacted if these symptoms occur.

2.6 DSM-TACE" (transarterial chemoembolisation)

Our current experience has shown that the anatomy of the liver arteries in up to 1/2 of patients is of such a nature that it can prevent uncomplicated intrahepatic treatment. It can be a double liver artery supply or meandering vessels, which provide unstable catheter deposit. Although there are methods to address these challenges (internal fixation of the catheter, extensive liver artery embolization, catheter and porting via A.subclavia), these also carry the risk of new complications. An alternative method of regional chemoinfusion consists in repeated catheterizations of the hepatic artery, whereby one or more

chemotherapeutics are applied as bolus, and are followed by the injection of occluding material into the hepatic artery, so called TACE.
The rationale of treatment is that the decreased perfusion achieved through embolization increases intracellular uptake of the chemotherapy. TACE for the treatment of hepatocellular carcinoma (HCC) is well described.
TACE for the treatment of liver metastases has been less studied, but promising recent results are available from German centers. The experience from this suggests that the chemoembolization can be made less selective than when treating HCC, when the embolization is carried out gently and with resorbable material.
This so-called DSM-TACE consists of intrahepatic infusion of chemotherapeutic followed by degradable glycogen particles (Degradable Starch Microsperes, DSM). The particles (EmboCeptS 450mg/7½ ml, Pharmacept Gmbh, Berlin) of about 50 µm cause hemostasis in the hepatic arteries for 1-2 hours, thereby exposing the tumor cells to a greater concentration of chemotherapeutics than with the usual intravenous or intraarterial infusion. The particles dissolve with a half-life of 50 min.
The administration takes place in the radiological department by a radiologist, first performing selective arteriography of A. hepaticae to identify any lateral branches that need to be occluded before chemoembolization, in order to avoid chemoembolization of the stomach and intestines. After any coil embolization of the side branches, chemotherapeutics are injected. Optionally, several liver artery branches are to be treated separately. Experienced nurse with chemo course will be present.
Side effects of TACE are predominantly described in the treatment of HCC and consist in abdominal pain, nausea and vomiting for 2-7 days. Further, fever appears immediately after treatment. Further, cases of intraperitonal hemorrhage have been described. The risk of side effects is correlated to the disease status, liver function (cirrhosis of the liver) and the volume treated. In the protocol, for this reason, the first 3 patients are treated at the lowest recommended dose. If none of these patients experience serious side effects, the dose will be increased.
In cases where a permanent catheter cannot be installed (Herlev and Aarhus) or in all cases in Odense, the patient is offered DMS-TACE. As a total of 6 treatments with DMS-TACE will be given, the treatment regimen will be:
DMS-TACE with oxaliplatin:

Oxaliplatin 70 mg/m^2 with infusion over 1/2 hour followed by EmboCeptS til flow stop, max 3 ml (3 patients).

Oxaliplatin 85 mg/m^2 with infusion over 1/2 h followed by EmboCeptS til flow stop, max 7½ ml (3 patients).

Oxaliplatin 85 mg/m^2 with infusion over 10 minutes followed by EmboCeptS til flow stop, max 7½ ml (3 patients).

If this treatment is tolerated in the latter patients, this treatment regimen will be continued.
Treatment will be evaluated closely.
Each time, treatment will be evaluated after 3 patients who have each received at least 1 treatment. If Grade 4 (NCI CTC version 3.0) adverse events related to intrahepatic therapy are observed, this part of the trial will be discontinued immediately. In the event of death, the trial is immediately discontinued. If no grade 3 side effects are seen that cannot be treated with standard supportive treatment, the dose will be increased to: Oxaliplatin 85 mg/m^2 followed by a maximum of 7½ ml of EmboCeptS intrahepatically every 4 weeks.
Oxaliplatin 85 mg/m2 intravenously over 1/2 hour every 4 weeks (alternating) (6 times).
Capecitabine 1300 mg/m2 continuously.
After catheter insertion/TACE:
Patients are observed according to the instructions of the wards.
Pain management, supportive treatment and observation in general will be done in accordance with the departments' instructions for intrahepatic treatment.
Due to little experience with DMS-TACE, the treatment will initially only be offered at Herlev Hospital.

2.7 Local treatment

As part of the evaluation of tumor response, all patients will be assessed for indication for local treatment (radiofrequency ablation (RFA) = microwave destruction). These treatments are outside of current protocol and are therefore not described, but the combination of chemotherapy-treated liver metastases and surgery/RFA is well known from the treatment of colorectal cancer. Patients found suitable for local treatment will be treated according to the standard procedures of the wards, and patientinformation will be dispensed accordingly.

2.8 HAI – XELOX

Based on the above pharmacological data, clinical studies and previous experience with intrahepatic chemotherapy, we will use the following regimen:

By permanent catheter:

1) Oxaliplatin intrahepatic with 85 mg/m^2 over 1/2 hour every 2 weeks.

2) Peroral capecitabine (Xeloda ®) 1300 mg/m^2 daily divided into 2 doses continuously.

3) In HER2-positive tumour in combination with trastuzumab (Herceptin ®) 8 mg/kg day 1 followed by 6 mg/kg every 3 weeks.

4) Evaluation after every 4. treatment according to RECIST 1.1.

5) After discontinuation of intrahepatic chemotherapy and possibly local treatment, capecitabine 2000 mg/m^2 day 1-14 every 3 weeks, and in HER2-positive disease in combination with trastuzumab until progression.

6) Collection of tissue from primary tumor, liver metastasis and other metastases, blood cells, serum and plasma at the start of treatment.

7) Collection of blood cells, serum and plasma at the evaluation times.

8) Collection of tissue from metastasis at a possible later operation.

DMS-TACE/treatment in non-permanent catheter

1) Oxaliplatin 70 mg/m^2 with infusion over 1/2 hour followed by EmboCeptS til flow stop, max 3 ml (3 patients).

Oxaliplatin 85 mg/m^2 with infusion over 1/2 h followed by EmboCeptS til flow stop, max 7½ ml (3 patients).

Oxaliplatin 85 mg/m^2 with infusion over 10 minutes followed by EmboCeptS til flow stop, max 7½ ml (3 patients).

If this treatment is tolerated in the latter patients, this treatment regimen will be continued. Treatment will be evaluated closely.
Oxaliplatin is given in the latter case at a concentration of 5mg/ml.

Or:

Treatment is given intrahepatically over 1/2 hour (not followed by microspheres). This treatment modality will be used in Odense and Herlev until it is demonstrated that it is safe to give oxaliplatin as a bolus. Patients (at Herlev Hospital) who do not wish to receive EmboCeptS in the initial phase of the study will also be offered this treatment

2) Oxaliplatin intravenously with 85 mg/m^2 over 1/2 hour every 4 weeks.

3) Peroral capecitabine (Xeloda ®) 1300 mg/m^2 daily divided into 2 doses continuously.

4) In HER2-positive tumour, in combination with trastuzumab (Herceptin ®) 8 mg/kg day 1 followed by 6 mg/kg every 3 weeks.
5) Evaluation after every 4th treatment according to RECIST 1.1.
6) After discontinuation of intrahepatic chemotherapy and possibly local treatment, capecitabine 2000 mg/m^2 day 1-14 every 3 weeks, and in HER2-positive disease in combination with trastuzumab until progression.
7) Collection of tissue from primary tumor, liver metastasis and other metastases blood cells, serum and plasma at the start of treatment.
8) Collection of blood cells, serum and plasma at the evaluation times.
9) Collection of tissue from metastases at a possible later operation.

3 Pharmacokinetics

In order to investigate the difference between intrahepatic and intravenous treatment, 4 patients will also carry out pharmacokinetics, taking 2 ml of blood on timepoints 0, 15, 30, 60 min, 2, 2½, 3, 4, and 6 hours, for the purpose of determining oxaliplatin concentration (this determination will be done in collaboration with Anders Johnsson, Lund Sweden). Each patient will have the analysis performed a total of 3 times (for IV treatment and for intrahepatic treatment with and without EmboCeptS). A total of 54 ml of blood will be taken, which is of no significance for the health of the patient.
Once the first samples have been assessed, the timing of pharmacokinetics may be changed, no more samples will be taken.

4 Tumor biology

It is essential to find the patients who could benefit from intrahepatic chemotherapy, which is why material (tumor tissue, whole blood, serum and plasma) will be collected for studies for new and future potential prognostic and predictive biomarkers.
A better understanding of the biology of the tumours will eventually lead to better cancer treatment and probably better selection and individualisation of the treatment. It is not yet clear which prognostic or predictive biomarkers in tumor tissue, the surrounding normal tissue and in blood tests in patients with breast cancer can predict the course of the disease or the effect of the chosen treatment.
A biobank of blood and tissues will be established. The biological material will only be used for a new research project after obtaining approval from the Research Ethics

Committee. The material will be stored for 15 years, and thereafter it will be destroyed. A code is kept for the identification of the biological material that is therefore personally identifiable. Together with the protocol itself, the research biobank has been notified to the Danish Data Protection Agency.
See Appendix 2.

5 Endpoints

5.1 Clinical endpoints

5.1.1 Primary endpoint

Response rate.
Fraction of patients who achieve complete or partial response in the liver according to RECIST 1.1.

Secondary endpoints

a) Time to Intrahepatic progression (PFS).

b) Time to Extrahepatic progression (PFS).

c) Number of patients who can have the tumor burden reduced so much that they become suitable for local treatment (radiofrequency ablation).

d) Survival.

e) Toxicity.

5.2 Paraclinical endpoints

Exploring the interaction between a number of biomarkers in cancer tissue and blood and their relation to relevant clinical parameters such as treatment effect (response), toxicity, time to progression and survival.

6 Trial design

Phase II study. A maximum of 12 intrahepatic treatments are given. The patient may then, at the discretion of the investigator, continue with systemic treatment. Treatment is

continued to progression, unacceptable toxicity or until the patient does not want to continue treatment.

6.1 Expected number of patients

Up to 50 evaluable patients with a permanent catheter and up to 50 evaluable patients with microcathether, recrutied over 3 years period (see statistical calculations).
Initiated October 2009 it is planned that the last patient will begin treatment in October 2012.

6.2 Duration of treatment

Intrahepatic: Maximally 12 series of intrahepatic chemotherapy (equivalent to ½ year of treatment).
Discontinuation: Patients discontinue treatment in case of progressive disease (PD) or unacceptable toxicity or until the patient does not wish to continue treatment.
Cardiotoxicity: If during treatment the development of cardiomegaly, heart failure (CHF) or arrhythmia occurs that require treatment, treatment with trastuzumab should be stopped. A decrease in LVEF $\underline{\geq}$ 0.20 (absolute units) and/or decrease in LVEF $\underline{\geq}$ 0.10 to < 0.50 will result in the patient's discontinuation of med trastuzumab.

7 Selection of patients

7.1 Inclusion criteria:

- Informed written and oral consent.
- Age over 18 years.
- Performance status 0-1 and life expectancy ≥ 3 months.
- Histologically or cytologically documented breast adenocarcinoma
- Liver metastases where local treatment is is assessed as not suitable.
- Patients with bone metastases or lymphnode metastases where there is no progression in the extrahepatic sites within 6 months and whose tumor burden is predominantly hepatic.
- The presence of liver metastases and limited extrahepatic disease must be documented by PET-CT scan.
- No progression on treatment with capecitabine.

- Previous treatment with taxane either adjuvant or for metastatic disease. If treatment has been given for metastatic disease, treatment should have continued until maximum response or cessation due to toxicity.
- Metastases < 70 % of the liver
- Neutrophil granulocytes 1.5 x $\geq 10^9$/l and platelets 100 x $10^9 \geq$/l
- Bilirubin $\leq$2.0 x UNL (upper normal limit).
- Creatinine clearance$\geq$ 30 ml/min.
- INR < 2.
- It is possible to perfuse the metastases through A. hepatica (possibly angiography).
- The patient is approved by the Second Opinion Committee.

If the patient is HER2 positive:

- Baseline LVEF ≥ 50 % measured by either echocardiography or MUGA.

7.2 Exclusion criteria:

- Chemotherapy with drugs other than capecitabine within 4 weeks.
- Concomitant or other prior malignant disease other than basal cell carcinoma and carcinoma in situ cervicis uteri.
- Previous treatment with oxaliplatin.
- Cytotoxic treatment or experimental treatment within 14 days before inclusion.
- The patient may not participate in other clinical studies.
- Pre-existing polyneuropathi more than or equal to grade 2 NCI CTC version 3.
- Serious medical conditions that are assessed to prevent treatment.
- Other serious medical illness (eg. severe heart disease or AMI within 1 year).
- Physical or mental illnesses that can prevent the administration of oral treatment.
- Patients with uncontrolled infection.
- Pregnant or lactating women. In fertile women, this is ensured with a negative pregnancy test.
- Female patients of childbearing age who do not use contraceptive (not hormonal) or breastfeed. The Danish Medicines Agency considers spiral to be adequate contraceptives.
- Signs of active CNS metastases. If there is clinical suspicion of brain metastases, a CT scan or MRI of the brain should be performed within 4 weeks prior to inclusion.

- Patients who, for linguistic, intellectual or cultural reasons, will not be able to fully understand the treatment concept and respond to any complications.
- Previous severe and unexpected reactions to treatment with fluoropyrimidine.
- Hypersensitivity to one or more of the known active substances, excipients or fluorouracil.

If the patient is HER2 positive:

- Dyspnea at rest due to complications of advanced malignancy (e.g., pulmonary metastases with lymphangitis) or other conditions requiring supportive oxygen therapy.

8 Treatment regimens and procedure

Patients are referred to EFEK/EEK, Department of Oncology.
Patients who meet the inclusion criteria and are approved at the multi-discipline conference ("HAI-konf"), are referred to Radiological Department for the purpose of establishment of port-a-kath. with catheter in A. hepatica.

8.1 Feasibility studies

8.1.1 Caranatomy.

For the purpose to localise of the arteries in the liver an angiography of the hepatic and abdominal vessels may be performed. The arterial and venous portal anatomy of the liver is also clarified by the reconstruction of thin slices from CT scans.
Variations in anatomy can change the surgical strategy for catheter placement.

8.1.2 Application of arterial Port A Cath. (HAI catheter)

The patient must meet the inclusion criteria and the anatomy of arteria hepatica must be detemined. The patient is referred to the Radiological department for X-ray guided catheter insertion through a peripheral artery branch, most frequently A. femoralis. An arterial catheter system (Port A Cath.) is implanted in A. hepatica. The aim is to place the catheter tip in A. hepatica without sticking into the lumen of A. gatroduodenalis A. hepatica. 5000 units of heparin are given in the catheter before each closure and after each infusion.

8.1.3 Side effects, as well as observation.

Patients should be informed of the general, albeit very small, risk of fatal outcome associated with catheter insertion, e.g. due to hemorrhagic shock. At the pump pocket, a hematoma/seroma or inflammation/infection, erosion or ulcers can be seen, or it can pop up. Incision pain may occur. At the catheter can be seen crack or dislocation such that one cannot draw or inject into the catheter port. Complete or partial catheterocclusion may occur. In addition, the patient should be observed for systemic side effects from chemotherapy specially elevated bilirubin, pancreatitis (amylase), cholecystitis and bleeding from the gastrointestinal tract. The patient is observed for 1 day with hospitalization after the first catheter insertion (permanent catheter), moreover, according to the procedure of the department.

8.2 HAI-XELOX

Treatment can be started from the day after catheter placement. Dosing is made at a maximum equivalent to 2 m^2.

8.2.1 Oxaliplatin

Catheter placement is checked before each treatment according to the procedure of the department. Through port-a-cath to A. hepatica is given oxaliplatin 85 mg/m^2 over 1/2 hour day 1 every 2 weeks. Pre-treatment and supportive treatment for nausea are given according to the department's standard procedure for intrahepatic therapy.

8.2.2 Capecitabine (Xeloda®)

Xeloda® is dispensed as tablets in dosing boxes. The tablets are taken in conjunction with a meal, or no later than 30 minutes after. The dose is distributed over 2 roughly equal doses. The first dose is taken in the evening day 1. Daily dose is 1300 mg/m^2.

8.2.3 EmboCeptS

EmboCeptS are microspheres with a diameter of 50 µm. The substance has a half-life of 50 minutes. Manufactured by PharmaCept Gmbh, Berlin, distributed by Nextpharma. The substance is routinely used to treat hepatocellular carcinoma. EmboCeptS is CE certified and is used on the approved indication.

8.3 Visit schedule and observations

Assessment	Screening (number of days before inclusion)	During treatment	End of treatment	Follow-up until progres-sion
Anamnesis and physical examination (+ PS)	8 days	Before each treatment (every 2 weeks) (physical examination may be omitted at the discretion of the investigator)	X	If indicated
Hematology*	8 days	Before each treatment (every 2 weeks)	X	If indicated
Biochemistry**	8 days	every 2 weeks	X	If indicated
Blood tests to determine biomarkers (Appendix 2)	8 days	For all evaluations up to progression	X	For all evaluations for progression
ECG	8 days			
Tumor assessment¤ For PET/CT followed by CT	28 days	After every 4th HAI-XELOX Then every 3 months **Assessment of extrahepatic disease of the bones after every 6th treatment (MRI or x-ray depending on baseline examination)**	X	Until PD
Ultrasound of the liver with biopsy	28 days			
Side Effects/ Adverse Events# (SAE within 24 hours)		Before each treatment (every 2 weeks) and in case of the SAE	X	Followed until recovery
MUGA, ECG (HER2 positive disease)	28 days	every 3 months		According to department standard

* Hematology: leukocytes, neutrophil granulocytes, thrombocytes, hemoglobin.
** Biochemistry: INR, alkaline phosphatase, bilirubin, LDH, ALT/AST, amylase, serum creatinine, sodium, potassium and s-Ca ion.
Hematology and biochemistry are assessed before treatment series to ensure satisfactory values and to allow for necessary dose modifications if indicated. Side effects are reported in CRF.
Side effects are recorded and graded according to NCI CTC version 3.0. If no relevant NCI CTC is found, the adverse reaction should be recorded as 0 = none, 1 = mild, 2 = moderate, 3 = severe, 4 = life-threatening.
Biomarkers are described in Appendix 2.

8.4 Registration

After recording the anamnesis, patient information is provided. The investigator conducts clinical, radiological and laboratory assessments to confirm that the patient meets all inclusion criteria. For patient information and ICF procedure, see Appendix 1.

If the patient meets the eligibility criteria, data will be faxed to the Clinical Research Unit, Department of Oncology, Herlev Hospital Fax. no. 4453 3076. The phone number for this is 4488 4000 + 89438.

8.4.1 Before each treatment series

The patient is assessed for the following parameters within 72 hours before each treatment series.

- Performance status
- Clinical assessment
- Assessment of side effects
- Blood tests according to the schedule.
- Any reporting of SAE is carried out accordingly.

8.4.2 Blood samples for the determination of biomarkers

- Before starting treatment og in connection with each evaluation. See Appendix 2.

8.5 Assessment of response

Tumor evaluation is done after every 4th series (every 8 weeks)

Thoraco-abdominal CT scan, clinical assessment and biochemical status as at baseline.

8.5.1 Definition of response

Made according to RECIST version 1.1.

8.5.1.1 Biomarkers

Blood tests to determine tumor markers (Appendix 2).

The samples will be stored in a biobank, in anonymized form. See also point 3.

8.6 Other concomitant medication

Patients on oral anticoalgulation therapy should be monitored regularly with INR due to possible interaction with Xeloda®.

Vitamin B6 (Pyridoxine) must not be used to treat hand-food syndrome, as there is a risk of reduced efficacy of oxaliplatin.

Prophylactic antiemetics including corticosteroids are allowed. Prophylactic antibiotics are allowed if it is deemed beneficial for the patient. G-CSF is not recommended as a prophylaxis but can be given if it is found beneficial for the patient.

8.6.1 Other medication

All other symptomatic treatment of the patient to perform optimal care is allowed as long as the name, route of administration and duration of treatment are documented in the patient's medical record.
No other antineoplastic treatment is allowed during treatment.
Patients are allowed to be given bisphosphonate.

8.7 Follow-up

To determine any timepoint of progression, patients will be followed with clinical assessment and CT scan every 3 months after treatment cessation until progression. At the same time, relevant routine blood samples are taken on indication and blood for project biomarkers are taken at each evaluation up to progression (Appendix 2).
After the finding of progression, treatment/control continues according to local guidelines, but subsequent treatment and any date of death must be documented.

9 Storage, mixing and dispensing of investigational products

In all cases, these are medications that are used as standard in the department. Storage and mixing as well as handling in the department are carried out on the principles of current standard guidelines. All dispensed investigational product will be labeled and handled according to ICH-GCP. Patients return unused medication and keep a diary.

10 Dose reductions

Adverse reactions are graded according to NCIC-CTC criteria (version 3.0), except for neurological adverse events, which are assessed according to Table 2.
As a rule, side effects of capecitabine and oxaliplatin are handled separately.
In the case of DMS-TACE, any dose reductions should always be made based on the dose given.
In the event of a reduction in the dose, the dose should not be increased again later.

Treatment postponement should be based on the day 15 observation (= the day of the scheduled day 1 of the following treatment) and the prescription should be based on the worst toxicity observed during the previous treatment. If hematological and non-haematological toxicity of the same degree coincides, dose reduction shall be based on the non-haematological rules for dose reduction.

10.1 Treatment delay

The next treatment with capecitabine and oxaliplatin is postponed until
1: Neutrophil leukocytes (ANC) > 1.5 x 109/l and or platelets > 100 x109/l
2: Any non-haematological toxicity such as mucositis, diarrhoea and PPE has decreased to ≤ Grade 2 or baseline toxicity level.

10.2 Dose reduction due to hematological toxicity

Three consecutive dose reductions are allowed. If, at the 3rd dose reduction, grade 3/4 haematological toxicity is still observed, the patient should stop treatment.
Dose reductions for subsequent treatments are based on the worst side effects.
The dose of capecitabine and oxaliplatin shall be reduced by 25% (see table) in all of the following series in the case of:

- febril neutropenia (temperature ≥ 38,5 °C and ANC < 1.0 x 10^9/l).
- neutropenia grade 4 (ANC < 0.5 x 10^9/l).
- thrombocytopenia grade 4 (platelets < 25 x 10^9/L).
- thrombocytopenia grade 3 and concomitant hemorrhage grade 2.
- if treatment is postponed more than 2 weeks due to haematological toxicity.
- grad 3 or 4 mucositis, diarrhea or nausea and vomiting despite appropriate medical treatment.

An additional 25% dose reduction can be made if the side effects persist.

For hematological toxicity, three consecutive dose reductions are allowed (only in isolated hematological toxicity):

Dose reduction:	Dose reduction: oxaliplatin	Dose reduction: capecitabine
Step 1	25 %	No dose reduction

Step 2	25 %	25 %
Step 3	50 %	25 %

In the case of chemotherapy related anemia grade 1-2 either transfusion of red blood cells or erythropoetin may be used to correct this side effect but dose reduction is not allowed.

10.3 Liver function

At the beginning of a series, serum bilirubin should be ≤ 2.0 x UNL. If an elevated bilirubin persists 2 weeks after postponed treatment, treatment is stopped. In the case of a bilirubin increase of ≥ 2.0 x UNL that is not due to disease progression or obstructive icterus, treatment with corticosteroids is allowed (e.g. 100 mg daily for 5 days). If bilirubin returns to ≤ 2.0 x UNL within 2 weeks of postponed treatment, treatment may be continued at a lower level according to the rules of dose reduction in case of non-haematological toxicity.

10.4 Dose reduction in oxaliplatin related sensory polyneuropathy (sPNP)

Any neurological side effects should be assessed according to Table 1 before each oxaliplatin treatment.

Table 1 Grading neurological side effects

Grade	**Symptoms**
Grade 0	None
Grade 1	Short-duration paresthesias/dysesthesias with complete recovery before the next treatment
Grade 2	Paresthesias/dysesthesias, which persist between 2 treatment series, but without affecting function
Grade 3	Functional impairment (eg. reduced force)

Table 2
Dose of oxaliplatin and neurological side effects

Side effects	**Duration of side effects**		**Persistent side effects at the next treatment 14 days**
	1-7 days	**>7 days**	
1) Cold-triggered dysesthesias	100 %	100 %	100 %
2) Paresthesias	100 %	100 %	1st time 75%

			2nd time 50% 3rd time stop
3) Paresthesias + pain	100 %	1st time 75% 2nd time 50% 3rd time stop	Stop
4) Paresthesias with functional impairment	100 %	1st time 50% 2nd time stop	Stop

No reduction

For dysesthesias regardless of whether they are painful or not or are cold-related or not after an oxaliplatin infusion, the dose should not be modified. In case of persistent non-painful paresthesias (other than due to cold exposure) or in painful paresthesias only in case of cold exposure without functional influence, the dose should not be reduced.

10.5 Hypersensitivity, allergies or laryngopharyngeal dysesthesia (LPD)

Allergic or hypersensitive reactions (e.g. rash, hypotension, flushing, dyspnea) are seen, albeit rarely, in patients treated with platinum derivatives. If hypersensitivity cannot be ruled out, pre-treatment with steroid is recommended at the next series of chemotherapy.
In case of suspicion of allergies or similar reactions, pre-treatment before the next series of chemotherapy is advised according to the department's standard for re-treatment.
If the oxaliplatin dose is reduced due to side effects, the dose should not be increased again.
If intrahepatic treatment is discontinued due to side effects or in cases where a catheter cannot be used, the patient is withdrawn from the protocold treatment and the patient can continue treatment in accordance with the department's usual guidelines.

10.6 Dose reduction for capecitabine

- In the case of febril neutropenia (temperature ≥ 38.5 degrees and ANC < 1.0 x 109/L), interuption of capecitabine treatment until the next scheduled series.

Table 4

Dose of capecitabine and non-hematological side effects (diarrhea and PPE)

	Grade 2	Grade 3	Grade 4
1st time	Pause until grade 0-1 Continue with 100%	Pause until grade 0-1 Continue at 75%	Pause until grade 0-1 Continue at 75%
2nd time	Pause until grade 0-1 Continue at 75%	Pause until grade 0-1 Continue at 50%	Stop*
3rd time	Pause until grade 0-1 Continue at 50%	Stop*	
4th time	Stop*		

- Treatment is discontinued unless the investigator considers it in the patient's interest to continue with reduced-dose treatment.
- If the investigator finds it in the patient's interest to switch from capecitabine to infusion therapy with 5-FU/LV as continuous pump therapy in the de Gramont regimen, this can be done. The investigator may deem it in the patient's interest to continue intrahepatic treatment with oxaliplatin, but the patient will be withdrawn from the study.

10.7 Non-hematological toxicity other than sensory polyneuropathy

Side effects will be graded using CTC. If side effects occur that are not described in the CTC, they should be graded on the following scale:

1 = mild, 2 = moderate, 3 = severe and 4 = life-threatening.

In cases of non-hematological toxicity grade 3 or 4, the following consecutive dose reductions may be used:

Dose reduction:	Dose reduction: oxaliplatin	Dose reduction capecitabine
Level 1	25 %	No dose reduction
Level 2	25 %	25 %
Level 3	50 %	25 %

A maximum of two consecutive dose reductions are allowed.

10.7.1 Adjustments and postponements of trastuzumab dose due to toxicity

There are no dose modifications for trastuzumab and it is not necessary to interrupt trastuzumab due to haematological toxicity. Treatment with trastuzumab should be hold for

related non-hematological toxicity (other than cardiac dysfunktion) of Grade 3 or 4 until recovery to ≤ Grade 2. If the same non-haematological toxicity again occurs with a grade 3 or 4, treatment with trastuzumab should be permanently discontinued.
Postponements due to trastuzumab-related toxicity is unusual. If a dose is postponed by less than one week, the usual dose of 6 mg/kg should be given as soon as possible and subsequent dosing continued according to the original dosing schedule.
If a dose is postponed by more than one week, treatment should be restarted as soon as possible with a loading dose of 8 mg/kg, followed by a dosage of 6 mg/kg.

10.7.2 Cardiac dysfunction

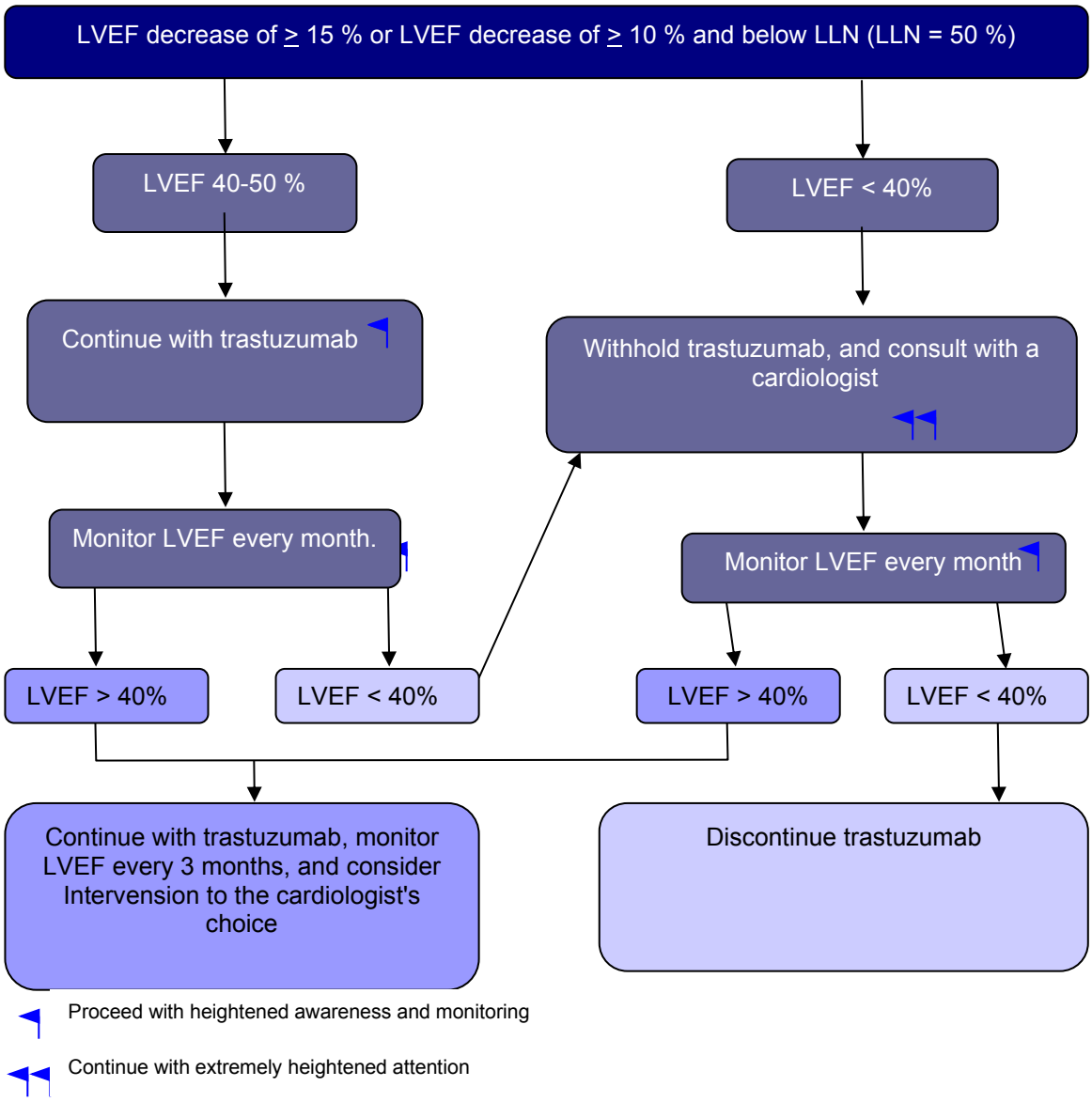
Treatment with trastuzumab should be discontinued if the patient develops clinical heart failure, i.e. is in NYHA grade II, III or IV.

All patients should have a baseline LVEF of ≥50%. LVEF is regularly monitored according to the department's standard. Trastuzumab is discontinued in any patient who develops clinical signs and symptoms suggestive of CHF (to be confirmed by LVEF).

CHF is treated and monitored according to medical standards.

There are currently insufficient data to assess the prognostic significance of asymptomatic declines in LVEF. However, in order to ensure the safety of patients in the trial, trastuzumab should be discontinued for all patients where a decrease in LVEF to below 40% is documented and confirmed with a new assessment within 4 weeks of initial assessment using the same assessment method.
For patients whose LVEF falls to values between 40% and 50%, the decision to discontinue or discontinue trastuzumab is based on the algorithm in the figure below.
Patients who develop symptom-giving cardiotoxicity will be referred to the cardiology department.

Figure. Algorithm for handling treatment with trastuzumab, based on LVEF assessments



10.7.3 Dose interruption due to reactions associated with infusion

Patients who experience a life-threatening infusion reaction at the first dose (e.g. tacypnea, bronchospasm, hypotension, hypoxia) should discontinue treatment.

Patients experiencing severe or moderate infusion symptoms can be treated by lowering the infusion rate or stopping the trastuzumab infusion, or by treatment with, for example, oxygen, beta agonists, antihistamines or corticosteroids.

Patients who experience mild, moderate or severe infusion reactions at the first dose may be re-treated with trastuzumab. Premedication with corticosteroids, antihistamines and antipyretics may be used before subsequent trastuzumab infusions.

If the starting dose with trastuzumab is discontinued due to an infusion reaction, the following guidelines apply:

- If the patient has received ≥ 75% of the starting dose, an additional load is unlikely to be necessary. However, the investigator may choose to administer the remainder of the load dose, either at any time before the next scheduled dose or together with the next scheduled dose.
- If the patient has received ≥ 50% and < 75% of the dose, the patient should receive the remainder of the dose before the next scheduled dose and preferably within the first 2 weeks after the interrupted load dose.
- If the patient has received < 50% of the starting dose, the patient should preferably receive the remainder of the starting dose within 1 week of the interrupted starting dose.

10.8 Treatment of complications

All complications will be treated according to the standard procedures of the wards. Patients are informed to contact the wards in case of side effects.

10.9 Duration of treatment

A maximum of 12 series of chemotherapy are planned unless there is progression or unacceptable side effects.
Patients are evaluated after every 4th series by CT scans; MRI/x-ray of extrahepatic foci is performed after 6 and 9 treatments. In case of response, the evaluation scans will be assessed in consultation with the Gastrounit, Department of Surgery to evaluate for possible local treatment.

10.9.1 Causes of discontinuation of treatment.

Disease progression at any stage during treatment.

Complications of treatment either from the catheter, or unexpectedly severe adverse reaction to the chemotherapy, or by signs of development of hepatic insufficiency.

The patient's own wish: the patient can at any time, at his own request, discontinue treatment.

10.10 Discontinuation of the study before scheduled time

The study will be stopped for the following reasons:

- If arrising adverse events are of such a serious nature that the continuation of the investigation becomes unacceptable
- If the recruitment rate is too low to expect completion of the trial in its current form within an acceptable period of time
- If, for administrative reasons, the number of drop-outs is too high and this situation cannot be improved

11 Patient safety

11.1 Adverse events

11.1.1 Definition

An adverse event is any symptom, sign or disease that occurs or worsens while the patient is participating in the study. The investigator shall assess whether there is a causal relationship between adverse events and investigational medicines.

Serious adverse events (SAE) is any adverse event that regardless of dose meeting one of the following:

- Results in death.
- Any life-threatening event - the patient, at the discretion of the investigator, was at immediate risk of dying from the adverse event when it occurred.
- Hospitalization or prolongation of existing hospitalization.
- Results in permanent or significant disability/incapacity for work.
- Congenital anomaly/malformation.
- Any significant medical event.

11.1.2 NOT SAE

The following will not be considered serious adverse event for the purposes of this study: An event that results in hospitalization or prolongs an existing hospitalization if the sole reason for admission or extension is for the following:

1. Death as caused by the progression of the patient's cancer.
2. Hospitalization is secondary to expected morbidity due to chemotherapy:
- chills and temperature elevation
- nausea and vomiting
- bone marrow suppression
– fever
– anemia
- obstipation
- diarrhea
– abdominal pain related to treatment

3. Hospitalization is secondary to expected morbidity due to cancer:
– weight loss
– fatigue
– electrolyte disorders
– pain management
– uneasiness/anxiety
– admission due to palliative care
- administering chemotherapy
- Catheter problems
– transfusion with blood products
– in conjunction with any of the study procedure
– placement of a permanent intravenous catheter
- hospice stays for terminal care;

All admissions are recorded on an excel sheet.
Any patient death must be recorded on the CRF.
Usual side effects to chemotherapy, progressive disease and events secondary to progressive disease should not be reported.

11.1.3 Suspected, unexpected, serious adverse reaction – SUSAR

Serious and unexpected side effects, i.e. a reaction that has not been described previously (in the Investigator's Brochure or in the Summary of Product characteristics) and where a probable or possible causal relationship with the medicinal product is assessed. There is a special obligation on the investigator to report these, cf. section 11.1.5.

11.1.4 Adverse Event Registration

All adverse events (AEs) occurring during the study period shall be recorded and documented in the relevant section of the CRF.
Patients are followed with adverse event registration until discontinuation of intrahepatic therapy (maximum 12 series). The side effects will be followed until 1 month after the last treatment, or until the side effects are ≤ grade 1, or as at baseline or assessed as stationary.
The events are described, graded and assessed for causality.
The grading is assessed by the investigator according to the definitions in NCI-CTC, version 3.0: grade 1 – 5. If the AE is not listed, graded according to the following description:
Grade 1 = mild
Grade 2 = moderate
Grade 3 = severe
Grade 4 = life-threatening or disabling
Grade 5 = death related to AE

Causality in relation to the investigational treatment is assessed by the investigator. The decisive factor in the documentation is the temporally dependent possible relationship between AE and the investigational product (IP). The following assessments of a causal relationship to the IP or trial procedure should be used:
Unrelated: There is no temporal relationship with the administration of the IP (pre-administration, too late, or the patient has not received the IP), or there is a reasonable causal relationship between another drug, underlying disease, other conditions, and AE.
Not likely: There is a temporal relationship to the administration of the IP, but there is not a reasonable causal relationship between the IP and the AE.
Possible: There is a reasonable causal relationship between the IP and AE.

Probable: There is a reasonable causal relationship between IP and AE. Upon stopping IP, the incident ceases. There is no need for re-introduction of IP.
Sure/definitive: There is a reasonable causal link between IP and AE. The event responds to the cessation of IP and is recurred upon re-introduction of IP (when clinically possible).

11.1.5 Reporting of serious adverse events (SAE/SAR/SUSAR)

All SAEs must be reported to the sponsor on the SAE form within 24 hours of the investigator's knowledge of the event (however, see section 11.1.2 for exceptions).
It is then the responsibility of the sponsor to assess whether the SAE is unexpectedly and presumably related and thereby = SUSAR and then further report to the Danish Medicines Agency.
In cases where SUSAR is life-threatening, the Danish Medicines Agency is informed within 7 days, otherwise within 15 days.
It is also the responsibility of the sponsor to prepare annual lists of all SAEs and SARs in the trial for the Research Ethics Committee and the Danish Medicines Agency, in accordance with the applicable rules and at the end of the investigation.

11.2 Early discontinuation of the study

The patient is withdrawn from the examination in case of death, progression, unacceptable side effects, other severe medical disorder or if the patient wishes. The date and reason are given in the journal and CRF.

12 Data analysis and statistics

12.1 Definition of populations

Intention-to-treat population: All patients who have received at least 1 series of intrahepatic chemotherapy. Patients with missing and/or illegitimate data will be included in the intent-to-treat population.

Evaluable-for-response population: All patients who have received at least 4 series of chemotherapy unless progression has been established prior to that.

12.2 Efficacy

12.2.1 Primary analysis (response rate)

The response rate with confidence interval will be calculated on all evaluable patients. The response rate will be based on RECIST 1.1. Criteria.

12.2.2 Secondary analysis (PFS)

Time to progression or death will be calculated based on the date of start of chemotherapy to the date of documented progression or death. Median time to progression or death with confidence interval will be calculated.

12.3 Sample size

Patients will be stratified according to treatment regimen i.e. with permanent catheter/DMS-TACE. The two groups will analysed seperately.
Rationale for sample size for endpoint response rate for which treatment is considered to have sufficient clinical efficacy (The analysis was performed according to "Flemming's Multiple Testing Procedure" (Ref. "One-Sample Multiple Testing Procedure for phase II clinical trials" T.R. Flemming, Biometrics, March 1982):
H_0: p=5%. Ineffective rate, response rate for which treatment is considered to have insufficient clinical efficacy.
H_1: p=20%. Minimum aim of effective rate, response rate above which treatment is considered to have sufficient clinical efficacy.
With alpha = 5 % (one-sided test) and power = 92 %.
If 5 or more patients out of 40 have clinical efficacy, the treatment regimen should be further investigated.
As 40 evaluable patients are targeted, i.e. patients who have received at least 4 series of chemotherapy, 50 patients will be included, as it is to be expected that 20% (10 patients) will not meet the criteria for evaluability.
Changes to the original statitistical plan will be notified to the Research Ethics Committee and the Danish Medicines Agency.

12.4 Statistical methods

PFS and survival will be estimated with the Kaplan-Meier method and compared with a log-rank test. Categorical variables are indicated by median followed by the range. A significance level of 5% will be used. The response rate is assessed in the evaluable population.

12.5 Side effects

Side effects are recorded among patients who have received at least 1 series of intrahepatic chemotherapy.
Patients are given pain diary for the registration of pain with intensity and time, as well as treatment of these.

12.6 Data management and archiving

Conducted by the Clinical Research Unit, Department of Oncology at Herlev University Hospital.
Monitoring is carried out by the University of Copenhagen's GCP unit.
The trial has been notified to the Danish Data Protection Agency. and the subjects are protected under the Act on the Processing of Personal Data and the Health Act (Section 3 on the Legal Status of Patients).

13 Ethics

This study is carried out in accordance with the globally accepted standardes of GCP (Good Clinical Practice) and in accordance with the latest revision of the Helsinki Declaration (version 8) and according to the national regulations. All patients will be informed both orally and in writing about the purpose of the study and after a reflection period, patients must give both oral and written consent before inclusion. The patient's consent form will be kept in the medical record. The study is submitted to the Research Ethics Committee and the Danish Medicines Agency and it must be approved before the start of the study. At the end of the study, the study report is prepared. The trial will be registered in Clinicaltrial.gov.

13.1 Ethical considerations

In this study, incurable patients with breast cancer are treated. In breast cancer, the consensus is that as soon as patients have developed liver metastases, treatment can

only be palliative. Hepatic intraarterial chemotherapy has so far only been very sparsely studied for the treatment of liver metastases in breast cancer. Thus, in most cases, only "case stories" (often in the media) have been reported. Only one phase I/II trial has been reported, using a combination of adriamycin and 5-fluorouracil. The overall response rate was 63% with a remarkably long median survival of 25 months. However, since patients with breast cancer bone and lymphatic metastases often have an indolent course, while patients with living metastases have an exceedingly poor prognosis, a number of these patients will potentially benefit from intrahepatic treatment. Today, the treatment is offered in Germany and many patients have a great desire to be able to receive the treatment in Denmark. The protocol has been approved by the National Committee for Experimental Cancer Treatment.

The primary purpose of this study is to investigate whether combining the injection of - oxaliplatin directly into the hepatic artery that supplies the liver metastases with blood and concomitant tablet treatment with capecitabine can cause the metastases in the liver to dwindle. At the same time as the patient still has routine blood tests, extra blood samples will be taken to determine various project biomarkers, which hopefully in the future can be used to individualize the treatment or designate patients who would benefit from such treatment (the patient can waive this part of the study, see patient information). The possible side effects are, in our own experience and what has been previously reported from similar studies, moderate and tolerable and will probably be outweighed by the possible beneficial effect of the treatment.

There is no experience with oxaliplatin as bolus intrahepatic. Treatment will therefore be evaluated after 3 patients who have each received at least 1 treatment. If Grade 4 (NCI CTC version 3.0) adverse events related to intrahepatic therapy are observed, this part of the trial will be discontinued immediately. In the event of death, the trial is immediately discontinued.

13.2 Patient safety, ethics

The responsible investigator will ensure that the study is conducted in accordance with the Helsinki Declaration and the laws and statutes of the country. The protocol will be approved by the local Research Ethics Committee, the Danish Medicines Agency and the Danish Data Protection Agency. The leaflet "*The subject's rights in a biomedical research project*" is handed out to each participating patient. Guidelines for providing oral

information and obtaining consent, see Appendiks 1. As this appendix also states how the first contact with the subject will take place. The patient's consent statement will be kept in the medical record. If a patient does not want to participate in the study, she will be treated according to the usual standards at the departments (systemic chemotherapy).

14 Subject identification

The patient's name should not be disclosed, nor will it be noted at the data center. Each patient will automatically be assigned an identification number when the patient is included in the study. This number will identify the patient, and the number should be noted on all Case Report Forms. To avoid identification problems, the patient's initials (maximum 4 letters), date of birth, as well as hospital number will also be noted on each Case Report Form.

15 Informed consent

All patients will be informed orally and in writing about the purpose of this study, possible side effects, the methods and possible risks to which she will be exposed. Patients will be informed of the strict protection measures on their data, but they will also be informed that their medical information may be viewed by other auhtorised persons in addition to the attending physician.

It will be emphasized that participation in the study is voluntary, as well as that the patient can withdraw from further treatment in the protocol at any time. This will not affect the further treatment of the patient. If the patient does not want treatment according to the protocol, she will receive treatment according to the department's usual guidelines. The investigation is therefore found to be ethically sound.

A signed informed consent form must be received from all patients included in the study, and consent must be received prior to registration at the data center.

16 Insurance

Patients participating in the study are covered by the hospital's liability insurance.

17 Timescale

Inclusion of the first patient is scheduled for 1.10.2009. The recruitment period is 3 years. Last patient will be included October 2012.

18 Sponsorship and finances

The study was initiated by the investigators. The Danish Cancer Society has given DKK 1.2 million for expenses for project nurse, database and data processing as well as for bioanalyst or nurse associated with the blood sampling/piping, data recording and data calculation. Additional financial support is being sought from the Danish Cancer Society, the Danish Health Science Research Council and private Danish foundations for salaries for bioanalysts, for the analysis of the biomarkers and for the analysis of the immunohistochemical examination, expenses for ELISA kits and immunohistochemical kits. Roche has supported the project with DKK 20,000 for the analysis of VEGF. There is no financial gain for the departments or staff in connection with this trial.

19 Publication

Upon completion of the study, publications on the study will be prepared as well as study reports of the various potential biomarkers (singly or in combination), which will be published in international jounals. Both positive and negative results will be published. Coordinating principal investigator prepares manuscript (in case of Congress: abstract) and is 1st author on the primary publication. Co-authorship as well as author order are determined according to Vancouver rules.

Articles emanating from the project with predominantly basic-medical, radiological or nuclear medicine results can be extracted from the person/department responsible for the majority of the work and the project group then discusses co-authorship at all clinical departments.

20 References

Background

Black RJ, Bray F, Ferlay J, Parkin DM. Cancer incidence and mortality in the European Union: Cancer registry data and estimates of National incidence. Eur J Cancer 1997; 33: 1075-1107.

Intrahepatic treatment

Arai Y, Sone Y, Inaba T, Ariyoshi Y, Kido C. Hepatic arterial infusion chemotherapy for liver metastases from breast cancer. Cancer Chemother Pharmacol 1994; 33: 142-4.

Ducreux M, Ychou M, Laplanche A, et al. Hepatic arterial oxaliplatin infusion plus intravenous chemotherapy in colorectal cancer with inoperable hepatic metastastases: a trial of the Gastrointestinal Group of the Fédération Nationale des Centres de Lutte Contre le Cancer. J Clin Oncol 2005; 23: 4881-7.

Elias D, Maisonnette F, Druet-Cabanac M, et al. An attempt to clarify indications for hepatectomy for liver metastases from breast cancer. Am J Surg 2003; 185: 158-64.

Ikeda T, Adachi I, Takachima S, et al. A phase I/II study of continuous intra-arterial chemotherapy using an implantable reservoir for the treatment of liver metastases from breast cancer: a Japan Clinical oncology Group (JCOG) study 9113. Jpn J Clin Oncol 1999; 29: 23-7.

Kemeny N, Huang Y, Cohen AM, et al. Hepatic arterial infusion of chemotherapy after resection of hepatic metastases from colorectal cancer. N Engl J with 1999; 341:2039-48.

Kemeny N, Eid A, Stockman J, et al. Hepatic arterial infusion of floxiridine and dexamethasone plus high-dose mitomycin C for patients with unresectable hepatic metastases from colorectal carcinoma. J Surg Oncol 2005; 91: 97-101.

Kemecy NE, Niedzwiecki D, Hollis DR, et al. Hepatic arterial infusion versus systemic therapy for hepatic metastases from colorectal cancer: a randomized trial of efficacy, quality of life and molecular markers (CALGB 9481). J Clin Oncol 2006; 24: 1395-1403.

Kemeny N, Jarnagin W, Paty P, et al. Phase I trial of systemic oxaliplatin combination chemotherapy with hepatic arterial infusion in patients with unresectable liver metastases from colorectal cancer. J Clin Oncol 2005; 23: 4888-96.

Lencioni L, Crocetti D, Cioni D, Della Pina C, Bartolozzi C. Percutaneous radiofrequency ablation of hepatic colorectal metastases. Technique, indications, results, and new promises. Invest Radiol 2004; 39: 689-97.

Lencioni L, Crocetti D, Cioni D, et al. Early-stage hepatocellular carcinoma in cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. Radiology 2005; 234: 961-7.

Okaro AC, Durkin DJ, Layer GT, et al. Hepatic resection for breast cancer metastases. An R Coll Surg Engl 2005; 87: 167-70.

Ricke J, Hildebrandt B, Mierch A, et al. Hepatic arterial port systems for treatment of liver metastases: factors affecting patency and adverse events. J Vasc Interv Radiol 2004; 15: 825-33.

Vogl TJ, Schwartz W, Eichler K, et al. Hepatic intraarterial chemotherapy with gemcitabine in patients with unresectable cholangiocarcinomas and liver metastases of pancreatic cancer. a clinical study on maximum tolerable dose and treatment efficacy. J Cancer Res Clin Oncol 2006; 132: 745 – 55.

Vogl TJ, Zangos S, Eichler K, Yakoub D, Nabil M. Colorectal liver metastases: regional chemotherapy via transarterial chemoembolization (TACE) and hepatic chemoperfusion: an update. Eur Radiol 2007; 17: 1025-34.

Vogl TJ, Zangos S, Eichler K, Selby JB, Bauer RW. Palliative hepatic intraarterial chemotherapy (HIC) using a novel combination of gemcitabine and mitomycin C: results in hepatic metastases. Eur Radiol 2008; 18: 468-76.

Vogl TJ, Bauer R, Eichler K, Gruber T. Repeated transarterial chemoembolization (TACE) in the treatment of patients with liver metastases of breast cancer: local tumor control and survival. J Clin Oncol 2008; 26 (May 20 suppl): abstract 549

Capecitabine

Barton G, Ponting CP, Spraggon G, et al. Human platelet derived growth factor is homologous to E. Coli thymidine phosphorylase. Protein Sci 1992; 1: 688-90.

Budman DR, Meropol NJ, Reigner B, et al. Preliminary studies of a novel oral fluoropyrimidine carbamate: capecitabine. J Clin Oncol 1998; 16: 1795–802.

Folkman J. What is the role of thymidine phosphorylase in tumor angiogenesis? J Natl Cancer Inst1996; 88: 1091-2.

GriffithsL, Dachs GU, Bicknell R, et al. The influence of oxygen tension and pH on the expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast tumor cells grown in vitro and in vivo. Cancer Res 1997; 57: 570-2.

Miwa M, Ura M, Nishida N, et al. Design of a novel oral fluoropyrimidine carbamate, capecitabine, which generates 5-fluorouracil selectively in tumors by enzymes concentrated in human liver and cancer tissue. Eur J Cancer 1998; 34: 1274-1281.

Moghaddam A, Zhang HT, Fan TP, et al. Thymidine phosphorylase is angiogenic and promotes tumor growth. Proc Natl Acad Sci 1995; 92: 998-1002.

Sumizawa T, Furukawa T, Haraguchi M, et al. Thymidine phosphorylase activity associated with platelet derived endothelial cell growth factor. J Biochem 1993; 114: 9-14.

Toi M, Hoshina S, Taniguchi T, et al. Expression of platelet-derived endothelial cell growth factor thymidine phosphorylase in human breast cancer. Int J Cancer 1995; 64: 79-82.

Twelves C, Glynne-Jones R, Cassidy J, et al. Effect of hepatic dysfunction due to liver metastases on the pharmacokinetics of capecitabine and its metabolites. Clin Cancer Res 1999; 57: 1696-702.

Oxaliplatin

Dean-Colomb W, Esteva FJ. Emerging agents in the treatment of anthrcycline- and taxane-refractory metastatic breast cancer. Semin Oncol 2008; 35 (2 suppl 2); S31-8.

Fasano J, Muggia F. Breast cancer arising in a BRCA-mutated background: therapeutic implications from an animal model and drug development. Ann oncol 2009 Jan 15 (epub ahead of print).

Nagourney RA, Flam M, Link J et al. Carboplatin plus gemcitabine repeating doublet therapy in recurrent breast cancer. Clin Breast Cancer 2008; 8: 432-5.

Nielsen D, Dombernowsky P, , Hansen OP, Skovsgaard T. Epirubicin or epirubicin and cisplatin as first-line therapy in advanced breast cancer. A phase III study. Cancer Chemother Pharmacol 2000; 46: 459-66. Larsen SK

Raymond E, Chaney SG, Taamma A, Cvitkovic E. Oxaliplatin: a review of preclinical and clinical studies. Ann Oncol 1998; 9: 1053-71.

Scønnemann KR, Jensen KA, Yilmas M, et al. Phase II study of short-time oxaliplatin, capecitabine and epirubicin (EXE) as first-line therapy in patients with non-resectable gastric cancer. Br J Cancer 2008 Aug 26 Epub ahead of print.

Qvortrup C, Yilmas M, Ogreid D, et al. Chronomodulated capecitabine in combination with short-time oxaliplatin: a Nordic phase II study of second-line therapy in patients with metastatic colorectal cancer after failure to irinotecan and 5-flourouracil. Ann Oncol 2008; 19: 1154-9.

Simpson DDunn C, Curran M, KL. Oxaliplatin: a review of its use in combination therapy for advanced metastatic colorectal cancer. Drugs 2003; 63: 2127-56.Goa

Thomas RR, Quinn MG, Schuler B, Grem JL. Hypersensitivity and idiosyncratic reactions to oxaliplatin. Cancer 2003; 97:2301-07.

Oxaliplatin Intrahepatic

Kern W, Beckert B, Lang N, et al. Phase I and pharmacokinetic study of hepatic arterial infusion with oxaliplatin in combination with folinic acid and 5-fluorouracil in patients with hepatic metases from colorectal cancer. Ann Oncol 2001; 12:599-603.

Trastuzumab

Cobleigh MA. Multinational Study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999: 17; 2639-48.

Jensen BV, Johansen JS, Price PA. High Levels of Serum HER-2/neu and YKL-40 Independently Reflect Aggressiveness of Metastatic Breast Cancer. Clin.Cancer Res 2003; 9: 4423-34.

Konecny G, Pegram MD, Beryt M et al: Therapeutic advantage of chemotherapy drugs in combination with Herceptin against human breast cancer cells with HER-2/neu overexpression. Breast Cancer Res Treat 1999; 57: abstr 467.

Slamon DJ. Human Breast Cancer: Correlation of Relapse and Survival with Amplification of the HER-2/neu Oncogene. Science 1987; 9: 177-82.

Soriano A, Helfrich B, Holden S et al: Synergistic effects of chemotherapy (CT) and radiation (RT) and Herceptin against human non-small cell lung cancer (NSCLC) expressing the HER-2/neu receptor. 12th International Conference on Monoclonal Antibodies for Cancer, , (abstr 40).San Diego CAOctober 14-16, 1999

Pegram M, Hsu S, Lewis G et al: Inhibitory effects of combinations of HER-2/neu antibody and chemotherapeutic agents used for treatment of human breast cancers. Oncogene 1999; 18:2241-51.

Pegram MD. Trastuzumab and chemotherapeutics: drug interactions and synergies. Semin Oncol 2000; 27(6 Suppl 11): 21-5.

Nielsen DL, Andersson M, Kamby C. HER2-targeted therapy in breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors. Cancer Treat Rev 2008; 112: 275-85.

Trastuzumab beyond progression

Bartsch R, Wenzel C, Hussian D et al. Analysis of trastuzumab and chemotherapy in advanced breast cancer after the failure of at least one earlier combination: an observational study. BMC Cancer 2006, 6:63.

Fountzilas G, Razis E, Tsavdaridis D et al. Continuation of trastuzumab beyond disease progression is feasible and safe in patients with metastatic breast cancer: a retrospective analysis of 80 cases by Hellenic cooperative oncology group. Clin Breast Cancer 2003; 4:120-5.

Gelmon KA, Mackey J, Verma S et al. Use of trastuzumab beyond disease progression: observations from a retrospective review of case histories. Clin Breast Cancer 2004 Apr; 5(1): 52-8; discussion 59-62.

Stemmler HJ, Kahlert S, Siekiera W et al. Prolonged survival of patients receiving trastuzumab beyond disease progression for HER2 overexpressing metastatic breast cancer (MBC). Oncology 2005; 28: 582-6.

Von Minckwitz G, Vogel P, Schmidt M et al.,Trastuzumab treatment beyond progression in patients with HER2 positive metastatic breast cancer - interim report. San Antonio Breast Cancer Symposium, 2007, Poster Presentation.

Combination therapy with trastuzumab and capecitabine

Bartsch R, Wenzel C, Altorjai G, et al. Capecitabine and trastuzumab in heavily pretreated patients with metastatic breast cancer. J Clin Oncol 2007; 25: 3853–8.
Cameron D, Casey M, Press M, et al. A phase III randomized comparison of lapatinib plus capecitabine versus capecitabine alone in women with advanced breast cancer that has progressed on trastuzumab: updated efficacy and biomarker analysis. Breast Cancer Res Treat 2008 (Epub ahead of print)

Ishida T, Kiba T, Takeda M, et al. Phase II study of capecitabine and trastuzumab combination chemotherapy in patients with HER2 overexpressing metastatic breast cancers after failure of both anthracyclines and taxanes. J Clin Oncol, 2008 ASCO Ann Meet Proc Part in 2008; 26 (18S): [abstract 1117].

Schaller G, Bangemann N, Gonsch T, et al. Capecitabine and trastuzumab: a phase II study in HER2-overexpressing metastatic breast cancer (MBC) pretreated with anthracyclines and/or taxanes. Breast Cancer Res Treat 2005; (Suppl 1): [abstract 2033].

Schaller G, Fuchs I, Gonsch T et al. Phase II study of capecitabine plus trastuzumab in human epidermal growth factor receptor 2-overexpressing metastatic breast cancer pretreated with anthracyclines or taxanes. J Clin Oncol 2007; 25: 3246-50.

Yamamoto D, Iwase S, Kitamura K, Odagiri H, Yamamoto C, Nagumo Y. A phase II study of trastuzumab and capecitabine for patients with HER2-overexpressing metastatic breast cancer: Japan Breast Cancer Research Network (JBCRN) 00 trial. Cancer Chemother Pharmacol 2008; 61: 509–14.

TACE
DasGupta S, Murumkar PR, Giridhar R, Yadar MR. Current perspective of TACE inhibitors: a review. Bioorg with Chem 2009; 17:444-59.

Li CP, Chao Y, Chen LT, et al. Fever after transcatheter arterial chemoembolization for hepatocellular carcinoma: incidence and risk factor analysis. Scand J Gastroenterol 2008; 43: 992-9.

Pohlen U, Mansmann U, Berger G, et al. Multicenter pilot study of 5-fluorouracil, folinic acid, interferon alpha-2b and degradable starch microspheres via hepatic arterial infusion in patients with nonresectable colorectal liver metastases. Anticancer Res 2004 ;24: 3275-82.

Pohlen U, Rieger H, Mansmann U, Berger G, Buhr HJ. Hepatic arterial infusion (HAI). Comparison of 5-fluorouracil, folinic acid, interferon alpha-2b and degradable starch microspheres versus 5-fluorouracil and folinic acid in patients with non-resectable colorectal liver metastases. Anticancer Res 2006;26: 3957-64.

Reso A, Ball CG, Sutherland FR et al. Rupture and intraperitoneal bleeding of hepatocellular carcinoma after transarterial chemoombolization procedure: a case report. Cases J 2009; 2: 68.

Schwartz M, Weintraub J. Combined transarterial chemoembolization and radiofrequency ablation for hepatocellular carcinoma. Nature Prac Oncol 2008; 5: 630-1.

Pharmacokinetics
Guthof I, Lotspeich E, Fester C, et.al.
Hepatic artery infusion using oxaliplatin in combination with 5-fluorouracil, folinic acid and metomycin C: Oxaliplatin pharmacokinetics and feasibility. Anticancer Res 2003; 23: 5203-5208

Recist
Therasse, P., et al. "New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of ." CanadaJ Natl Cancer Inst 2000; 92:205-16.

Eisenhaue EA, Therasse P, Bogaerts J et al. New response evaluation criteria in solid tumors: revised RECIST guideline (version1.1). Eur J Cancer 2009; 45: 228-47.

Appendix 1
First contact with the subject and guidelines for oral information and obtaining consent

First contact

The patient will be referred to an Experimental Unit in the appropriate departments for intrahepatic treatment. Prior to referral, the patient will be approved by the Second Opinion Committee. This means that even before the information session, the patient is informed about the treatment at the referring oncology department.

Information and consent process

1. Before the information interview

- an agreement must be made on the time and place of the conversation.
- information shall be provided that it is a request to participate in a scientific trial
- information must be provided about the right to a period of reflection after information and the possibility of bringing a family member/friend/other relevant person to the interview.

2. The Information Conversation

- must be carefully planned
- must take place in an undisturbed setting
- the participant must be given sufficient time to read the written information, listen to the oral information and ask questions (the written information is provided after the oral information is provided)
- the investigator must inform the participant of the right to waive knowledge of his or her own health conditions
- the information is provided by the investigator responsible for the study or by the person authorised to do so (sub-investigator = doctor) associated with the trial;

3. Obtaining consent

- the patient's consent to trial participation is given as soon as possible after the information interview, taking into account the necessary reflection period, which is at least one day

APPENDIX 2

Biomarkers background

The strategy in the fight against cancer is currently undergoing a paradigm shift. The goal is no longer to find a universal treatment, but to develop methods and treatments that, based on the latest technology and knowledge, focus on the individual patient. There is a great desire for better and more individualized treatments "personalized medicine" - via so-called translational research. The individual patient will thus benefit the maximum from the research by bridging the gap between the laboratory and the clinic.

A "Biomarker" is defined as "a character trait that can be objectively measured and evaluated as an indicator of a normal biological process, a pathological process, or a pharmacological response to a therapeutic intervention". Biomarkers include a spectrum of molecules with different characteristics but that share association with diseases and can be used for clinical detection of diseases (screening, diagnosis) and/or clinical follow-up (prognosis, treatment effect, monitoring) for early signs of disease worsening in patients and individualised treatment.

Genetic variation is thought to be a major cause of difference in treatment effect of a given medication in the individual. Predictive and prognostic genetic biomarker profiles have gained ground in recent years in, among other things, the treatment of haematological and oncological patients, where it is currently possible for several diseases to identify patient groups that will respond to a given treatment. Genetic biomarkers can be defined as an objectively measurable characteristic e.g. DNA, single nucleotide polymorphisms (SNPs) and copy number variation (CNV), RNA, and micro(mi)RNA that is related to normal biological processes, pathological processes, and/or effect on treatment (www.fda.gov/cder/guidance/6400fnl.htm/ September 2004). Prognostic biomarkers can predict disease worsening independent of treatment, while predictive biomarkers can predict treatment response. Some biomarkers can be both prognostic and predictive. New analytical methods make it possible to examine patients' genome profile and thus obtain information specific to the individual patient. There is great potential in using the information from these advanced gene and miRNA analyses to tailor a treatment plan for the individual patient.

New research has further demonstrated a complex interaction between a number of growth factors and their receptors, cytokines, metalloproteinases and their inhibitors, as well as matrix proteins produced by cancer cells and by the inflammatory cells and fibroblasts/myofibroblasts localized around the cancer cells. This interaction is partly responsible for cancer cell growth, differentiation, migration, and metastasis and is of great importance for patient survival. In recent years, there has been a significant development of biochemical analyses, which now enable serum/plasma concentration determinations of a number of potential biomarkers reflecting angiogenesis, growth factors and their receptors, inflammation, and the metabolism of extracellular tissue.

It is likely that disease severity, treatment efficacy and prognosis of patients with breast cancer and spread to the liver before, during and after treatment with various forms of chemotherapy (e.g. HAI treatment) and new biologic treatment (e.g. Herceptin) can be better assessed by analysing a combination of a number of potential tumor biomarkers than by using only conventional blood tests (haematology and liver counts), X-ray, Ultrasound, MRI and CT scans.

There is no consensus yet on the use of new biomarkers in these patients. Hayes et al. (1996, 1998) have described a number of guidelines "Tumor Marker Utility Grading System" (TMUGS) to facilitate the implementation of potential new cancer biomarkers from the laboratory to daily clinical practice. It remains open whether the knowledge of these potential biomarkers, which may reflect mechanisms of breast cancer with spread to the

liver, has a real clinical applicability in the individual patient, so that clinical decisions can be made that will ultimately improve the prognosis and quality of life of the individual patient compared to not having been known about the biomarker. All new biomarkers are on a "Utility scale +" (defined as: "Assay probably associated with process or end point, but additional confirmatory studies are required") or a "Utility scale +/-" (defined as: "Preliminary data are suggestive that assay correlates with process, or with end point, but substantially more definitive studies are required"). No marker is on a "Utility scale ++" (defined as: "Definitive studies demonstrate that assay reflects process or end point"). According to "TMUGS", a number of validation requirements must be met for a biomarker before it can be assumed to have achieved "Level of evidence I (LOE I)", after which clinical implementation is feasible. "LOE I" studies are either large prospective studies that specifically ask the question whether a biomarker has a clinical applicability to, say: 1) predict prognosis for the patient; 2) to predict the treatment effect of, for example, surgery, chemotherapy, or new biological treatment; or 3) that the biomarker provides better information about disease activity than the methods currently used. "LOE I" studies may also involve a review of meta-analyses of biomarker studies. The published studies on potential biomarkers in patients with breast cancer are on "LOE III". In particular, these are retrospective studies in which the blood samples are not primarily collected from patients with the intention of testing the value of the marker as a prognostic marker of time to progression of the disease or death, or to test their predictive value to assess the efficacy of treatment. Individual studies are at the middle level "LOE II", which consists of accompanying studies with prospectively collected blood samples as part of a therapeutic study, with pre-established endpoints and an evaluation of both the biomarker and the therapeutic treatment.
In the current project, blood samples (whole blood, blood in PAXgeneRNA tubes, serums and various types of plasma) are stored for later analysis of known potential new biomarkers and for later analysis of future biomarkers of breast cancer with spread to the liver. Tissue biopsies of primary tumor as well as liver metastases and other metastases will also be stored for later relevant in situ, immunohistochemical, miRNA and gene analyses.

The following tumor markers are today established in cancer mammae:

Estrogen and progesterone receptor: Expression of these steroid hormone receptors predicts response to endocrine therapy and the receptors are well-established predictive markers that are currently used routinely. Studies have shown that the expression can be altered so that the primary tumor and metastasis do not have the same expression and it is recommended today internationally to repeat the study in patients who have developed relapse and have a focus available for biopsy (standard).
HER2 (Human epithelial growth factor receptor, Neu, c-erbB2) is a transmembranous glycoproteine with an extracellular ligand-binding domain, a transmembranous part, and an intracellular domain with tyrosine kinase activity. Is a Tyrosine kinase receptor belonging to the IGF family. These receptors are an integral part of the signaling pathways that regulate cell growth, and they are closely related to the development of cancer. The expression of HER2 in breast cancer tissue is today routinely used as a predictive and prognostic marker, and can predict response to treatment with trastuzumab. The expression of HER2 in breast cancer tissue can be altered so that primary tumor and metastasis do not have the same expression and it is recommended today internationally to repeat the analyses in patients who have developed relapse and have a focus available for biopsy (standard). Further, there seems to be an interaction between HER2 and the hormone receptors, so that the receptors interact (cross talk).

Topo IIα: Topoisomerase II, a nuclear enzyme involved in cell division. Overexpression of the enzyme causes increased sensitivity to anthracyclines. The enzyme is localized on the same amplicon as HER2 and is often overexpressed in cells with overexpression of HER2.

In addition to the above biomarkers, the following will be analyzed in the current project:

Gene and microRNAarray expression: The use of gene arrays to measure gene expression profiles has great potential in cancer research. For example, cluster algorithms can be used to group tumors on the basis of genprofiles and statistical methods to identify genes based on their relevance in relation to different clinical characteristics and treatment responses. With these methods, new classes of hematological diseases and solid tumors have been identified. The development of gene signatures for prognosis, progression and respons to treatment (chemotherapeutics and antibodies) has also advanced but is still only used in a few cases in daily clinical practice. Genarray profiles can discriminate between normal and cancerous tissue, or can distinguish between different histopathological stages, and is likely to become useful in identifying patients with a poor prognosis and different treatment responses.
MiRNAs are small non-coding 19-25 nucleotides that regulate gene expression post-transcriptionally by binding to 3'UTR on mRNA. MiRNA is not translated into protein, but instead the primary product, pri-miRNA, is processed into short structures called pre-miRNA and finally into mature miRNA. The mature miRNA, together with the protein complex RISC, has a regulatory function on protein synthesis. In cancer, altered expression of miRNA is observed. Dysregulation of miRNA in the cell is associated with cancer development and changes in miRNA are related to survival. Currently, about 700 different human miRNAs are known, which regulate genes that are important for cancer, angiogenesis, stem cell differentiation, and inflammation. Several studies have recently been published on interesting microRNAs (e.g. mir-21, mir-22, mir-155, mir-191, mir-196, mir-200, mir-205, mir-221, mir-342, mir-375, mir-520) in patients with breast cancer, and hopefully it will be possible to find specific gene and miRNA array profiles in these patients to assess diagnosis, disease severity, treatment effect and prognosis.
p53: a Tumor suppressor gene. Experimental studies suggest that mutations in this gene may be predictive of resistance to anthracyclines. A few studies have shown that mutated p53 is correlated to a poor prognosis.
Thymidine Phosphylase (TP): TP is a key enzyme in the metabolism of 5-FU and capecitabine. TP is also known as tumor-associated angiogenic factor in that it has a high vascular formation potential and has anti apoptotic properties. The concentration is 3.2 times higher in tumor tissue than in normal tissue. High concentration of TP is correlated to rapid malignant growth, aggressive invasive potential, and a poor prognosis. TP is upregulated by radiotherapy as well as by a variety of chemotherapeutics (e.g. paclitaxel, docetaxel, mitomycin C, gemcitabine and vinorelbin) as well as oxaliplatin. The modulation of TP during chemotherapy varies among patients. Studies are ongoing on whether, on the basis of TP status in the tumor and its modulation by chemotherapy, it will be possible to design specific treatment measures for different patients.
EGFR: The epidermal growth factor receptor (EGFR) is a central transmembranous growth factor receptor that is commonly found in many normal human tissues. Immunohistochemical analysis of EGFR protein in cancer tissue biopsis is highly dependent on fixation and time for immunohistochemical staining, and a validated scoring system has not yet been established regarding the degree of EGFR protein occurrence in cancer biopsis. EGFR can be determined in serum by ELISA, but the method has not been

validated. In non-small-cell lung cancer treated with the EGFR inhibitor Tarceva, response has been found almost exclusively in never smokers, and it is postulated that smoking can induce a mutation in EGFR that causes this difference. It is unknown whether mutations in EGFR in breast cancer patients have predictive or prognostic value.
PTEN: The PI3K-Akt signaling pathway is one of the signaling pathways activated by the HER family and is important for, among other things, cell survival. PTEN (Phosphatase and tensin homologue deleted on chromosome 10) is an enzyme that has an inhibitory effect on this particular signaling pathway. PTEN was first clarified as being a tumor suppressor for a particular brain tumor, but it has since been seen impaired function of this enzyme in several other cancers and it has an important regulatory function in cancer development. PTEN expression can be examined immunohistochemically from tissue samples.
Interleukin-6: Interleukin-6 (IL-6), an immunomodulatory cytokine, is produced both by cancer cells (including breast cancer cells) and normal cells (pmacrophages, lymphocytes, fibroblasts, and endothelial cells). IL-6 plays an important role in the immune system, in the acute phase response, acts as a paracrine and autocrine growth factor for cancer cells, increases tumor growth and angiogenesis *in vivo*, and inhibits radiation and chemotherapy induced apoptosis and response. IL-6 expression in breast cancer is elevated compared to normal tissue and some patients with metastatic breast cancer have elevated plasma IL-6 compared to healthy individuals, and high plasma IL-6 is associated with poor prognosis. Plasma IL-6 is an independent predictor of treatment response.
C-reactive proteine: C-reactive proteine (CRP) is an acute phase reactant produced by hepatocytes and upregulated by pro-inflammatory cytokines, including IL-6 in association with inflammation, particularly infection. Serum CRP is correlated to IL-6 protein expression in the tissues of cancer patients as well as to serum IL-6. Preoperative serum CRP in patients with cancer has been found in some studies to be significantly related to tumor size, stage, and detected lymphatic and liver metastases. It has been found that elevated CRP is significantly related to relapse after curative resection of gastrointestinal cancer. A high pre- and postoperative serum CRP is associated with shorter survival in patients with many types of cancer, including breast cancer.
Vascular endothelial growth factor: Vascular endothelial growth factor A (VEGF-A, et 45 kDa glycoproteine) has a key role in the formation and maintenance of cancerous tissue blood vessels (tumor angiogenesis). VEGF stimulates the proliferation and migration of endothelial cells to form new vessels, protects endothelial cells from apoptosis, increases the vascular permeability of plasmaroteins and is stimulated, among other things, by hypoxia. VEGF is formed by cancer cells, macrophages, monocytes, leukocytes, thrombocytes, lymphocytes and fibroblasts. Overexpression of VEGF and its receptors has been found in most solid tumors (including breast cancer). High VEGF-A expression in tumor tissue is in some studies related to poor prognosis. In normal tissue, expression of VEGF is observed in epithelial tissues in areas with hypoxia. In patients with several different types of cancer, a high expression of VEGF-A is related to advanced disease stage and poor prognosis, and patients with high tumor burden have higher serum VEGF than patients with low tumor burden. After curative resection of primary colon cancer, serum VEGF decreases to normal values, whereas no changes in serum VEGF have been found in patients with colorectal cancer who have only had palliative resection. High serum VEGF in cancer patients is associated with a poor response to chemotherapy, rapid relapse and short survival, and serum VEGF is an independent prognostic variable. Due to the release of VEGF from leukocytes and thrombocytes, VEGF should be measured in plasma.
YKL-40: YKL-40 (CHI3L1, a 40 kDa glycoprotein) is formed by various cancer cells (including breast cancer) and may have implications for cancer cell proliferation and

survival, angiogenesis, and metastasis/invasive growth. *In vitro* studies of glioblastoma cell lines have shown increased YKL-40 production by stress influences, such as hypoxia and radiotherapy. High expression of YKL-40 protein in breast cancer is not associated with the prognosis. A high serum YKL-40 (compared to healthy persons) is related to a short time to disease progression and death in patients with localized or metastatic breast, colorectal, small cell lung, prostata, ovarian, head-neck, cervix, and renal cell cancer, malignant melanoma, and acute myeloid leukemia. Serum YKL-40 is an independnet biomarker that is independent of serum HER2, estrogen receptor status, CEA, CA125, PSA, and LDH. A relationship has been found between high serum YKL-40 and chemoresistance in patients with metastatic breast and ovarian cancer.

PINP and PIIINP: Cancer cells are involved in extracellular matrix remodeling processes and regulate both the degradation and formation of the surrounding extracellular matrix. Tye I and III collagen, which are the most abundant collagens in the connective tissue, are synthesized as prokollagens with proprptides at both ends (PINP and PIIINP, respectively). After cleavage, these are released into the blood. Serum PINP and PIIINP concentrations therefore reflect the synthesis of type I and III collagen. There is an increased amount of type I and III collagen in the connective tissue around the cancer cells, the desmoplastic reaction, and this has an impact on the malignant phenotye of the cancer cells. A relationship has been found between serum PINP and chemoresistence and aggressiveness of the disease in patients with metastatic breast cancer, and in patients with colorectal cancer, serum PIIINP was an early prognostician for postoperative recurrence.

TIMP-1: Tissue inhibitor of metallorpoteinase (TIMP-1) regulates metalloproteinase activity and is a growth factor for cancer cells in vitro, stimulates angiogenesis and inhibits apoptosis. The expression of TIMP-1 mRNA and protein in various cancerp tissues such as breast, colorectal, ventricular and pulmonary cancer, as well as malignant melanoma is elevated relative to normal tissue, and high expression is associated with distant metastases and poor prognosis. Cancer cells stimulate surrounding connective tissue cells to produce TIMP-1, and genarray has shown several times increased exression of TIMP-1 in cancer tissue compared to normal tissue. TIMP-1 is localized in the connective tissue and basal membranes, primary at the invasive edge of the tumor. Plasma TIMP-1 concentration is elevated in patients with various solid tumors such as metastatic breast, colorectal, bladder, prostata, and renal cancer and related to localization, stage, and prognosis. In patients with colorectal cancer, plasma TIMP-1 is also a sensitive biomarker for identifying both early stages (Dukes' A and B) and right-sided colon cancer, and in combination with serum CEA, the sensitivity of plasma TIMP-1 for the identification of patients with colorectal cancer increased. High pre-operative plasma TIMP-1 in these patients is the strongest prognostic biomarker for short survival and independent of Dukes' stages and other prognostic biomarkers.

Proteases: Plaminogen activator system, plays an important role in remodulation of extracellular matrix as well as metastasis and invasive processes. Preliminary studies have shown that high expression of the uPA receptor (uPAR) may be a poor prognostic factor. Further, urokinase-type plasminogen activator (uPA) and plasminogen activator inhibitor type-1 (PAI-1) are key components of the tissue-degrading processes associated with cancer, and their expression likewise correlated with poor prognosis in several cancer types.

Protein array: Only a few% of the proteins circulating in the blood are known today, and it is likely that several new biomarkers will be identified in the near future. Serum, plasma and blood cells will therefore be collected and stored for the determination of future as yet unidentified biomarkers that could later be studied with protein array.

Metabolomic: There are many thousands of metabolites (molecules with molecular weight <1500 Da) in humans. "Metabolomics" is the study of metabolites in biological fluids, tissues, and isolated cells. "Metabolome" represents a dynamic unit that is related to the genome of a particular organism and reflects the sustained interaction of metabolic and signaling pathways in relation to interaction with the environment, including diet, medication, pathophysiological characteristics, etc. Metabolomic studies result in complex data sets that require statistical methods for understanding.
Nuclear Magnetic Resonance (NMR) spectroscopy is an accurate method based on the spin of atomic nuclei. NMR can be used to investigate the structure of molecules and their spatial and electronic structure and requires minimal sample preparation. This is of importance when examining biological fluids from patients. NMR spectra (normal 1H NMR spectra) of biological samples contains signals of many small molecules present in the sample at a concentration of > 1 μM. NMR spectra or profiles of biological samples (blood samples, tissues) have been found informative in terms of diagnosis and prognosis of various diseases, including cancer diseases, allowing the division of patients into different categories (healthy vs. diseased, patients who either have vs. do not have the effect of the treatment). The purpose of these procedures in the current study is to find relationships between metabolic parameters and disease status as well as efficacy of treatment in patients with breast cancer and liver metastases treated with intrahepatic chemotherapy and in some additionally in combination with trastuzumab.

Material

A biobank will be established with blood from patients before, during and after treatment and with tissues from both primary tumor and metastases.
Blood samples: Whole blood is collected in PAXgene™ Blood RNA Tubes (Qiagen) and stored at -80°C. Serum, EDTA, citrate and heparin plasma are collected and stored at -80°C. Cells (Buffycoat) from the EDTA glass are stored at -80°C. Extraction of DNA, mRNA and miRNA is carried out using Tri-X, which is a method where DNA, mRNA and miRNA can be isolated simultaneously from a PAXgen tube. miRNA is further purified from EDTA plasma and/or serum.
Tissue samples: Tissues from primary tumour (formalin fixed/parafine-embedded) and tissues from liver metastases, and other metastases (fresh frozen tissue and formalin fixed/parafine-embedded) are stored for examination of any changes in the expression of the biomarkers. This is considered standard study, as several studies have shown that biomarkers can change in the course and the treatment offer (e.g. +/- trastuzumab) will be dependent on the expression of HER2 and partly for the purpose of biobanking. Patients can also be included in the protocol if they do not want a biopsy from the liver.
The biological material will only be used for a new research project after obtaining permission from the Research Ethics Committee. The material will be stored for 15 years.

Methods

Routine methods: The expression of estrogen receptor, progesterone receptor, HER2, topoIIα, p53, VEGF, EGFR, YKL-40, TIMP-1 and PTEN in tumor biopsies is determined with routine methods at the pathological department, Herlev Hospital.
ELISA: Comercial ELISA is used to determine plasma VEGF (R&D Systems, UK), plasma IL-6 (R&D Systems, UK), serum EGFR (R&D Systems, UK), serum YKL-40 (Quidel Corporation, USA), serum HER2 (Dako, Denmark), plasma TIMP-1 (Abbott, USA), and serum P1NP (in-house). Serum P3NP is determined with RIA (Orion Diagnostica, Espoo, Finland). It applies to all these biomarkers that the validity of the methods is well

researched. CRP is measured as a routine blood test at the Department of Clinical Biochemistry.
Genarray profiles are determined with Affymetrix U133 plus 2.0 Gene Chip System at Mogens Kruhøffer, AROS Applied Biotechnology A/S in Aarhus.
MiRNAarray profiles are determined with Low Density Array (LDA) assays (Applied Biosystems) at Mogens Kruhøffer, AROS Applied Biotechnology A/S in Aarhus. If there is a better miRNA array method in the future, it will be used.
Metabolomic: The studies are carried out at Mogens Kruhøffer's collaborators at the NMR laboratory at CIRMMP, CERM, Italy (www.cerm.unifi.it), where there is a 600 MHz NMR spectrometer (Bruker BioSpin) with a 5 mm 1H-31P-15N cryoprobe and include an automatic tuning-matching (ATM) and an automatic sample switching and used primarily for metabolomic analyses. A 900 MHz NMR spectrometer (Bruker BioSpin) equipped with a 5 mm TXI cryoprobe also exists and can be used for experiments needed for the identification of biomarkers. AROS and CERM have the necessary statistical competence to assess these analyses.

Statistical methods

The statistical analyses are carried out in collaboration with professional statisticians, among others, at AROS and CERM.
Relevant demographic, clinical, histo-pathological and clinical chemical data, metastasis localization, medication, co-morbidity, as well as treatment repons, time to disease progression and time to death will be recorded in a data bank.
The level (high versus low/normal) of potential biomarkers will relate to time to progression of disease and survival and will be estimated with the Kaplan-Meier method and compared with log-rank testing. Catagory variables are indicated by median followed by the span.
The response rate is assessed in eluable population. Univariate and multivariate analyses will be made.
The calculations of the gene and microRNA array studies will use, among other things, two-dimensional unsupervised hierarchial clustering, "The prediction Analysis of Microarrays(PAM)" version 2.1, R-software package (www.r-project.org) and Plink software (http://pngu.mgh.harvard.edu/~purcell/plink/), and "The Significance Analysis of Microarrays (SAM)" version 3.0.

Risk assessment

Project blood samples will only be taken in conjunction with other scheduled routine blood sampling. Likewise, the tissue samples necessary for the project will be taken at the same time as the patient has a biopsy taken to determine changes in conventional biomarkers. Thus, there is no independent risk in the project.
After the biopsy, there may be discomfort in the form of soreness due to blood accumulation and there may be a risk of bleeding. Performing a biopsy is a routine function.
Before treatment and at the planned scans for disease evaluation after every 4th series, 63.5 ml of blood will be taken for project blood samples along with 10 ml of blood for routine blood tests. Project blood samples will be taken up to and including the time of disease progression. In most patients, a total of between 254 ml and 381 ml of blood will be taken over a period of 6 to 10 months, which does not pose a risk to the patient.

Ethical considerations

One of the aims of the project is to investigate whether determining a number of new potential biomarkers can improve the determination of treatment response and prognosis, as well as the monitoring (for early signs of disease progression) of patients with metastatic breast cancer. The patients will have the extra blood samples taken at the same time as the patient will still have routine blood tests. 63.5 ml of extra blood will be taken, which does not pose a risk to the patient. Examination of the primary tumor does not pose any risk to the patient. Patients included in this study will not be informed of the results of the studies and the study results will not have therapeutic consequences. The basis for this decision is that the sensitivity and specificity of the analytical techniques used, as well as the significance of any findings, have not been clarified and that the average life expectancy for this patient group is 2 years. In addition, the analyses will not necessarily be carried out in the immediate aftermath of the sampling. After the first 2 years, in connection with the evaluation of the methods, a decision will be made as to whether the methods should be established as standard analyses. At this stage, it will also be assessed whether future subsequent patients should be informed of the results.

References

Biomarkers in general

Atikinson AJ, JR. Biomarkers and surrogate endoints: Preferred definitions and concetual framework. Clin Pharmacol Ther 2001;69:89-95.pp

Duffy MJ, et al. Clinical utility of biochemical markers in colorectal cancer: Euroean grou on tumour markers (EGTM) guidelines. Eur J Cancer 2003;39:718-27.pp

Hayes DF, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456-66.

Hayes DF. Determination of clinical utility of tumor markers: a tumor marker utility grading system. Recent Results Cancer Res 1998;52:71-85.

The American Society of Clinical Oncology. Clinical ractice guidelines for the use of tumor markers in breast and colorectal cancer. pJ Clin Oncol 1996;14:2843-77.

Gen- and microRNAarray

Bartels CL, et al. MicroRNAs: novel biomarkers for human cancer. Clin Chem 2009;55:623-31.

Chin KV, et al. Alication of exression genomics for redicting treatment resonsis in cancer. Ann NY Acad Sci 2005;1058:186-95.ppppp

Christensen K, et al. What genome-wide association studies can do for medicine. N Engl J med 2007;356:1094-7.

Croce CM, et al. miRNAs, cancer, and stem cell division. Cell 2005;122:6-7.

Cummins JM, et al. Implications of micro-RNA profiling for cancer diagnosis. Oncogene 2006;25:6220-7.

Dy GK, et al. Systemic cancer therapy: evolution over the last 60 years. Cancer 2008;113:1857-87.

Frazer KA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature 2007;449:851-61.

Garber K. Genomic Medicine. Gene exression tests foretell breast cancers future. Science 2004;303:1754-5.p

Glinsky GV. Disease phenocode analysis identifies SNP-guided microRNA maps (MirMaps) associated with human "master" disease genes. Cell Cycle 2008;7:3680-94.

Hardy J, et al. Genomewide association studies and human Disease. N Engl J med 2009;360:1759-68.

Hoffman AE, et al. MicroRNA mirR-196a-2 and breast cancer: a genetic and epigenetic association study and functional analysis. Cancer Res 2009, June 30. Epub ahead of print.

Huang TH, et al. Up-regulation of miR-21 by HER2/neu signaling promotes cell invasion. J Biol Chem 2009;284:18515-24.

Hui AB, et al. Robust global micro-RNA profiling with formalin-fixed paraffin-embedded breast cancer tissues. Lab Invest 2009;89:597-606.

Iorio MV, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Res 2005;65:7065-70.

Iorio MV, et al. MicroRNA-205 regulates HER3 in human breast cancer. Cancer Res 2009;69:2195-2200.

Israel A, et al. Increased microRNA activity in human cancers. Plus one 2009;4:e6045.

Khoshnaw SM, et al. MicroRNA involvement in the pathogenesis and management of breast cancer. J Clin Pathol 2009;62:422-8.

Kruhøffer M, et al. Isolation of microarray -grade total RNA, microRNA, and DNA from a single PAXgene blood RNA tube. J Mol Diagn 2007;9:452-8.

Lowery A, et al. MicroRNA signatures predict oestrogen receptor, progesterone receptor and HER2/neu receptor status in breast cancer. Breast Cancer Res 2009;11:R27.

Lu J, et al. MicroRNA expression profiles classify human cancers. Nature 2005;435:834-8.

McCafferty MP, et al. Interactions between the estrogen receptor, its cofactors and microRNAs in breast cancer. Breast Cancer Res Treat 2009, June 9. Epub ahead of print.

Mitchell PS, et al. Circulating microRNAs as stable blood-based markers for cancer detection. Proc Natl Acad Sci USA 2008;105:10513-8.

Nelson KM, et al. MicroRNAs and cancer: past, present, and potential future. Mol Cancer Ther 2008;7:3655-60.

Nygaard S, et al. Identification and analysis of miRNAs in human breast cancer and teratoma samples using deep sequencing. BMC Medical Genomics 2009; 2:35.

Pandey DP, et al. MiR-22 inhibits estrogen signalling by directly targeting the estrogen receptor alpha mRNA. Mol Cell Biol 2009;29:3783-90.

Pearson TA, et al. How to interpret a genome-wide association study. JAMA 2008;19:1335-44.

Qi L, et al. Expression of miR-21 and its targets (PTEN, PDCD4, TMI) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma. BMC Cancer 2009;9:153.
The International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299-320.
The Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;447:661-78.
Volinia S, et al. A microRNA exression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 2006;103:2257-61.p
Waldman SA, et al. Translating microRNA discovery into clinical biomarkers in cancer. JAMA 2007;297:1923-5.
Yang Y, et al. Identification of miR-21 targets in breast cancer cells using a quantitative proteomic approach. Proteomics 2009;9:1374-84.
Zhu W, et al. Circulating microRNAs in breast cancer and healthy subjects. BMC Research Notes 2009;2:89.

VEGF

Adams J, et al. Vascular endothelial growth factor (VEGF) in breast cancer: comarison of lasma, serum, and tissue VEGF and microvessel density and effects of tamoxifen. Cancer Res 2000;60:2898-905.pp
Davies MM, et al. Plasma vascular endothelial but not fibroblast growth factor levels correlated with colorectal liver metastasis vascularity and volume. Br J Cancer 2000;82:1004-8.
Dvorak HF. Vascular ermeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a otential target for diagnosis and theray. J Clin Oncol 2002;20:4368-80.ppp
Ferrara N, et al. The biology of VEGF and its recetors. Night with 2003;9:669-76. p
Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Night with 1995;1:27-31.
Fuhrmann-Benzakein E, et al. Elevated levels of angiogenic cytokines in the lasma of cancer atients. Int J Cancer 2000;85:40-5.pp
Force A, et al. Vascular endothelial growth factor in the sera and effusions of atients with malignant and nonmalignant disease. Cancer 1999;85:178-87.p
Lissoni IP, et al. Changes in circulating VEGF levels in relation to clinical resonse during chemotheray for metastatic cancer. Int J Biol Markers 2003;18:152-5.pp
Nicholas J, et al. Vascular endothelial growth factor (VEGF) is released from latelets during blood clotting: imlications for measurement of circulating VEGF levels in clinical disease. Clin Science 1998;xx:395-404.pp
Ointment P, et al. Serum vascular endothelial growth factor is often elevated in disseminated cancer. Clin Cancer Res 1997;3:647-51.
Ointment P, et al. Leukocytes and latelets of atients with cancer contain high levels of vascular endothelial growth factor. Clin Cancer Res 1999;5:487-91.pp
Seo Y, et al. High exression of vascular endothelial growth factor is associated with liver metastasis and a oor rognosis for atients with ancreatic adenocarcinoma. Cancer 2000;88:2239-45.ppppp
Takahashi Y, et al. Vessel counts and exression of vascular endothelial growth factor and its recetor, KDR, correlates with vascularity, metastasis, and oliferation of human colon cancer. pppCancer Res 1995;55:3964-8.
Tokunaga T, et al. Vascular endothelial growth factor (VEGF) mRNA isoform exression attern correlated with liver metastasis and oor rognosis in colon cancer. Br J Cancer 1998;77:998-1002.pppp
Zebrowski BK, et al. Markedly elevated levels of vascular endothelial growth factor in malignants ascites. Ann Surg Oncol 1999; 6:373-8.
Van den Eynden GG, et al. Differential expression of hypoxia and (lymph)angiogenesis-related genes at different metastatic sites in breast cancer. Clin Exp Metastasis 2007;24:13-23.
Warren RS, et al. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of exerimental liver metastasis. pJ Clin Invest 1995;95:1789-97.

EGFR and HER2

Andersen TI, et al. Detection of c-erbB-2 related rotein in sera from breast cancer atients. Relationshi to ERBB2 gene amlification and c-erbB-2 rotein overexression in tumour. Acta Oncol 1995;34:499-504.pppppp
Arteaga CL. Overview of eidermal growth factor recetor biology and its role as a theraeutic target in human neolasia. Semin Oncol 2002;29 (sul 14):3-9.pppppp
Atkins D, et al. Immunohistochemical detection of EGFR in arafin-embedded tumor tissues: Variation in staining intenstity due to choice of fixative and storage time of tissue sections. J Histochem Cytochem 2004;52:893-901.p
Baselga J, et al. Clinical udate and emerging trends in eidermal growth factor recetor targeting in cancer. J Clin Oncol 2005;23:2445-59.ppp

Fehm T, et al. The rognostic significance of c-erbB-2 serum rotein in metastatic breast cancer. ppOncology (Huntingt) 1998;55:33-8.
Harris L, et al.c-erbB- serum of atients with breast cancer. Int J Biol Markers 1999;14:8-15.2 inp
Isola JJ, et al. Elevated erbB-2 oncorotein levels in reoerative and follow-u serum samles define an aggressive disease course in atients with breast cancer. Cancer 1994;73:652-8.pppppp
Italiano A, et al. Eidermal growth factor recetor (EGFR) status in rimary colorectal tumors correlates with EGFR exression in related metastatic sites: biological and clinical imlications. Ann Oncol 2005;16:1503-7.ppppp
Jensen BV, et al. High levels of serum HER-2/neu and YKL-40 indeendtly reflect aggressivenes of metastatic breast cancer. Clin Cancer Res 2003; 9:4423-34. p
Kandl H, et al. Soluble c-erbB-2 fragment in serum correlates with disease stage and redicts for shortened survival in atients with early-stage and advanced breast cancer. Br J Cancer 1994;70:739-42.pp
Leitzel K, et al. Elevated serum c-erbB-2 antigen levels and decreased resonse to hormone theray of breast cancer. J Clin Oncol 1995;13:1129-35.pp
Mansour OA, et al. Tissue and serum c-erbB-2 and tissue EGFR in breast carcinoma: three years follow-u. Anticancer Res 1997;17:3101- 6.p
Molina R, et al.c-erbB-2 oncorotein, CEA, and CA atients with breast cancer: rognostic value. Breast Cancer Res Treat 1998; 51:109-19. p15.3 inpp
Mehta RR, et al. Plasma c-erbB-2 levels in breast cancer atients: rognostic significance in redicting resonse to chemotheray. J Clin Oncol. 1998;16:2409-16.ppppp
Olayioye MA, et al. The ErbB signaling network: recetor heterodimerization in develoment and cancer. EMBO J 2000;19:3159-67.pp
Polychronidis AC, et al. Serum levels of c-erbb-2 correlate with advanced stage and liver metastasis in colorectal cancer. Folia Med () 2003;45:12-6.Plovdiv
Ross JS, et al. The HER-2/neu oncogene in breast cancer: Prognostic factor, redictive factor, and target for theray. The Oncologist 1998;3:237-52.pp
Slamon DJ, et al. Use of chemotheray lus a monoclonal antibody against HER2 for metastatic breast cancer that overexresses HER2. N Engl J med 2001;344:783-92.ppp
Slamon DJ, et al. Human breast cancer: correlation of relase and survival with amlification of the HER-2/neu oncogene. Science 1987;235: 177-82.pp
Slamon DJ, et al. Studies of the HER-2/neu roto-oncogene in human breast and ovarian cancer. Science 1989;244:707-12.p
Stern DF. Tyrosine kinase signaling in breast cancer: ErbB family recetor tyrosine kinases. Breast Cancer Res 2000;2:176-83.p
Sugano K, et al. Combined measurement of the c-erbB-2 rotein in breast carcinoma tissues and sera is useful as a sensitive tumor marker for monitoring tumor relase. Int J Cancer 2000;89:329-36.pp
Vogel CL, et al. Efficacy and Safety of Trastuzumab as a Single Agent in First-Line Treatment of HER2-Overexressing Metastatic Breast Cancer. J Clin Oncol 2002;20:719-26.p
Willsher PC, et al. Prognostic significance of serum c-erbB-2 rotein in breast cancer atients. Breast Cancer Res Treat 1996;40:251-5.pp
Wu JT, et al. Detection of the extracellular domain of c-erbB-2 oncorotein in sera from atients with various carcinomas: correlation with tumor markers. J Clin Lab Anal 1993;7:31-40.pp
Yamauchi H, et al. Prediction of resonse to antiestrogen theray in advanced breast cancer atients by retreatment circulating levels of extracellular domain of the HER-2/c-neu rotein. J Clin Oncol 1997;15:2518-25.ppppp
Zabrecky JR, et al. The extracellular domain of 185/neu is released from the surface of human breast carcinoma cells, SK-BR-3. J Biol Chem 1991;266:1716-20.p

YKL-40

Jensen BV, et al. High levels of serum HER-2/neu and YKL-40 indeendently reflect aggressiveness of metastatic breast cancer. Clin Cancer Res 2003;9:501-12. p
Johansen JS, M et al. Serum YKL-40: a new otential marker of rognosis and location of metastases of atients with recurrent breast cancer. Eur J Cancer 1995;31A:1437-42.ppp
Johansen JS, et al. High serum YKL-40 levels in atients with rimary breast cancer is related to short recurrence free survival. Breast Cancer Res Treat 2003;80:15-21.pp
Johansen JS, et al. Review. Serum YKL- new rognostic biomarker in cancer atients? Review. Cancer Eidemiol Biomarkers Prev 2006;15:194-202.40, appp
Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodeling, fibrosis and cancer. Dan with Bull 2006;53:172-209. Review.
Lee CG, et al. Role of breast regression protein 39 (BRP-39)/chitinase 3-like-1 in Th2 and IL-13-induced tissue responses and apoptosis. J Exp Med, Epub a head of print .May 4, 2009

Recklies AD, et al. The chitinase 3-like rotein human cartilage 39 (HC-g39) stimulates roliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase-and rotein kinase B-mediated signalling athways. Biochemist J 2002;365:119-26. ppppp
Rehli M, et al. Molecular characterization of the gene for human cartilage g-39 (CHI3L1), a member of the chitinase rotein family and marker for late stages of macrohage differentiation. Genomics 1997;43:221-5. ppp
Rehli M, et al. Transcritional regulation of CHI3L1, a marker gene for late stages of macrohage differentiation. J Biol Chem 2003;278:44058-67.pp
Renkema GH, et al. Chitotriosidase, a chitinase, and the 39-kDa human cartilage glycorotein, a chitin-binding lectin, are homologues of family 18 glycosyl hydrolases secreted by human macrohages. Eur J Biochem 1998;251:504-9.pp
Volck B, et al. YKL- mammalian member of the chitinase family, is a matrix rotein of secific granules in human neutrohils. 40, apppProc Assoc Am Phys 1998;110:351-60.

IL-6

Castell JV, et al. Acute-hase resonse of human heatocytes: regulation of acute–hase rotein synthesis by interleukin-6. Heatology 1990;12:1179-86.pppppp
Chung YC, et al. Serum interleukin-6 levels reflects the disease status of colorectal cancer. J Surg Oncol. 2003;83: 222-6.
Cohen T, et al. Interleukin 6 induces the expression of vascular endothelial growth factor. J Biol Chem. 1996;271:736-41.
DeMichele A, et al. Host genetic variants in the interleukin-6 promoter predict poor outcome in patients with estrogen receptor-positive, node-positive breast cancer. Cancer Res 2009;69:4184-91.
Drachenberg DE, et al. Circulating levels of interleukin- atients with hormone refractory rostate cancer. Prostate 1999;41:127-33.6 inpp
Esfandi F, Mohammadzedah G, Basati G. Interleukin-6 level in patients with colorectal cancer. Cancer letters 2006; 244:76-78.
Ferdeghini M, Gadducci A, Prontera C et al. Serum interleukin-6 levels in uterine malignancies. Preliminary data. Anticancer Res 1994;14:735-7.
Galizia G, et al. prognostic significance of circulating Il-10 and Il-6 serum levels in colon cancer patients undergoing surgery. Clin Immunol. 2002;102: 169-78.
George DJ, et al. The rognostic significance of lasma interleukin-6 levels in atients with metastatic hormone-refractory rostate cancer: results form cancer and leukaemia grou B 9480. Clin Cancer Res 2005;11:1815-20. ppppp
Heinrich PC, et al. Interleukin-6 and the acute phase response Biochem J 1990; 265:621-36.
Heinrich PC, et al. Princiles of interleukin (IL)-6-tye cytokine signalling and regulation. Biochem J 2003;374:1-20. pp
Kamimura D, et al. IL-6 signal transduction and its hysiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 2003;149:1-38.p
Kinoshita T, et al. Serum interleukin-6 reflects the tumor proliferative activity in patients with colorectal carcinoma. Cancer 1999;85:2526-31.
Knűpfer H, et al. Significance of interleukin-6 (IL-6) in breast cancer (review). Breast Cancer Res Treat 2007;102:129-35.
Lyon DE, et al. Cytokine comprisons between women with breast cancer and women with a negative breast biopsy. Nurs Res 2008;57:51-8.
Miki C, et al. C-reactive protein as a prognostic variable that reflects uncontrolled up-regulation of the IL-1-IL-6 network system in colorectal carcinoma. Dig Dis Sci 2004; 49: 970-6.
Nilsson MB, et al. Interleukin-6 secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. Cancer Res. 2005;65: 10794-10800.
Penson RT, et al. Cytokines IL-1beta, IL-2, IL-6, IL-8, MCP-1, GM-CSF and TNFalha in atients with eithelial ovarian cancer and their relationshi to treatment with aclitaxel. pppppInt J Gynecol Cancer 2000;10:33-41.
Premkumar VG, et al. Serum cytokine levels of interleukine-1beta, -6, -8, tumour necrosis factor-alpha and vascular endothelial growth factor in breast cancer patients treated with tamoxifen and supplemented with co-enzyme Q(10), riboflavin and niacin. Basic Clin Pharmacol Toxicol 2007;100:387-91.
Scambia G, Testa U, Panici PB et al. Interleukin-6 serum levels in atients with gynecological tumors. Int J Cancer 1994;57:318-23.p
Srivani R, et al. A rognostic insight on in vivo exression of interleukin- uterine cervical cancer. Int J Gynecol Cancer 2003;13:331-9. pp6 in
Trikha M, et al. Targeted anti-interleukin-6 monoclonal antibody theray for cancer: a review of the rationale and clinical evidence. pClin Cancer Res 2003;9:4653-65.

Walter M, et al. Interleukin 6 secreted from adipose stromal cells promotes migration and invasion of breast cancer cells. Oncogene 2009, June 1 Epub ahead of print.

TIMP-1

Birkedal-Hansen H, et al. Matrix metalloroteinases: a review. pCrit tore oral biol with 1993; 4:197-250.
Clavel C, et al. Immunolocalization of matrix metallo-proteinases and their tissue inhibitor in human mammary pathology. Bull Cancer 1992;79:261-70.
Cornelius LA, et al. Matrix metalloroteinases generate antiostatin: effects on neovascularization. J Immunol 1998;16:6845-52.p
Dien J, et al. Signal transducers and activators of transcription-3 up-regulates tissue inhibitor of metalloproteinase-1 expression and decreases invasiveness of breast cancer. Am J Pathol 2006;169:633-42.
Hayakawa T, et al. Growth-romoting activity of tissue inhibitor of metalloroteinases-1 (TIMP-1) for a wide range of cells. A ossible new growth factor in serum. FEBS Lett 1992;298:29-32.ppp
Holten-Andersen MN, et al. Quantitation of TIMP- lasma of healthy blood donors and atients with advanced cancer. 1 inppBr J Cancer 1999;80:495-503.
Li G, et al. Tissue inhibitor of metalloroteinase-1 inhibits aotosis of human breast eithelial cells. Cancer Res 1999;59:6267-75.pppp
Luparello C, et al. Tissue inhibitor of metal lorotease (TIMP)-1 and roliferative behaviour of clonal breast cancer cells. Breast Cancer Res Treat 1999;54:235-44.pp
Nakopoulou L, et al. The favourable prognostic impact of tissue inhibitor of matrix metalloproteinses-1 protein overexpression in breast cancer. APMIS 2003;111:1027-36.
Newell KJ, et al. Exression and localization of matrix-degrading metalloroteinases during colorectal tumorigenesis. ppMol Carcinog 1994;10:199-206.
Polette M, et al. Detection and localization of mRNA's encoding matrix metalloproteinases and their tissue inhibitor in human breast pathology. Invasion Metastasis 1993; 13:31-7.
Ree AH, et al. High levels of messenger RNAs for tissue inhibitors of metalloroteinases (TIMP-1) and TIMP-2) in rimary breast carcinomas are associated with develoment of distant metastases. Clin Cancer Res 1997;3:1623-8.ppp
Sieuwerts AM, et al. Concentrations of TIMP1 mRNA splice variants and TIMP-1 protein are differentially associated with prognosis in primary breast cancer. Clin Chem 2007;53:1280-8.
Yoshiji H, et al. Mammary carcinoma cells over-exressing tissue inhibitor of metalloroteinases-1 show enhanced vascular endothelial growth factor exression. Int J Cancer 1998;75:81-7.ppp
Yukawa N, et al. Plasma concentration of tissue inhibitor of matrix metalloroteinase atients with colorectal carcinoma. Br J Surg 2001;88:1596-601.p1 inp
Zeng ZS, et al. Distinct attern of matrix metalloroteinase 9 and tissue inhibitor of metalloroteinase 1 mRNA exression in human colorectal cancer and liver metastases. ppppBr J Cancer 1995;72:575-82.
Zeng ZS, et al. Elevated tissue inhibitor of metalloroteinase 1 RNA in colorectal cancer stroma correlates with lymh node and distant metastases. ppClin Cancer Res 1995;1:899-906.

PINP and PIIINP

Jensen BV, et al. Extracellular matrix building marked by the N-terminal roetide of rocollagen tye I reflect aggressiveness of recurrent breast cancer. Int J Cancer 2002;98;582-9.ppppp
Plebani M, et al. N-terminal etide of tye III rocollagen. A ossible redictor of colorectal carcinoma recurrence. Cancer 1997;79:1299-303.pppppp
Risteli J, et al. Raid equilibrium radioimmunoassay for the amino-terminal roetide of human tye III rocollagen. ppppppClin Chem 1988; 34:715-8.
Örum O, et al. Procollagen tye I N-terminal roetide (PINP) as an indicator of tye I collagen metabolism: Elisa develoment, reference interval and hyovitaminosis D induced hyerarathyroidism. pppppppppBone 1996;19:157-66.

Metabolomic

Assfalg M, et al. Evidence of different metabolic phenotypes in humans. Proc Natl Acad Sci USA 2008;105: 1420-4.
Claudino WM, et al. Metabolomics: available results, current research projects in breast cancer, and future applications. J Clin Oncol 2007;25:2840-6.
De Leo A, et al. New strategies to identify molecular markers predicting chemotherapy activity and toxicity in breast cancer. Ann Oncol 2007;18 (Supll 12):xii8-14.
Spratlin JL, et al. Clinical applications of metabolomics in oncology: a review. Clin Cancer Res 2009;15:431-40.

Appendix 2.
Guidelines for blood sample forms for HAI Mammae

Upon inclusion, a patient specific blood sample form is prepared

Flowchart of blood sample form 1

The patient is registered on form 1 with Hospital, Patient Initials, and Patient No. as well as date of inclusion.
In the form, the number of greiner tubes and Paxgen RNA tubes stored in the freezer are noted.
The patient is given his own chartek, in which the blood sample forms are stored together with a copy of informed consent.

Freezer boxes

Each visit has its box.
Each sample type has its box.
It is noted in scheme 1 in which box (no.), where the greiner tubes are placed.

If it is difficult to retrieve all blood samples from the patient, priority is given as follows:

PURPLE EDTA glass
RED dry glass
PAXGEN tube
BLUE citrate glass
GREEN heparin glass

HAI Mammae contact names for blood sample handling

[redacted]
Bioanalyst
Clinical Research Unit 65C8
Herlev Hospital
Herlev Ringvej 75
2730 Herlev
[redacted]

[redacted]
Bioanalyst
Clinical Research Unit 65c8
Herlev Hospital
Herlev Ringvej 75
2730 Herlev
[redacted]

[redacted]
Consultant
Oncology Dept.
Herlev Hospital
Herlev Ringvej 75
2730 Herlev
[redacted]

HAI Mammae flowchart for blood samples

Hospital:
Patient initials:
Patient number:

For blood sample management – see Laboratory folder

Table 1

VISIT	A	B1	B2	B3	B4	B5	B6	B7
	Before treatment	1. Scan after 4. series	2. Scan after 8. series	3. Scan after the 12. series	4. Scan follow-up	5. Scan follow-up	6. Scan follow-up	7. Scan follow-up
Blood sample taken Date								
3 X 6 ml Red/Black Dry glass for SERUM Greiner tube 5 pcs **RED**								
3 X 6 ml Purple/Black EDTA glass for EDTA plasma Greiner tube 5 pcs **BLUE**								
3 X 3.5 ml Light Blue/Black Citrate Glass for CITRATE Plasma Greiner tube 5 pcs **WHITE**								
3 X 4 ml Green/Black Heparin Glass for HEPARIN Plasma Greiner pipe 3 pcs **GREEN**								
Buffycoat from 3 EDTA glasses Greiner tube 3 pcs **YELLOW**								
2 Paxgene RNA tubes								
Signature								

HAI Mammae handling blood samples

Sampling ALL VISITS	3 X 6 ml **RED** DRY GLASS	3 X 6 ml **PURPLE** EDTA GLASS	3 X 3.5 ML **BLUE** CITRATE GLASS	3 X 4 ml **GREEN** HEPARIN GLASS	3 tubes with ***Buffy coat***	2 X PAXGEN RNA tube
PREPARING	At least 30 min. Centrifuged at 2330g 10 min 4° C Serum is transferred to 5 x cryo tube with **red** lid minimum 500 µl	At least 30 min. Centrifuged at 2330g 10 min 4° C Plasma is transferred to 5 x cryo tube with **blue** lid minimum 500 µl	At least 30 min. Centrifuged at 2330g 10 min 4° C Plasma is transferred to 5 x cryo tube with **white** lid minimum 500 µl	At least 30 min. Centrifuged at 2330g 10 min 4° C Plasma is transferred to 3 x cryo tube with **green** lid minimum 500 µl	Use the glass from the EDTA plasma Remove plasma to approximately. over cell layers.5 mm Take all the white blood cells from 1 glass and put them in 1 cryo tube with **yellow** lid 1 glass = 1 cryo tube So you have a total of 3 cryo tubes.	**Should be turned over gently 8-10 times, *IMMEDIATELY* after blood sampling!** Left for 2 - 48 hours at room temperature. Turn over before freezing the pipe.
STORAGE	-80°C freezes	-80°C freezes	-80°C freezes	-80°C freezes	-80°C freezes	Freeze at -20° C for 24 - 72 hours. Move to -80°C freeze.

Appendiks 3.

New York Heart Association (NYHA) Classification System

Class I:	Patients with heart disease but without accompanying restrictions on physical activity. Ordinary physical activity does not cause excessive fatigue, palpitations, dyspnea or anginas pain.
Class II:	Patients with heart disease, which results in slight restriction in physical activity. They feel good at rest. Regular physical activity causes fatigue, palpitations, dyspnea or anginas pain.
Class III:	Patients with heart disease, which results in marked restriction in physical activity. They feel good at rest. Less than regular physical activity causes fatigue, palpitations, dyspnea or anginas pain.
Class IV:	Patients with heart disease that results in the inability to perform any physical activity without discomfort. Symptoms of heart failure or angina may also be present at rest. Discomfort increases with any physical activity.

Ref.: Textbook Of Medicine. Vol 2, pp2228, Oxford University Press, 1997.Oxford