Clinical Investigation Plan

A mono-centric, first in-human (FIH), safety and preliminary efficacy study of (neo)adjuvant, model-based, whole-body hyperthermia (WBHT) treatment in advanced solid cancer patients or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma patients

MATTERS

Version ~~7~~8.0, ~~20 January~~17 August 2023

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Document history

Document version	Document Date	Author	Reason for change
1.0	27 March 2020	Ivana Gorbaslieva	First version signed off
2.0	9 Nov 2020	Ivana Gorbaslieva	Addition of urine collection for exosome analysis. Use of trademarks TempoCure® and TempoSensor® Some minor updates in text to clarify items.
3.0	7 May 2021	Ivana Gorbaslieva	Use of waist circumference instead of BMI as inclusion criterion. Update coagulation parameters in inclusion criteria. Addition of requirements on anti-platelet and anticoagulant medication in inclusion criteria.
4.0	18August 2021	Ivana Gorbaslieva	Addition of enema, body lotion (or equivalent), anti-decubitus measures and pre-heated fluids. Readjustment of the blood sampling during treatment. Addition of the procedure on postponed treatment and patient on hold process
5.0	28February 2022	Ivana Gorbaslieva	SAE reporting to Competent Authorities according to new legislation. New cohort design. Chemotherapy according to the NCCN guidelines. Modification of existing inclusion criteria Quality of Life assessment (EORTC-QLQ-C30- version 3)
6.0	25July 2022	Ivana Gorbaslieva	Addition dosage and time of chemotherapy administration.

Document version	Document Date	Author	Reason for change
			Withdrawal of chemotherapy as monotherapy (cohort B/C/D).
7.0	20 January 2023	Ivana Gorbaslieva	Timing administration chemotherapy. Adapted blood sampling. Swan Ganz is now optional.
8.0	17 August 2023	Oleg Rudenko	Addition of optional Electromyography (EMG) at screening. Additional collection of tumor markers. Use of doppler echo to assess blood flow dynamics in the liver. Use of the AMPIR-01 fluid and blood warmer during study procedure. Collection of Standard of Care CT scan and relevant tumor markers after the End of Study. Explicit declaration of the optional use of propofol for maintenance of anesthesia.

In case the clinical investigation will be amended, an updated Clinical Investigation Plan version will be generated. Approval for this clinical investigation and any subsequent amendments will be obtained internally and externally.

Compliance statement

The clinical investigation will be conducted in accordance with this Clinical Investigation Plan, the Declaration of Helsinki, applicable good clinical practices and regulations and the appropriate local legislations.

The clinical investigation will not start before the required approvals of Ethics Committee (EC) of the involved investigation site and/or Regulatory Authorities/Competent Authority (CA) approval are obtained.

The sponsor will enter into an insurance contract according with article 29 of the Belgium Law relating to experiments on the human person of 7 May 2004.

CIP Signature page

Name Sponsor:	Signature	Date
ElmediX Chief Executive Officer **John-Paul Bogers**		
ElmediX Chief Medical Officer **Oleg Rudenko**		

I have read the Clinical Investigation Plan (CIP) and agree that it contains all necessary details for carrying out this study. I will conduct the study as outlined therein, in compliance with applicable good clinical practice (e.g. ISO14155) and all applicable regulatory requirements. In addition, I will conduct the study in accordance with the ethical principles of the Declaration of Helsinki.

I assume responsibility for the proper conduct of the study at this site and I will ensure that all persons assisting in the study under my supervision are adequately informed about the study, the device and their study related duties and functions as described in the CIP.

I will provide copies of the CIP and all information on the device relating to past non-clinical and clinical experience, which were furnished to me by the sponsor, to all physicians and other study personnel responsible to me who participate in this study, and will discuss this material with them to ensure that they are fully informed regarding the device and the conduct of the study.

I agree to keep records on all subject information (e.g. case report forms and informed consent statements), device shipments and return forms, and all other information collected during the study, in accordance with local and national regulations.

Local Principal Investigator	Signature	Date
Prof. Dr. Marc Peeters		
Prof. Dr. Dirk Ysebaert		
Dr. Vera Saldien		

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List of abbreviations

ADE	Adverse Device Effect
AE	Adverse Event
aPTT	Activated Partial Thromboplastin Time
ALT	Alanine Transaminase (SGPT)
AST	Aspartaat Transaminase (SGOT)
BMI	Body Mass Index
CA	Competent Authority
CA 19-9	Cancer Antigen 19-9
sCD40L	Soluble CD40-CD40 ligand
CE	Conformité Européenne
CEA	Carcino-embryonic antigen
CIP	Clinical Investigation Plan
Cmax	Maximum plasma concentration
CRA	Clinical Research Associate
CRC	Colorectal cancer
CRO	Contract Research Organization
CT	Computed Tomography
DCF	Data Clarification Form
DIC	Disseminate Intravascular Coagulation
DKK	Dickkoph-related protein
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
eGFR	Estimated Glomerular Filtration Rate
EMG	Electromyography
EN	European Standard is valid for all European Member States
FU	Follow up
FIH	First in-human
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
HCG	Human Chorionic Gonadotropin
HT	Hyperthermia
IB	Investigator Brochure
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IFU	Instructions for Use
IL	InterLeukin
INR	International Normalized Ratio
ISO	International Organization for Standardization
MD	Medical Device
MDR	Medical Device Regulation
NA	Not applicable
NCCN	National Comprehensive Cancer Network
OTC	Over the counter
PACU	Post Anesthetic Care Unit

RECIST	Response Evaluation Criteria in solid tumors
SADE	Serious Adverse Device Effect
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation
SOC	Standard of care
TGF	Transforming Growth Factor
tT	treatment Temperature
tD	treatment Duration
ULN	Upper Limit of Normal
USADE	Unanticipated Serious Adverse Device Effect
VEGF	Vascular Endothelial Growth Factor
WBHT	Whole-Body Hyperthermia / whole-body hyperthermia
WHO	World Health Organization
TMF	Trial Master File

Synopsis / Clinical study summary

Study Name	A mono-centric, first in-human (FIH), safety and preliminary efficacy study of (neo)adjuvant, model-based, whole-body hyperthermia (WBHT) treatment in advanced solid cancer patients or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma patients
Protocol code	MATTERS
Protocol version	~~7~~8.0, ~~20 January~~17 August 2023
Investigation device	The TempoCure® is a device to deliver a whole-body hyperthermia treatment to a patient. The device is designed to rise the temperature of the patient to a specific setpoint, defined as the treatment Temperature (tT) in Celsius (°C). Thereafter, the device finely regulates the temperature of the patient to the selected setpoint for a specific period, defined as the treatment Duration (tD) in minutes (min). The TempoCure® is composed of four main units: • Treatment unit: this is the insulated cabinet in which the patient is placed on the stretcher. • Sensing unit: this unit measures the temperature and/or heat flux of the patient who is placed in the treatment unit and serves as input module for the control unit. • Control unit: the control unit acts upon the input from the sensing unit to steer the mechanical unit to bring and keep the patient at the treatment temperature and hold the treatment temperature during the treatment duration. After treatment, the control unit returns the patient in a controlled way back to normothermic state. The power supply is part of control unit. • Mechanical unit: the mechanical unit consists of the ventilation fans (used to forcedly circulate warm air in the treatment unit) and active heating/cooling elements. The operation of all parts of the mechanical unit is directly regulated by the control unit.
Patient population	The TempoCure® is intended for use on adult patients with advanced solid cancer (cohort A1, A2) or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma (cohort B/C/D) previously treated or under standard of care (SOC) treatments. In total, 14 (up to 25 patients) will be treated in five different cohorts (A1, A2, B, C and D (2-5 patients per cohort)).

Objective	Primary objective: To assess the safety of different durations (2h, 4h and 6h) and repetitions (3 times, once a week on day 1, 8 and 15 respectively) of whole-body hyperthermia with the TempoCure® alone or in combination with standard of care (SOC) chemotherapy according to the NCCN guidelines, up to 4 weeks after last treatment in patients with advanced solid cancer or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma. Secondary objective: To evaluate the preliminary efficacy parameters of different durations of whole-body hyperthermia with the TempoCure® alone or in combination with SOC chemotherapy according to the NCCN guidelines, up to 10 weeks after last treatment in patients with advanced solid cancer or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma. As part of the preliminary efficacy, to evaluate the Quality of Life of different durations of whole-body hyperthermia with the TempoCure® alone or in combination with SOC chemotherapy according to the NCCN guidelines, up to 10 weeks after last treatment in patients with advanced solid cancer or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma.
Design	Potential patients are further screened, after consenting, to confirm the eligibility criteria during **the screening period**. Whole-body hyperthermia will be applied in a unit connected to the operating room at the University Hospital Antwerp (UZA). All the patients will undergo the treatment under deep anesthesia. **The hospitalization** starts the day before and ends two days after the whole-body hyperthermia treatment. The patient's body temperature will be monitored by ElmediX temperature sensors. Invasive temperature monitoring will be performed in the liver. The intrahepatic sensor will be placed by a surgeon under ultrasound guidance. This sensor is at the most temperature sensitive organ and it is essential for the treatment. In addition, other (oesophageal, rectal and cutaneous) temperatures will be monitored. Optionally, additional sensors can be employed such as ear, gastric,

	duodenal, urinary bladder and one or more non-invasive, dermal or intradermal sensors. 1. Cohort A1. Three patients with advanced solid cancer will be subjected to repetitive hyperthermia starting with 2 hours (day 1), 4 hours (day 8) and 6 hours (day 15) using the TempoCure® to keep the patient's body at a temperature (tT) of 41.5°C. A warming-up and a cool-down period (estimated time of 4 to 6 hours based on test models) needs to be added to the treatment duration of 2, 4 or 6 hours. 2. Cohort A2. Whole-body hyperthermia will be applied to two additional patients with advanced solid cancer, once a week and for 3 treatments in total. The first patient(s) in this cohort will receive 3 consecutive hyperthermia treatments of 4 hours per treatment. After the last patient received 3 times a 4-hour treatment, the next patient in this cohort will receive 3 hyperthermia treatments of 6 hours per treatment. 3. Cohort B. Three (up to five) pancreatic cancer patients will be subjected to three hyperthermia treatments (2 hours duration per treatment) alone (1st treatment) and in combination with (2nd and 3rd treatment) Standard of Care (SOC) chemotherapy according to the NCCN guidelines. During the hospitalization for the 2nd treatment, the patient will receive 50% of the standard chemotherapy dosage. During the hospitalization for the 3rd treatment, the patient will receive 100% of the standard chemotherapy dosage. 4. Cohort C. Three (up to five) pancreatic cancer patients will be subjected to three hyperthermia treatments (4 hours duration per treatment) alone (1st treatment) and in combination with (2nd and 3rd treatment) Standard of Care (SOC) chemotherapy according to the NCCN guidelines. During the hospitalization for the 2nd treatment, the patient will receive 50% of the standard chemotherapy dosage. During the hospitalization for the 3rd treatment, the patient will receive 100% of the standard chemotherapy dosage.

	5. Cohort D. Three (up to five) pancreatic cancer patients will be subjected to three hyperthermia treatments (6 hours duration per treatment) alone (1st treatment) and in combination with (2nd and 3rd treatment) Standard of Care (SOC) chemotherapy according to the NCCN guidelines. During the hospitalization for the 2nd treatment, the patient will receive 50% of the standard chemotherapy dosage. During the hospitalization for the 3rd treatment, the patient will receive 100% of the standard chemotherapy dosage. Patients will be followed-up for up to 10 weeks after last treatment (**follow up period**) to assess safety and preliminary efficacy. Patients will be treated under the current protocol and closely monitored during the treatment period. A Data Safety Monitoring Board (DSMB) will be installed to assess safety of the patients during the study. After each study cohort, a risk assessment will take place by the DSMB before starting the next cohort, using the data up to Follow up visit 1. As soon as 3 patients are treated with 2 hours of WBHT in cohort A1, and after a positive advice of the DSMB, the enrolment in Cohort B can be initiated. The risk assessment by the DSMB to officially open cohort B, will include patient's data up to 7 days after the 2 hour WBHT treatment. As soon as 3 patients are treated with 4 hours of WBHT in cohort A1, and after a positive DSMB advice, the enrolment in Cohort A2 can be initiated. Enrolment in Cohort C will start after completion of cohort B (3 patients received three 2-hour WBHT treatment), followed by a positive DSMB advice. Enrolment in Cohort D will start after completion of cohort C (3 patients received three 4-hour WBHT treatment, followed by a positive DSMB advice.

Optional	A patient may receive one additional WBHT treatment 8 weeks after the 3rd WBHT treatment at the discretion of the Investigator. The duration of this additional WBHT treatment will be the same as the duration of the 3rd treatment. For patients in cohort B, C and D, chemotherapy administration (regimen and timing) will be identical as during the third WBHT treatment. Medical information that may be relevant for the interpretation of the study data (tumor markers, CT imaging) performed as standard of care after the End of Study visit, may be collected.
Number of patients	Approximately 14 (up to 25) patients will be treated. In each cohort, 2 or 3 (up to 5) patients will be evaluated. Enrolment is defined as the day the patient signs the informed consent form (ICF).
Study duration	Upon signature of ICF and screening period (maximum 16 days), each eligible patient will participate for 15 days (or longer in case the WBHT treatments will be postponed) in the treatment period and for additional 10 weeks (after last treatment) in the safety follow up period. Total study duration for a patient including screening period will be minimal 14 weeks.
Eligibility – Inclusion Criteria	1. Patients between 18- and 75-years of age at time of signing the informed consent 2. Patients with advanced solid cancer (for cohort A1, A2 only) or metastatic pancreatic adenocarcinoma confirmed by histology (for cohort B/C/D only) 3. Patients previously treated or under treatment with standard of care treatment (cohort B/C/D only) or patients without treatment options 4. WHO performance status ≤ 1(see appendix V) 5. Maximum waist circumference ≤ 150 cm 6. Weight ≤ 100 kg 7. Height ≤ 1,90 m 8. Adequate liver structure (confirmed by CT scan) allowing the placement of the liver sensor 9. No (prostate) pathology that would interfere with the placement of the bladder catheter 10. Adequate bone marrow function defined as a. white blood cell count ≥ 2000/µl b. neutrophils ≥ 1500 cells/µL c. platelets ≥ 100 x 10^9/L

	d. hemoglobin ≥ 10 g/dl documented within 1 week prior to first treatment 11. Adequate coagulation defined as a. PT (%) ≥ 70% b. aPTT ≤ ULN c. Von Willebrand Factor Antigen ≥ LLN d. Von Willebrand Factor Activity ≥ LLN e. PFA COL/EPI CT ≤ 1.15 ULN f. PFA COL/ADP CT ≤ 1.15 ULN 12. Adequate liver function defined as a. Transaminases (AST, ALT) ≤ 2.5 x ULN or ≤ 5.0 in presence of liver metastasis b. bilirubin ≤ 2 x ULN documented 13. Adequate renal function defined as a. serum creatinine ≤ 1.6 mg/dL (male); ≤ 1.3 mg/dL (female); b. albumin ≥ 30g/L c. calculated eGFR ≥ 60 mL/min (CKD-EPI equation) documented within 1 week prior to randomization 14. No blood donation 3 months prior to the WBHT treatment 15. No participation in other clinical trial 4 weeks prior to the WBHT treatment 16. No biological therapy 4 weeks prior to the WBHT treatment or during WBHT treatment 17. No surgery 4 weeks prior to the WBHT treatment 18. No radiotherapy 3 weeks prior to the WBHT treatment or during WBHT treatment 19. No chemotherapy 1 week prior to the WBHT treatment (for cohort A and B/C/D) or during WBHT treatment (for Cohort A1, A2) 20. No anti-platelet aggregation medication intake from 5 days prior to the first WBHT treatment until 5 days after the last treatment 21. No anticoagulant medication intake between screening and last follow-up visit. However, if deemed necessary by the investigator, the patient may receive prophylactic Low Molecular Weight Heparin on the day prior to the first WBHT treatment until 10 days after the last WBHT treatment 22. No transdermal patches during participation in the study 23. No piercings (internally or externally) during WBHT treatment

	24. Life expectancy of at least 18 weeks 25. Effective contraception for both male and female patients if applicable. Women of childbearing potential must have negative blood pregnancy test at screening visit. 26. Written informed consent must be given according to good clinical practice and national/local regulations.
Eligibility- Exclusion criteria	1. Pregnant or breastfeeding women (based on HCG levels) 2. Presence of brain metastasis (known or suspected) 3. Other malignant diseases in the medical history during the last 5 years (exceptions: carcinoma in situ of the cervix or adequately treated basal cell carcinoma of the skin) 4. Serious medical risk factors involving any of the major organ systems, including high cardiovascular risk, coronary stenting or myocardial infarction in the last year 5. Clinically significant pulmonary disease which might interfere with mechanical ventilation 6. History of autonomic dysfunction (due to the influence on skin blood flow) 7. History of malignant hyperthermia or a positive diagnostic test (Caffeine-Halothane Contracture test) in case of family history of malignant hyperthermia. 8. History of untreated endocrine pathology (e.g. diabetes type II, hyper- or hypothyroidism). 9. Primary diabetes type I (due to vascular complications) 10. Known allergies to drugs that will be used during the trial (e.g. anesthetic, analgesic, (chemotherapy used in cohort B/C/D)) 11. Active infections not controlled by medication 12. Severe, non-healing wounds, ulcers or bone fractures 13. Organ allografts requiring immunosuppressive therapy 14. (History of) clinically significant (investigator decision) psychiatric disorder and/or psychosocial disorder that may interfere with adequate compliance to the protocol or signature of the informed consent

	15. Other clinically significant disease which could impair the patient's ability to participate in the study according to the investigator's opinion 16. Participation in another clinical trial during this trial
Primary Endpoint	Up to 4 weeks after last treatment (Follow up visit 4) • Incidence of adverse device events (ADEs) in relation to the medical device • Incidence of adverse events (AEs) related to WBHT treatment alone or in combination with SOC chemotherapy according to the NCCN guidelines. • Incidence of related clinically significant abnormalities on electrocardiogram (ECG), vital signs, physical examination and laboratory parameters
Secondary Endpoint	Up to 10 weeks after last treatment (End of study visit) • The evolution of clinically significant biological parameters (e.g. tumor specific markers) will be measured • Estimation will be performed of the recommended phase II dose of the combination therapy, guided by safety data. The recommended dose will be based on the anesthesiology procedure during the treatment and reported adverse events • Quality of Life assessment (EORTC-QLQ-C30- version 3)
Clinical endpoint	Up to 10 weeks after last treatment, efficacy parameters measured as per standard of care: • Tumor measurement – CT scan pre- and post WBHT treatment according to RECIST criteria defined as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) and not assessable (NA) • Progression free survival • Overall survival • Quality of Life assessment (EORTC-QLQ-C30- version 3)
Study Visits	See appendix I
Study Assessments	See appendix III

1 Introduction, background and rationale

1.1 Introduction pancreatic cancer

Pancreatic adenocarcinoma is an intractable malignancy and is the seventh leading cause of global cancer deaths in industrialized countries (1) and the third most common in the USA.(2) Based on GLOBOCAN 2018 estimates, pancreatic adenocarcinoma has ranked the 11th most common cancer in the world counting 458,918 new cases and causing 432,242 deaths (4.5% of all deaths caused by cancer) in 2018.(1) Worldwide incidence and mortality of pancreatic cancer correlate with increasing age and is slightly more common in men than in women.(1) To date, the causes of pancreatic carcinoma are still insufficiently known, although certain risk factors have been identified, such as tobacco smoking, diabetes mellitus, obesity, dietary factors, alcohol abuse, age, ethnicity, family history and genetic factors, *Helicobacter pylori* infection, non-O blood group and chronic pancreatitis. Because patients seldom exhibit symptoms until an advanced stage of the disease, pancreatic cancer remains one of the most lethal malignant neoplasms.(3) Upon progression of the tumor, it manifests as a gradual onset of non-specific symptoms including jaundice, weight loss, light-colored stools, abdominal pain and fatigue.(4)

Despite advancement in the knowledge of potential risk factors that cause pancreatic cancer and newly available tools for early diagnosis, its incidence is estimated to increase and will include 355,317 new cases by 2040. Pancreatic cancer is mainly divided into two types of pancreatic cancer: pancreatic adenocarcinoma (PDAC), which is the most common (85% of cases) arising in exocrine glands of the pancreas, and pancreatic neuroendocrine tumor (PanNET), which is less common (less than 5%) and occurs in the endocrine tissue of the pancreas.(5) Despite advancements in the detection and management of pancreatic cancer, the patients have a very poor prognosis, typically after diagnosis, only 24% of people survive 1 year, and 9% live for 5 years.(6)

Accurate staging and prognostic information are essential for treatment planning in pancreatic adenocarcinoma. Current methods include endoscopic ultrasound, CT scanning, and serum CA 19–9 levels.

Based on the clinical stage of the tumor, pancreatic adenocarcinoma is classified into four types: I (no spread or resectable), the cancer is limited to the pancreas and has grown 2 cm (IA) or greater than 2 cm but less than 4 cm (IB); II (local spread or borderline resectable), the cancer is > 4 cm and is limited to the pancreas, or there is spread locally to the nearby lymph nodes; III (wider spread or unresectable), cancer may have expanded to the nearby blood vessels or nerves, but has not metastasized to distant sites; IV (metastatic), cancer has spread to distant organs. The tumor can also be classified according to the TNM (8th edition). Stage IV cancer corresponds to TxNxM1.

Surgery, chemotherapy and radiotherapy are traditionally used to extend survival and/or relieve the patient's symptoms.

The chemotherapy regimen, or schedule, usually consists of a specific number of cycles given over a set period of time (with a rest period in between cycles). The following drugs are approved by the US Food and Drug Administration (FDA) for pancreatic

cancer: capecitabine, fluorouracil (5-FU), leucovorin, gemcitabine, nab-paclitaxel, oxaliplatin, irinotecan, erlotinib.
The choice of which combination to use varies, depending on the cancer location and often depends on the oncologist's experience with the drugs, as well as the different side effects. For pancreatic cancer, chemotherapy may be described by when and how it is given:

First-line chemotherapy: This is the first treatment used for people with locally advanced solid or metastatic pancreatic cancer.

Second-line treatment: When treatment does not work or stops working to control cancer growth, the tumor is called resistant. Sometimes, first-line treatment does not work at all (primary resistance), or the treatment worked well for a while and then stops being effective later (secondary or acquired resistance). In certain situation, the patients may benefit from additional treatment (second-line treatment) with different drugs in case the patient's overall health is good.
Despite the current methods of diagnosis and treatments, for advanced-stage cancer cases, there is still no definite cure and many patients die from the metastasis.(7)

1.2 Introduction advanced solid cancer

A solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumor may be benign (no cancer) or malignant (cancer). Examples of solid tumors are sarcomas, carcinomas and lymphomas. Leukemias (cancers of the blood) generally do not form solid tumors.

Solid tumors include cancers of the colon, ovary, breast, brain and other tissues. Colorectal cancer is introduced below as one of the most common types of solid tumors.

1.2.1 Colorectal cancer (CRC)

Colorectal cancer is the world's fourth most deadly cancer with almost 900,000 deaths annually. CRC accounts for approximately 10% of all annually diagnosed cancers and cancer-related deaths worldwide. It is second most common cancer diagnosed in women and third most in men. (1) The incidence of colorectal cancer worldwide is predicted to increase to 2.5 million new cases in 2035.(1,8)

Several environmental lifestyle factors increase colorectal cancer risk, such as bodyweight, smoking, excessive alcohol intake and red/processed meat intake.(9) Although type 2 diabetes and colorectal cancer share some of the same risk factors, such as physical inactivity and obesity, patients with type 2 diabetes mellitus maintain their increased risk after correcting for these factors.(10)

The pathogenesis of colorectal cancer arises from a polyp. This process begins with an aberrant crypt, which evolves into a neoplastic precursor lesion and eventually progresses to colorectal cancer over a period of 10-15 years. The majority of the colorectal cancers is assumed to be a stem cell or stem-cell-like cell. This profound cell type is the result of progressive accumulation of genetic and epigenetic alterations that

inactivate tumor-suppressor genes and activate oncogenes. The stem cells reside in the base of the colonic crypts and are absolutely essential for the initiation and maintenance of a tumor. In general, there are two major distinct precursor lesion pathways: the traditional adenoma-carcinoma pathway (referred to as the chromosomal instability sequence) leading to 70-90% of the colorectal cancers and the neoplasia pathway (involved in 10-20% of the colorectal cancers).(11,12)

The clinical symptoms of CRC diagnosed patients is a combination of rectal bleeding, change in bowel habits, abdominal pain and anemia.(13)

For diagnosing colorectal cancer, colonoscopy is the method of choice. In addition, CT colonography is used as a complementary imaging method. Distant staging of liver and lungs is done with CT, and an increasing role of MRI for further determination of liver lesions. In addition to the complete blood count, all international guidelines recommend checking carcinoembryonic antigen (CEA) concentrations at the time of diagnosis. An elevated CEA concentration is associated with worse prognosis, and CEA concentrations that do not normalize in the postoperative phase might indicate residual disease.(14,15)

The treatment management of colorectal cancer patients consists of endoscopic treatment (for resection of the malignant polyps) or surgical treatment (for resection of the colorectal carcinoma). Chemoradiotherapy is the most used therapy with a dose of 45-50 Gray in 25-28 fractions and with a fluoropyrimidine as a radiation sensitizer. Systemic therapy for metastatic colorectal cancer typically includes a chemotherapy backbone paired with a biological (anti-VEGF agents (such as bevacizumab), anti-EGFR drugs (such as cetuximab)).(16–18) Peritoneal metastases have long been considered as a untreatable condition. Hyperthermic intraperitoneal chemotherapy have resulted in improved survival in a subset of patients with limited disease .(19)

1.3 Correlative/biomarker study

Correlative studies, which are common secondary endpoints in (oncology) clinical trials, can be described as covering two broad and related aspects of clinical trial research: the evaluation of markers associated with (i) clinical activity and (ii) mechanism of action.

Well designed and performed early-stage correlative studies have the potential to strongly influence further clinical development of oncology clinical trials, and correlative data obtained from early stage trials has the potential to provide important guidance on the design and ultimate success of later stage trials.

As secondary endpoint for this study, known tumor markers (markers found in blood and specifically/a-specifically elevated by the presence of a particular tumor type) associated with the advanced solid cancer (to be defined according to the tumor type) or pancreatic cancer will be analyzed by the local laboratory as per standard of care.

In the future, preselection of patients will be based on predictive markers in order to predict whether a patient will respond to the therapy or not. In order to find biomarkers for hyperthermia treatment, patient blood samples will be collected, stored and further analyzed using next-generation sequencing methods.

The following parameters will be evaluated and are in our interest field from a clinical or scientific perspective;

CA19-9:
Serum CA19-9 is the most extensively studied, validated and clinically useful tumor marker for pancreatic cancer in terms of diagnosis, resectability, prognosis and recurrence. Unfortunately, CA19-9 serum level evaluation is limited by poor sensitivity of 81% and specificity of 82%.(20)

CEA:
Carcinoembryonic antigen (CEA) is a widely used tumor marker and is increased in 30%-60% of patients with pancreatic cancer. Beside the clinically validated tumor marker CA19-9, the diagnostic and prognostic value of CEA is gradually being recognized. One of the latest meta-analysis indicated a significantly higher diagnostic accuracy of CA19-9 and CEA combined when compared with CEA or CA19-9 alone. Moreover, there was also a significant association between high levels of CEA and worse overall survival. The elevated serum level of CEA, as a vital supplementary to CA19-9, can play an important role in the prediction of poor prognosis of pancreatic cancer patients. (21)

Survivin:
Survivin is an inhibitor of apoptosis which plays key roles in regulating cell division and inhibiting apoptosis by blocking caspase activation. Survivin is overexpressed in pancreatic ductal adenocarcinoma (PDAC). Aberrant survivin expression is associated with tumor cell proliferation, progression, angiogenesis and therapeutic resistance. Studies on the underlying molecular mechanisms indicate that survivin is involved in the regulation of cytokinesis and cell cycle progression, as well as its participation in a variety of signaling pathways such as the p53, Wnt, hypoxia, transforming growth factor and Notch signaling pathways. The aberrant expression of surviving in tumor cells is regulated by numerous factors including microRNAs (miRNAs) and receptor tyrosine kinases (RTKs), as well as in its downstream signaling cascades, such as phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt), mitogen-activated protein kinase kinase/mitogen-activated protein kinase (MEK/MAPK), mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) pathways (Figure 1).
The expression serum level of survivin in PDAC has its clinical significance as a prognostic factor.
High serum survivin levels are significantly associated with perineural invasion, venous invasion, lymph node status (N stage), cell differentiation and recurrence but not with age, gender of the patients or tumor location. A significant difference in overall survival

was shown to be associated with survivin expression. Detection of serum survivin expression may predict the prognosis of patients with PDAC. (22–24)

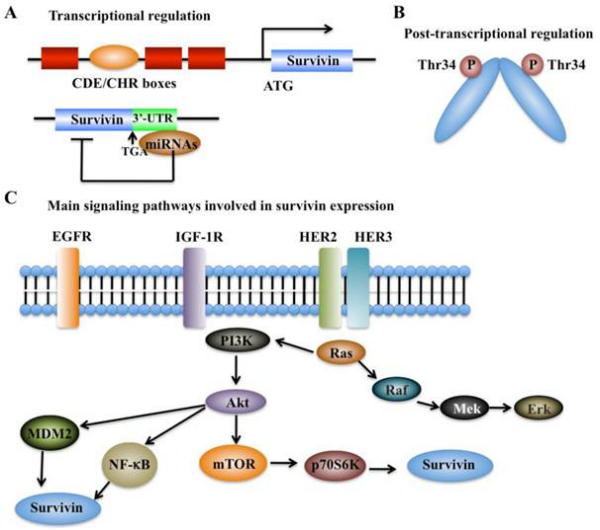


Figure1. Regulation of surviving expression (24)

IL-6, IL-8, IL-11:

Cytokines are key mediators of innate and adaptive immunity regulating a broad spectrum of processes including antigen presentation, cellular activation and bone marrow differentiation. Th1 response is mediated by proinflammatory cytokines, such as IL-6, IL-12, IFN-gamma and TNF-alpha and promotes cellular immune response against malignancy. Various cytokines produced in the tumor microenvironment by adaptive/innate immune cells and cancer cells, trigger Th2 response and promote the growth of cancer cells (Figure 2).

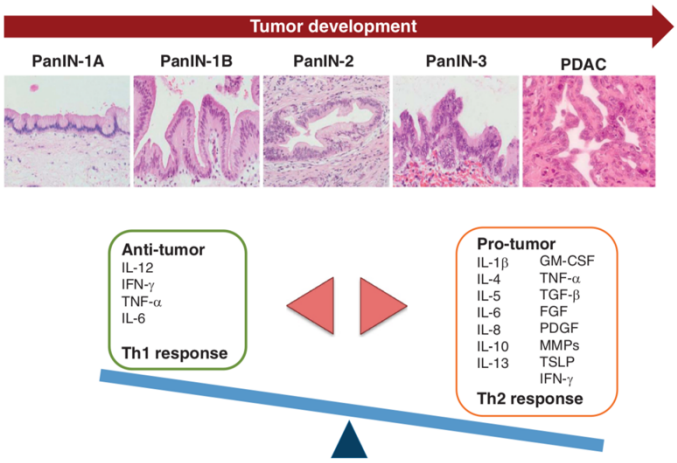


Figure 2. Cytokines involved in pancreatic tumorigenesis (25)

Several human case-control studies have linked high serum levels of interleukin (IL)-6 with pancreatic cancer. A strong positive correlation was found among cachexia, tumor stage and decreased survival. Multivariate analysis revealed that the elevated serum level of IL-6 was an independent risk factor for progression to extensive hepatic metastasis (odds ratio 1.928, 95% confidence interval 1.131-3.365, $P = 0.019$). In addition, serum IL-8 and IL-11 has been reported as a predictor for pancreatic cancer prognosis, increased tumor growth, angiogenesis and metastasis.

Importantly, most of the studies were able to justify the use of IL-6 as a prognostic factor for pancreatic cancer progression. (25–29)

VEGF-C:
For metastatic patients, the prognosis is often variable with limited treatment options. Vascular endothelial growth factor-C (VEGF-C) plays a crucial role in lymphangiogenesis and has been implicated in promoting lymph node metastasis in pancreatic adenocarcinoma. Serum VEGF-C levels appear useful in nodal staging and prognosis in pancreatic cancer. The finding that serum VEGF-C was elevated in N1 and M1 subjects, but not in N0 subjects, is consistent with a mechanism in which VEGF-C contributes to disease progression through increased metastasis. (30,31)

sCD40L:
CD40-CD40 ligand (CD40L) interaction is considered to contribute to the promotion of prothrombotic responses and production of angiogenesis-associated factor in addition to adaptive immune responses.
Serum soluble CD40L is clinically significant correlated with immunosuppression and angiogenesis in PDAC carcinogenesis/progression and is a promising prognostic biomarker for PDAC superior to CA19-9 and CEA. (32)

TGF- β1:
The transforming growth factor TGF-β signaling pathway is deregulated in many diseases, including cancer. In healthy cells and early-stage cancer cells, this pathway has tumor-suppressor functions, including cell-cycle arrest and apoptosis. However, its activation in late-stage cancer can promote tumorigenesis, including metastasis and chemoresistance.

Previous studies have demonstrated that increased TGF-β expression promotes disease progression in PDAC (33,34). Low circulating levels of TGF-β have been associated with prolonged survival in pancreatic cancer patients (35). Increased TβR1 and TβR2 expression has been observed in the majority of pancreatic cancer subtypes. Activation of TGF-β receptor signaling in PDAC cells resulted in increased Smad3 phosphorylation and nuclear translocation, leading to inhibition of cell growth. However, it also can result in activation of Smad7, which antagonizes Smad3, leading to activation of VEGF-A, vascularization and metastasis (Figure 3). (36–38)

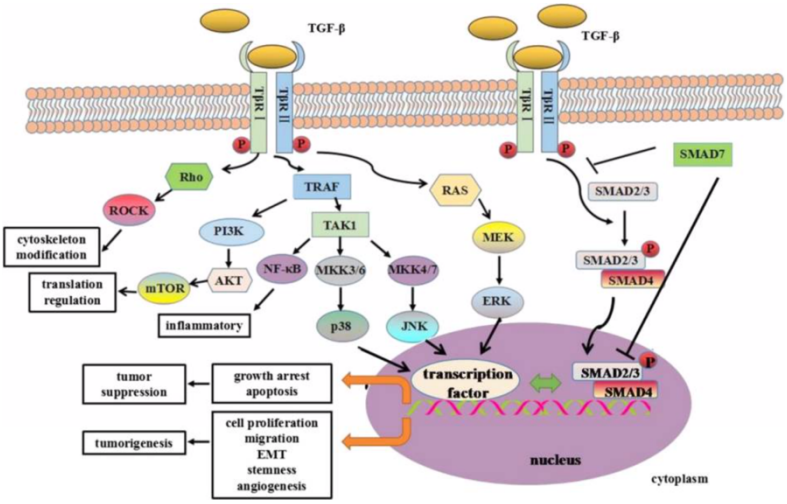


Figure 3. Schematic representation of TGF-β signaling as well as the role of TGF-β signaling pathway in cancer onset and progression (38)

DKK1:

Dkk1 (Dickkopf-related protein 1) is found increased in PDAC patients' specimens and serum. Dkk1 can be a promising biomarker specific to PDAC, which has the potential to increase PDAC survival rates through improving early stage detection and monitoring progression compared to current biomarker gold standards. In addition, recent studies suggest that Dkk1 could be an excellent target for cancer immunotherapy. Interestingly, Dkk1-CKAP4-PI3K/AKT signal pathway also plays role in pancreatic cancer cell proliferation. Recent studies show that Dkk1 is overexpressed in pancreatic cancer tissues and could be a potential biomarker for this disease, with a sensitivity of 89.3% and specificity 79.3% in PDAC. Dkk1 has been studied only in relation to the Wnt/β-catenin pathway and not the RANK/RANKL pathway in PDAC. (39)

Exosomes:

Exosomes facilitate cell-cell and cell-microenvironment communication and contain functional biomolecules such as DNA, RNAs, proteins, lipids and metabolites (Figure 4) and are discharged by all cells including tumor cells. The exosomes could be distinguished from apoptotic bodies and microvesicles by their heterogenous size, origin and composition. In comparison with the circulating tumor cells (CTC) and circulating tumor DNA (ctDNA), the exosomes are very stable over time. (40)

Pancreatic cancer is characterized by a dense stromal reaction, consisting of a desmoplastic reaction with the extracellular matrix, tumor cells, immune cells and cancer associated fibroblasts (CAF's). Exosomes released by pancreatic cancer cells enter the blood vessels and migrate preferentially to the liver (Figure 5). There, they are taken up by the Kupffer cells (macrophages), which react by increasing TGF- β signaling (Figure 6). Subsequently, the MIF (microphage inhibiting factor) is released from the exosomes through initiation of an immune-evasive response, resulting in the

creation of a metastatic niche for pancreatic cancer cells mediated by exosomes. This process is then followed by the establishment of liver metastases.

As shown in Figure 7, the exosomes contribute to several key processes in pancreatic cancer progression, including: metastasis promotion, evasion of tumor cells from the immune response, metabolism alteration, tumor-stromal cell communication and chemosensitivity reduction. Therefore, different exosomale-packed molecules, such as miRNA's (21-5P, 203, 212-3P, 451a,146a), transforming growth factor beta (TGFb), cancer associated fibroblasts (CAF), Snail (repressor of E-cadherin expression), microphage inhibiting factor (MIF), heat shock proteins (HSP27,70,90), Integrines (avb5, ITGB4) and pancreatic cancer-initiating cell markers (CD44v6, tetraspanin Tspan8, epithelial cell adhesion molecule EpCAm, CD104, microRNA (miRNA 4306,3976,4644,1246)) are planned to be analyzed by liquid biopsy. (40,41)

In particular, human urine has great potential as a non-invasive source of exosomes because it is easily accessible, can be obtained in large quantities, recurrently, and with minimal intervention.(42,43) Another advantage of urine is the reduced amount of non-exosome proteins that could complicate the isolation process. Thus, it has been reported that a sample of human urine contains about 3,400 different proteins distributed into exosomes and non-exosome polypeptides with little overlapping (44), making urine exosomes a very attractive source for biomarker discovery.

The mentioned biological parameters will be measured before during and after the treatment of the patients treated with whole-body hyperthermia alone (cohort A1, A2) or in combination with standard of care chemotherapy (cohort B/C/D).

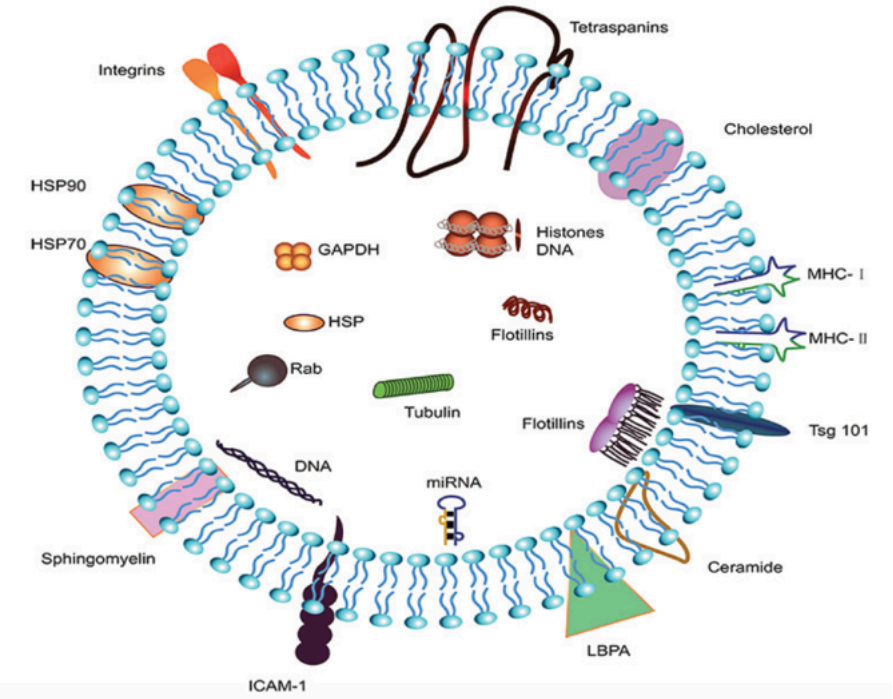


Figure 4. Components and structures of exosomes (40)

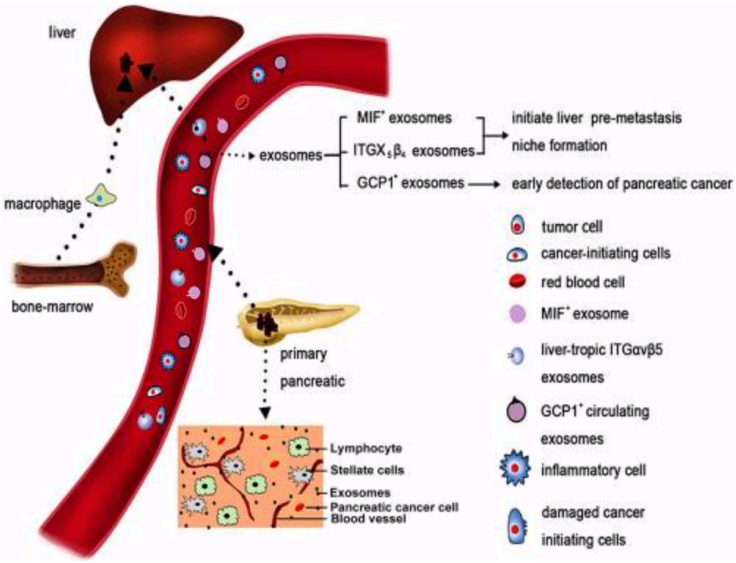


Figure 5. Pancreatic cancer, its microenvironment and metastatic route (40)

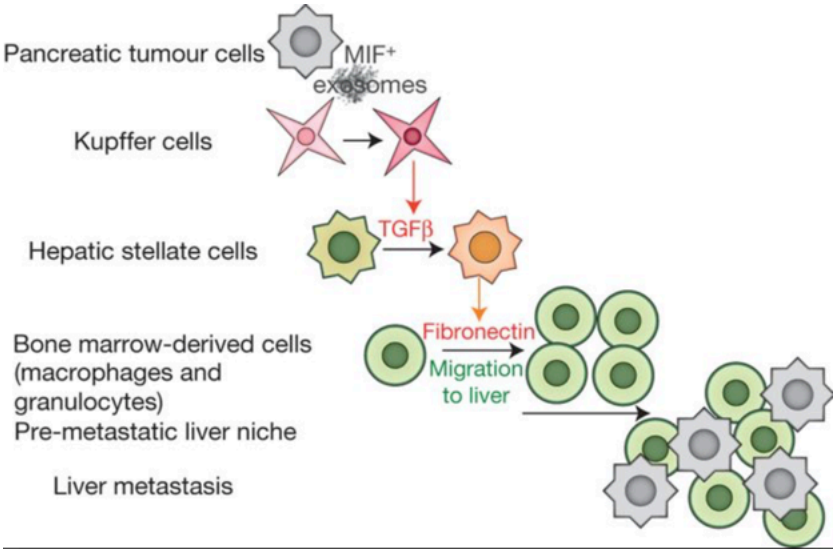


Figure 6. The hepatic pre-metastatic niche in pancreatic adenocarcinoma (45)

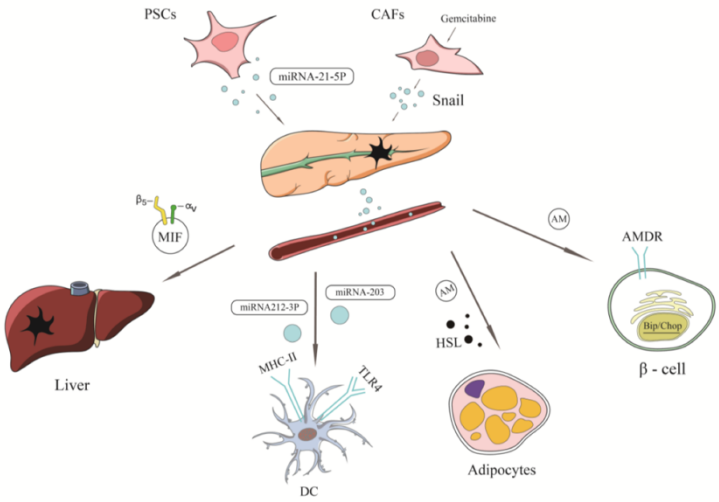


Figure 7. Sources and functions of exosomes in pancreatic cancer (41)

1.4 Introduction Whole-Body Hyperthermia (WBHT)

Hyperthermia, the procedure of raising the temperature of a part of the entire body above normal for a defined period of time, is applied alone or as an adjunctive treatment to various established cancer treatment modalities such as radiotherapy and chemotherapy. (46)
Clinical hyperthermia falls into three broad categories, namely, (1) localized hyperthermia, (2) regional hyperthermia, and (3) whole-body hyperthermia (WBHT).(47)
The term “hyperthermia” describes various applications of heat that may act in a synergistic way when applied synchronously to radio- and/or chemotherapy. (48)

Whole-Body Hyperthermia (WBHT), in contrast to local or regional hyperthermia, represents the only hyperthermia modality available for patients with disseminated malignancies. (49)
There is renewed growing interest in hyperthermia to become a potential treatment approach in cancer patients. (50)

1.4.1 Background Whole-Body Hyperthermia (WBHT)

Already in 1898, the Swedish gynecologist Westermark published a report on the use of long- term (48 hours) local (by virtue of intravaginal metal coil heated with circulated water to 42–44°C) and regional (hot tubs) heating for treatment of various gynecological diseases. Among others, he described several excellent results in inoperable cervix cancer. He was the first who had shown the ability of the long-term heating to destroy tumors without damaging healthy tissues.

In 1916, *Percy et al.* reported a 3–7 year survival of inoperable cancer of the uterus after local hyperthermia above 45°C and *Balfour et al*. confirmed these results. Attempts of hyperthermic radiosensitization started soon after the introduction and implementation of radiotherapy which lead to the development of radio-therm in 1931, the first hyperthermia device. (51)

In 1960, regional hyperthermia perfusion in combination with alkylating agents was performed in patients with head and neck tumors with 10% of complete response. (52)

From 1965 to 1975 hyperthermia experienced a considerable progress. Whole-body hyperthermia and regional hyperthermic perfusion technologies were developed. The first published paper of local and whole-body hyperthermia combination was published in 1977.
Ever since, whole-body hyperthermia has been induced in the patients by submersion in hot wax or liquid, encasement in a high-flow water perfusion suit, wrapping in plastic or by extracorporeal perfusion (53). However, whole-body hyperthermia has not been implemented in the clinics due to the uncontrolled way of intervention leading to a lack of safety.

1.4.2 Biological rationale

The biological rationale for the treatment of malignant disease by heat is driven by a number of reasons.

1. the survival of cells depends on the temperature and duration of heating in a predictable and repeatable way. (54)
2. the tumor cell environment (such as hypoxia, poor nutrition, and low pH) that negatively influences the tumor cell killing by ionizing radiation and some chemotherapy regimens, is beneficially influenced by heat therapy. (55)
3. the differential sensitivity of normal and tumor cells to heat is dependent on cell type and environmental conditions. (55)
4. heat treatment enhances the biological effect of both radiation and chemotherapy agents. (56,57)

The biological rationale is based on a direct cell-killing effect at temperatures in the range of 41– 42°C. (58)
However, the thermal-dose-response relation varies among cell types and depends on microenvironmental factors such as pH. (59)

The main molecular event underlying the biological effects of hyperthermia in the clinically relevant temperature range (39–45°C) is protein damage. The activation energies for protein denaturation and heat-induced cell death are within the same range. Cellular and tissue level studies, both *in vitro* and *in vivo*, indicate that tumor protein denaturation is the most likely thermal effect causing permanent irreversible damage whereas the healthy cells undergo reversible process of damage. (60)

Biophysical approaches as well as work with model proteins have directly shown that substantial protein denaturation occurs in a clinically still relevant temperature range. As a result of denaturation, proteins are prone to aggregation. Without chaperones, these aggregates can have destructive consequences for many macromolecular structures and their functions. (61)

The responses of tumors to hyperthermia involve both cellular and host-related factors. Frequently, it is not easy to separate these experimentally. (59)

When cells are exposed to elevated temperatures, they are inactivated in a time-and temperature-dependent fashion. Inactivation starts at about 40 to 41°C, at least, for murine cells and tumors. (62) At these low temperatures, cell inactivation continues for only a few hours; beyond that time, the surviving cells and healthy cells appear resistant to further exposure to such temperatures. (49)
Studies have shown that this is not a selection of heat-resistant subpopulations, but rather a consequence of the induction of a temporary resistance to heat. This transient phenomenon is referred to as thermo-tolerance. However, prolonged heating at mild temperatures (41– 42°C) overcomes this transient thermo-tolerance. (63)

1.4.3 General rationale

Whole-body hyperthermia, (to a limit of 42°C), is a distinctive and complex pathophysiological condition that has tremendous impact on tissue metabolism, blood flow, organ function, and tissue repair. (57)

WBHT has been investigated since the 1970s as an adjuvant to conventional chemo- or radio- therapy for the treatment of various malignant diseases. It is used to treat metastatic cancer. (64)
As mentioned earlier, WBHT can be combined with chemotherapy to increase tumor cell death without increasing bone marrow suppression. A newer approach is to increase the temperature to ~ 41.5°C for a longer period, which, in combination with cytokines and cytotoxic drugs, is expected to lead to a greater therapeutic index than WBHT at the maximum tolerated level. (65)

To ensure that the desired temperature is reached, but not exceeded, the temperature of the tumor and surrounding tissue is monitored throughout hyperthermia treatment. The tolerance of liver and brain tissue limits the maximum temperature for using WBHT to 41.5 – 41.8°C, but this temperature may be maintained for several hours. Heating can be accomplished with thermal conduction heat sources such as immersion in heated fluids, heated air, wrapping the patient in heated blankets, or using thermal chambers (similar to large incubators). (66,67)
However, these approaches of whole-body hyperthermia have not been implemented in the clinics due to lack of control and standardization, leading to unpredictable results and safety issues.

To ensure the safety of the patients, ElmediX has developed an investigational device including a sensor unit which controls the temperature at the most hyperthermia sensitive location (the liver).

1.5 The clinical significance of Whole-Body Hyperthermia in pancreatic cancer patients

The majority of the pancreatic cancer patients are diagnosed at late metastatic or advanced stages. However, only 10-15% of them are able to be resected surgically.(68) Majority of the patients are subjected to chemotherapy where gemcitabine is one of the baseline of treatment for more than a decade and still employed nowadays.(69) The standard of care chemotherapy combination of FOLFIRINOX, FOLFOX, gemcitabine/nab-paclitaxel or gemcitabine alone is able to improve the survival rates.(70) However, pancreatic adenocarcinoma has a weak response to all current treatment regimens and many clinical trials targeting specific molecular pathways failed to improve this situation.
The major failure of different clinical trials is the resistance to the therapies. This is due to the abundant and dense accumulation of non-tumor cells known as the stroma, which obstructs vascularization and the delivery of chemotherapeutics. In addition, it blocks the delivery of oxygen, causing hypoxia.

Mild hyperthermia, meaning raising the temperature of the tumor to approximately 40-44°C, for one hour, acts as a sensitizer for radiotherapy and chemotherapy. In this temperature range, improved drug delivery in the tumor enhances the effect of chemotherapy. When blood flow improves, hypoxia is decreased, which further enhances radiation effects through an increased production of oxygen radicals. In addition, DNA repair mechanisms are inhibited by hyperthermia, thus enhancing tumor cell killing.(71)

A systematic review of *van der Horst et al, 2018*, addressed clinical trials that used local or whole-body hyperthermia treatment (at variable temperatures) in pancreatic cancer patients. In those described trials, the weighted estimate of the treated population median overall survival was 11.7 compared to 5.6 for the control cohorts. However, only few control cohorts were included in these clinical trials. In addition, locoregional hyperthermia (42-44°C) clinical trials showed that the weighted estimate median overall survival of the treated population was 15 months compared to 9 months in control cohorts.(72)
The quality of the reviewed studies was limited in terms of temperature, duration and control. Therefore, future randomized controlled trials are needed to confirm the efficacy.

1.5.1 The clinical significance of hyperthermia in phase III trials

A number of phase III trials have been conducted involving the use of hyperthermia (HT) and radiotherapy (RT), HT and chemotherapy (CT), or trimodality therapy. These

trials have involved both superficial and the deep malignancies in various sites throughout the body and in patients in whom the treatment attempt was either curative or palliative. The reported trials have shown benefit to the addition of HT. The trials are summarized in Table 1 and 2.

Table 1. Selected phase III trials of CT with or without HT

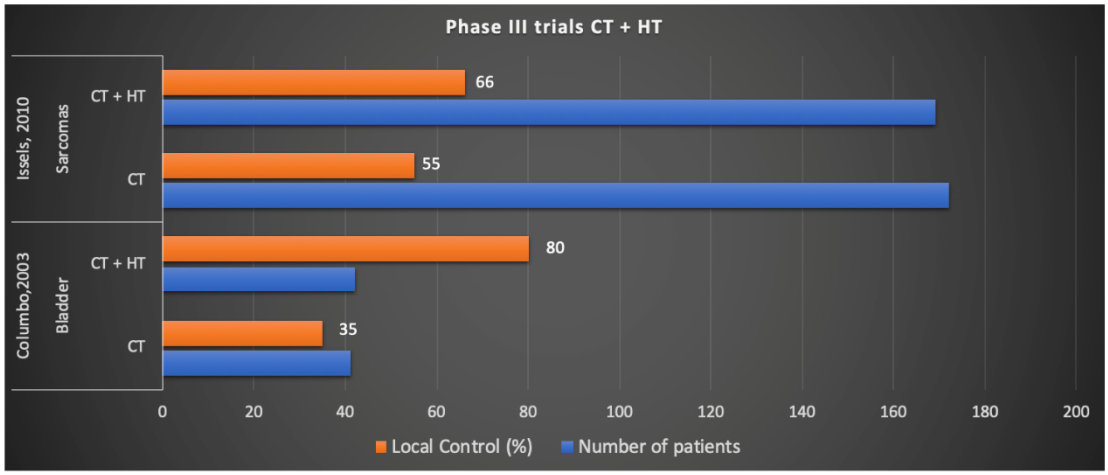
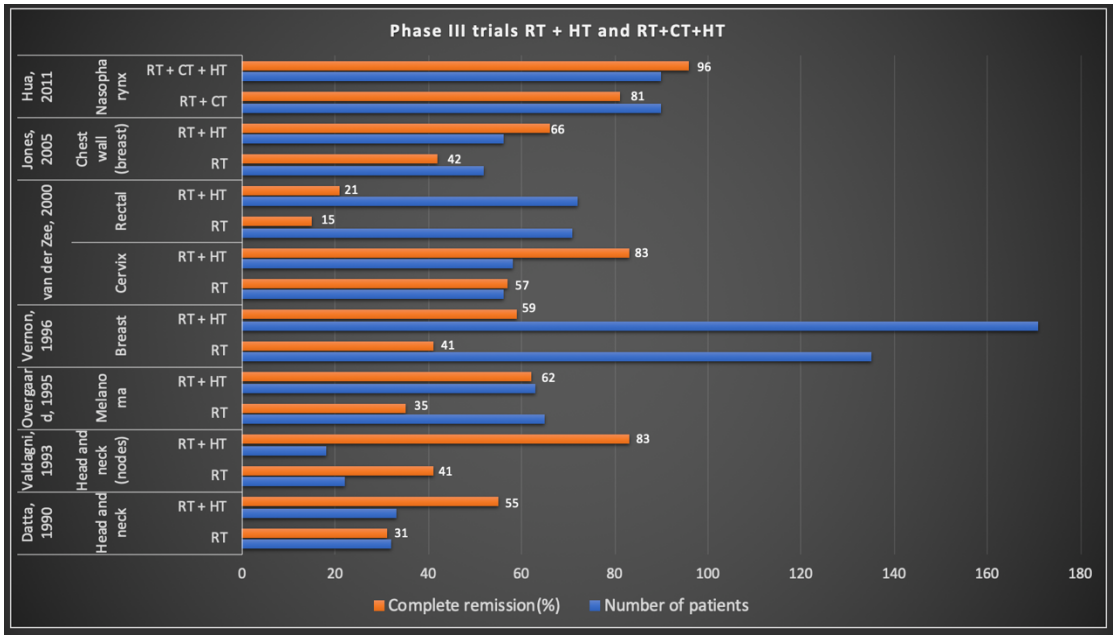


Table 2. Selected phase III trials of RT with or without HT



1.5.1.1 Soft tissue sarcoma

The use of CT and HT for soft tissue sarcomas was published by *Issels et al*.(73) These investigators, in a multi-institutional European and North American study, randomized 241 patients with high-risk soft tissue sarcomas to receive neoadjuvant CT alone (etoposide, ifosfamide, doxorubicin) or the same CT with deep HT. The primary endpoint was local progression-free survival. Treatment was given on days 1 and 4 of each CT cycle with a goal of achieving tumor temperatures of 42°C for 60 minutes. Four cycles of CT were planned, followed by definitive surgical resection when possible and typically followed by postoperative RT as well. Neither the surgery nor the RT was randomized but was given to all suitable patients. At 4 years, local progression-free survival was 66% in the CT+HT group, compared with 55% in those receiving CT alone. This difference was highly statistically significant.
This study provides high-quality evidence for the chemosensitization apparently achieved by locoregional HT.

1.5.1.2 Bladder carcinoma

Columbo et al. (74) investigated the use of intravesical CT alone compared with the same plus HT for bladder carcinoma. 83 patients were randomized to receive either mitomycin CT alone intravesically or the same plus HT. Each patient group, following complete transurethral resection of any bladder tumors, received 8 weekly sessions of intravesical therapy, followed by a maintenance regimen of four monthly sessions. The duration of each treatment was 60 minutes. HT was delivered via an intravesical applicator. The temperature goal was a median temperature of 42°C ± 2°C for at least 40 minutes per session. The primary endpoint was recurrence-free survival, which was 35% at 2 years in the CT-alone patients and 80% in the CT + HT patients, a highly statistically significant result.

1.5.1.3 Nasopharynx carcinoma

Hua et al. (75) randomized 180 patients with advanced nasopharynx cancer to receive HT + RT or RT alone. HT treatment was carried out with an intracavitary microwave applicator. The CR rate was 95.6% in the RT/HT group, compared with 81% in the RT-alone group, a highly statistically significant result. The RT dose was 70 Gy in 2-Gy fractions. HT treatments were once weekly for 30 minutes following RT. All patients received concurrent cisplatin/5-FU CT as well.

1.5.1.4 Breast cancer hyperthermia trials

Locally recurrent breast cancer on the chest wall is probably the primary clinical situation in which the use of HT has been investigated because of the frequency of this clinical scenario, as well as its superficial nature, which facilitates heating. Many phase II trials have been published, generally showing positive effect for the addition of HT to RT. Five separate phase III trials were combined for analysis in an international collaborative study by *Vernon et al*. (76) were randomized to either RT alone or RT combined with HT. Heating techniques differed somewhat among institutions. Nonetheless, significant improvement in the complete response rate of patients receiving HT + RT was demonstrated, with 59% complete response in the combined treatment group and 41% of those receiving RT alone. The differences were most pronounced in those patients who had been previously irradiated and were being re-treated.

A second important trial was carried out by *Jones et al*. (77), in which superficial tumors were randomly allocated to RT alone or RT+HT. One hundred eight patients were entered into the trial, approximately evenly divided between the two arms, with significant improvements in complete response rate.

1.5.1.5 Dutch deep hyperthermia trials (including Rectal cancer)

Among the more significant deep HT trials are those carried out by the Dutch group involving patients with cervical and rectal cancer. (78) In these trials, patients with previously untreated, locally advanced pelvic carcinomas were randomized to receive RT alone or RT+HT. HT treatments were generally given once weekly for a total of five treatments. For the rectal cancer group of patients, the CR rates were 21% and 15%, respectively, for the RT + HT group or RT-alone ($p > .001$). The observed benefits, however, were largely confined to patients with cervical carcinoma. The CR rate for this group was 83% with RT + HT compared with 57% for RT alone.

The Dutch trial is an important demonstration of the value of HT combined with RT for deep-seated malignancies.

1.5.1.6 Malignant melanoma trial

Overgaard and colleagues (79) randomized 129 melanoma superficial/skin lesions in 70 patients between RT alone and RT+HT. The heating was carried out with microwave applicators of 43°C for 60 minutes. CR rate was 35% for those treated with RT alone, compared with 62% for those treated with RT+HT. The differences were statistically significant.

1.5.1.7 Head and Neck carcinoma

Randomized trials investigating the efficacy of HT in head and neck carcinoma have been published over the last 20 years. The first, by *Datta et al*. (80) randomized 65 patients to receive RT alone or RT+HT for stage I-IV head and neck carcinoma of various primary sites. 52 of the 65 patients had stage III/IV disease. Although a benefit was seen for the addition of HT to RT, it was significant only in the stage III/IV patients, where the 2-year disease-free survival was 25% in the RT/HT group, compared with 8% in the RT-alone group. CR rate was 55% for those treated with RT+HT compared with 31% for patients treated with RT alone. The differences were statistically significant.

Another trial, guided by *Valdagni et al*. (81) evaluated the treatment of cervical lymph nodes in patients with locally advanced head and neck cancer. Patients were randomized to treatment with either RT alone or RT+HT. The CR rate was 83% for those treated with RT+HT compared with 41% for patients treated with RT alone. The differences were statistically significant. There was no enhancement of normal-tissue toxicity in the RT+HT group. Heat was delivered with an external microwave applicator.

1.6 Experience in preclinical and clinical model

ElmediX has developed a novel systemic treatment modality (TempoCure®), based on whole-body hyperthermia. This treatment can be used (neo)adjuvant to chemotherapeutics, radiation and immunotherapy. The device consists of different units (treatment, sensing, control and mechanical) responsible for the full operation during the treatment.

1.6.1 In-vitro experiments

Our very first results are based on *in-vitro* experiments. Different pancreatic cell lines have been tested at different temperatures. The best results are obtained when combining temperature with longer duration.

The range percentage of viability and proliferation cell index of different pancreatic cancer cell lines (BxPC-3, PanC-1, Capan-1, Capan-2 and MIA PaCa-2) is 50% at 41.5°C whereas the range percentage in healthy cell line (PWR-1E – addressed in orange) is 100% at 41.5°C (Figure 8).

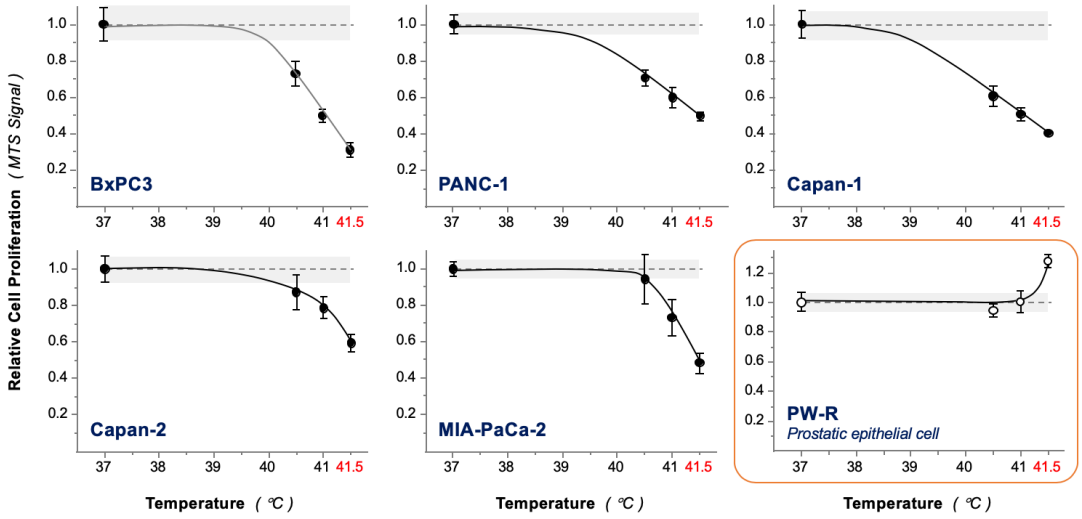


Figure 8. *In vitro* experiments

1.6.2 Minipig experiments

Objective
The only goal of our phase-I minipig study design was to prove the safety of whole-body hyperthermia (WBHT).

Method
Whole-body hyperthermia was applied in a specialized facility (Medanex BVBA, Diest, Belgium) on Aachener minipigs (approximately 25kg). The minipigs were subjected to single and repetitive hyperthermia, using the TempoCure®, generation II and III, at a temperature of 41.5°C (measured intrahepatically) and for a period of 6-8h. The experiments were done under full anesthesia. After the experiment, the minipigs were awake and in a veterinary facility for 2-3 weeks before necropsy.

Results
In total, 5 minipigs were subjected to whole-body hyperthermia. The first three minipigs were treated with a monotherapy at 41.5°C for 6-8h. A fourth minipig was sequentially treated with three sessions at 41.5°C for 8h with an interval of 6-8 days. The last minipig was a control/sham experiment where all the handling was done the same way as with the other minipigs, except the heating to 41.5 °C, where the animal stayed at 37°C during the total incubation.

During the experiment and the follow up, biochemical and hematological changes were detected. However, these changes were reversible and the animals were in good condition for 2-3 weeks, before necropsy. The analysis of the tissue did not show hyperthermia related changes.

1.6.3 Dog patient treatments

Objective
The first and most important goal of our phase-I veterinary (dog) clinical study was to prove the safety of whole-body hyperthermia (WBHT) treatment alone and in combination with standard of care therapy in dogs with cancer.

Method
Whole-body hyperthermia alone or whole-body hyperthermia combined with radiotherapy or chemotherapy were applied, using the TempoCure®, generation III and III+, at a temperature of 41.5°C and therefore, the dog patients (different breeds with different naturally occurring cancer types) were individually hospitalized in the small-animal department at the oncological center OncoVet in Lille, France. The size of the animal, the clinical condition (based on tumor type, imaging, blood results), the required monitoring and treatment determined the size of the loft in which each animal was hospitalized.
The procedure started with warming-up phase for approximately 2 hours followed by a plateau phase at 41.5°C for 8 hours. The procedure ended by the cooling-down phase for approximately 1 hour.
The duration of hospitalization was determined by the clinical condition of the patient animal. In general, dog patients were hospitalized for 3 days post treatment and 2 (to 3) WBHT treatments were performed per dog patient with an interval of 3 weeks (Figure 8).

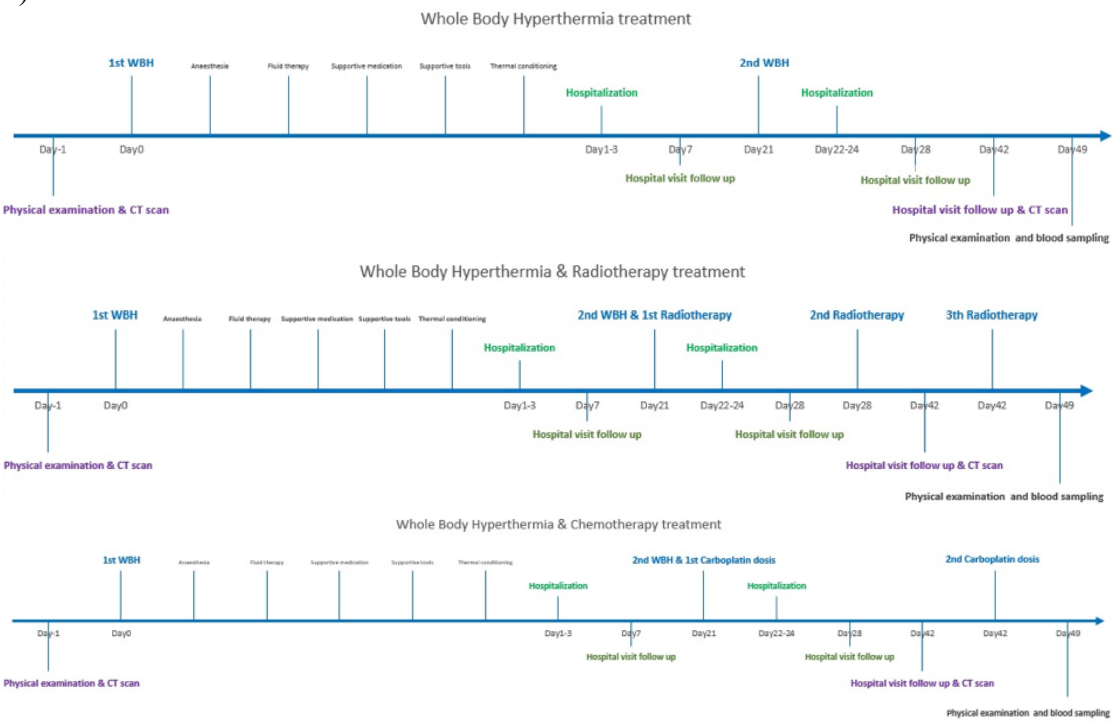


Figure 8. Design of the veterinary treatment strategies (whole-body hyperthermia, radiotherapy and chemotherapy)

Results
In total, six dogs were treated with whole-body hyperthermia. Three out of the six dog patients received additional radiotherapy and three dogs received additional chemotherapy (carboplatin).

In total, 14 sessions of whole-body hyperthermia were performed on the six dogs.
All the dogs treated with WBHT were anesthetized for a period of almost 12 hours per intervention. None of the patients showed anesthesia-related abnormalities. Additionally, the majority of the blood parameters (grouped as absolute leukocyte counts, blood values, platelet values, glucose & ionogram, multiple organs, proteins and coagulation) were stable. However, some of the coagulation values (APTT, PT, D-dimers) showed a disturbance while reaching the plateau phase of 41.5°C. Disturbance of these parameters was monitored during the entire treatment and in case needed, appropriate medication or fluid therapy was applied. The coagulation parameters stabilized after ending the treatment.

The patient's follow up after the treatment session was performed according to the standard of care protocol, and none of the dog patients showed abnormalities related to the whole-body hyperthermia treatment.

Remark:
The insertion of the liver sensor is a very essential and crucial step for temperature monitoring. During one treatment of a dog patient, the insertion of the liver sensor resulted in a liver rupture. The primary origin of this event was a combination of fibrotic liver of the patient and inappropriate insertion of the catheter needed for sensor placement.
The weight and height of the dog patient were also crucial. During two hyperthermia treatments, a dog with very thin skin (and even thinner fat layer) developed skin lesions, known as pressure points. These lesions were treated according to the clinician experience and the dog patient responded to the prescribed therapy.

Conclusion
According to the first results of our phase-I veterinary (dog) clinical study, the safety of whole-body hyperthermia is proven based on different breeds of dogs with different naturally occurring cancer types.

In addition, the dog treatment study gives us more insight on safety parameters to monitor and safety requirements to take as well as on improvements in device design.

2 Identification and description of the TEMPOCURE® investigational medical device

2.1 Summary

The HyperTherm will be trademarked under the name TempoCure®. The name HyperTherm is replaced by TempoCure® in new study related documents.
The HyperTemp sensor will be trademarked under the name TempoSensor®. The name HyperTemp is replaced by TempoSensor® in new study related documents.

The TempoCure® is a medical system based on whole-body hyperthermia technique. The operating principle of the TempoCure® is based on temperature conditioned air circulation. A control mechanism steers the temperature of the air (warmer or colder) in order to keep the patient at the desired treatment temperature during the full period of the treatment. The skin acts as heat exchanger between the temperature conditioned air and the patient body.
The patient's body temperature will be monitored by ElmediX temperature sensors. Invasive temperature monitoring will be performed in the liver. The intrahepatic sensor will be placed by a surgeon under ultrasound guidance. This sensor is at the most temperature sensitive organ and it is essential for the treatment. In addition, other (oesophageal, rectal and cutaneous) temperatures will be monitored. Optionally, additional sensors can be employed such as ear, gastric, duodenal, urinary bladder and one or more non-invasive, dermal or intradermal sensors.

The TempoCure® is intended for use on adult patients with cancer. The TempoCure® is a medical device/ system intended to administer whole-body Hyperthermia in patients, potentially in a (neo) adjuvant setting combined with chemotherapy and/or radiotherapy and/or immunotherapy for treatment of cancer.

The TempoCure® is intended to be used by physicians and medically qualified personnel in healthcare facilities. The product should only be used by physicians that have been adequately trained in this Hyperthermia technique.

The TempoCure® is intended to be used in the hospital or other appropriate clinical setting. The location of the unit within these facilities should be appropriate for the device, like an operating theatre, medium/intensive care unit or associated storage facilities. The TempoCure® system is mobile.

An ElmediX team member familiar with the device (e.g. a technical engineer, an TempoCure® operator) will be present during every treatment and will be responsible for the operation of the TempoCure® and for the real-time data capture.

An Investigator Brochure (IB) with technical information as well as Instructions For Use (IFU) for the TempoCure® are available.





Figure 9: TempoCure® Generation 5- Treatment unit

The TempoCure® is composed of four main units (with sub-systems- Figure 10):

- Treatment unit: this is the insulated cabinet in which the patient is placed on the stretcher (Figure 9).
- Sensing unit: this unit measures the temperature and/or heat flux of the patient who is placed in the treatment unit and serves as input module for the control unit.
- Control unit: the control unit acts upon the input from the sensing unit to steer the mechanical unit to bring and keep the patient at the treatment temperature and hold the treatment temperature during the treatment duration. After treatment, the control unit returns the patient in a controlled way back to normothermic state. The power supply is part of control unit.
- Mechanical unit: the mechanical unit consists of the ventilation fans (used to forcedly circulate warm air in the treatment unit) and active

heating/cooling elements. The operation of all parts of the mechanical unit is directly regulated by the control unit.

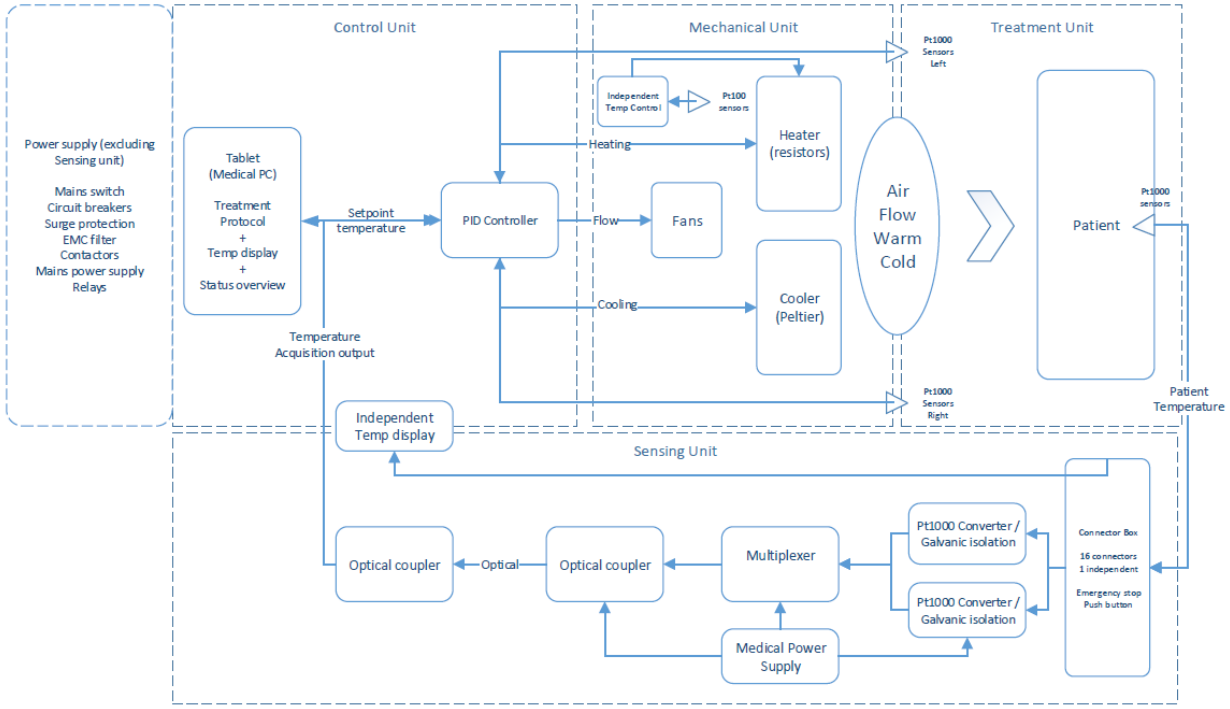


Figure 10: Sub-systems

2.2 The treatment of the patient

During the treatment of the patient, the following devices will be available (Figure 11):

- TempoCure® generation 5

  TempoCure® component: TempoSensor® in body temperature sensors

- Not part of the system:
  - Anesthesia equipment
  - Patient monitoring equipment;
    - ECG
    - SpO2
  - Continuous Intravenous infusion
    - NaCl 0,9 %

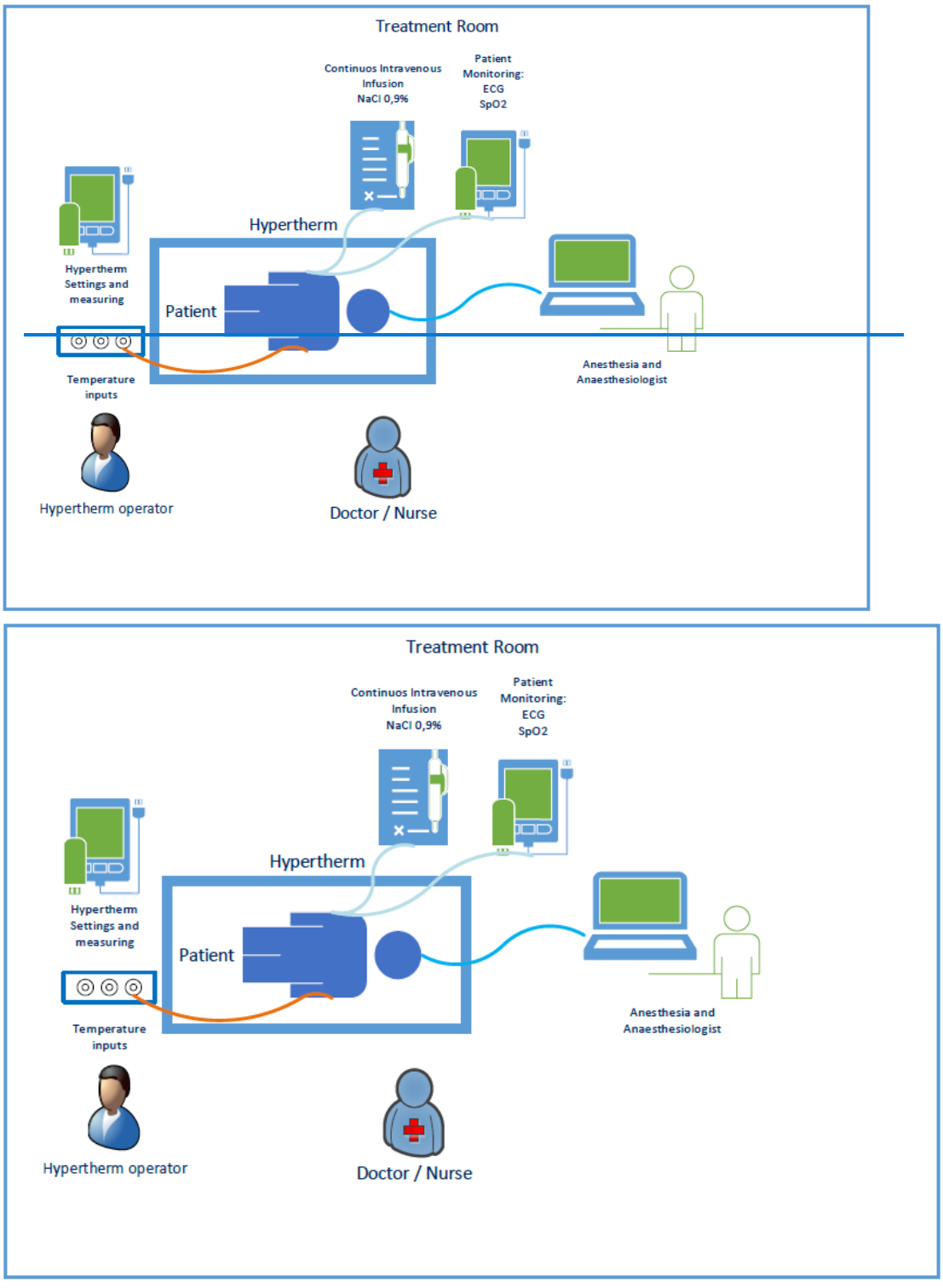


Figure 11: The treatment room overview

The general workflow for the TempoCure® consists of multiple steps which are shown in figure 12 below.

During the patient preparation phase, patient is placed on the specific stretcher. The patient is placed under deep anesthesia and, after recording his body weight, the temperature sensors are inserted in the body. The patient is finally transferred to the treatment chamber and coupled to the sensing unit. The patient is also connected to standard hospital equipment (e.g. for monitoring and infusion). Start of treatment is defined as the time the patient is under deep anesthesia and normothermic state. End of treatment is defined as the time the patient is under deep anesthesia and returned to normothermic state. Temperature sensors will be removed. The body weight is recorded in the same configuration as before the WBHT treatment.

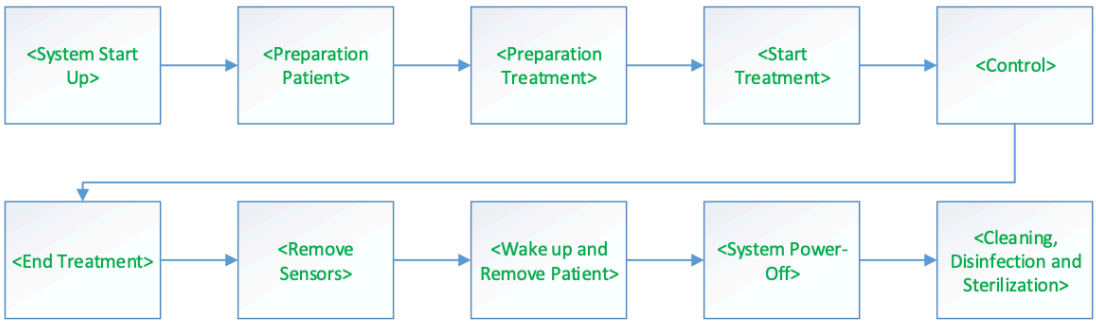


Figure 12: Workflow

2.3 TempoSensor® in body temperature sensor - Pt1000 Sensor

Pt1000 sensor "Pt" is the symbol for platinum, "1000" for the resistance in ohms at 0 °C (Figure 13).

The Pt1000-sensor is used for precise temperature monitoring applications, where errors in measurement have to be minimized. The linear relationship of the resistor to temperature, simplifies its use in many electronic applications.

The invasive patient temperature Pt1000 sensor (TempoSensor®) is part of the Sensing unit. The developed sensor is directly responsible for transducing the thermal changes of the patient into an electric signal. The signals recorded from multiple sensors are forwarded to an optical coupler to provide the required electrical insulation.

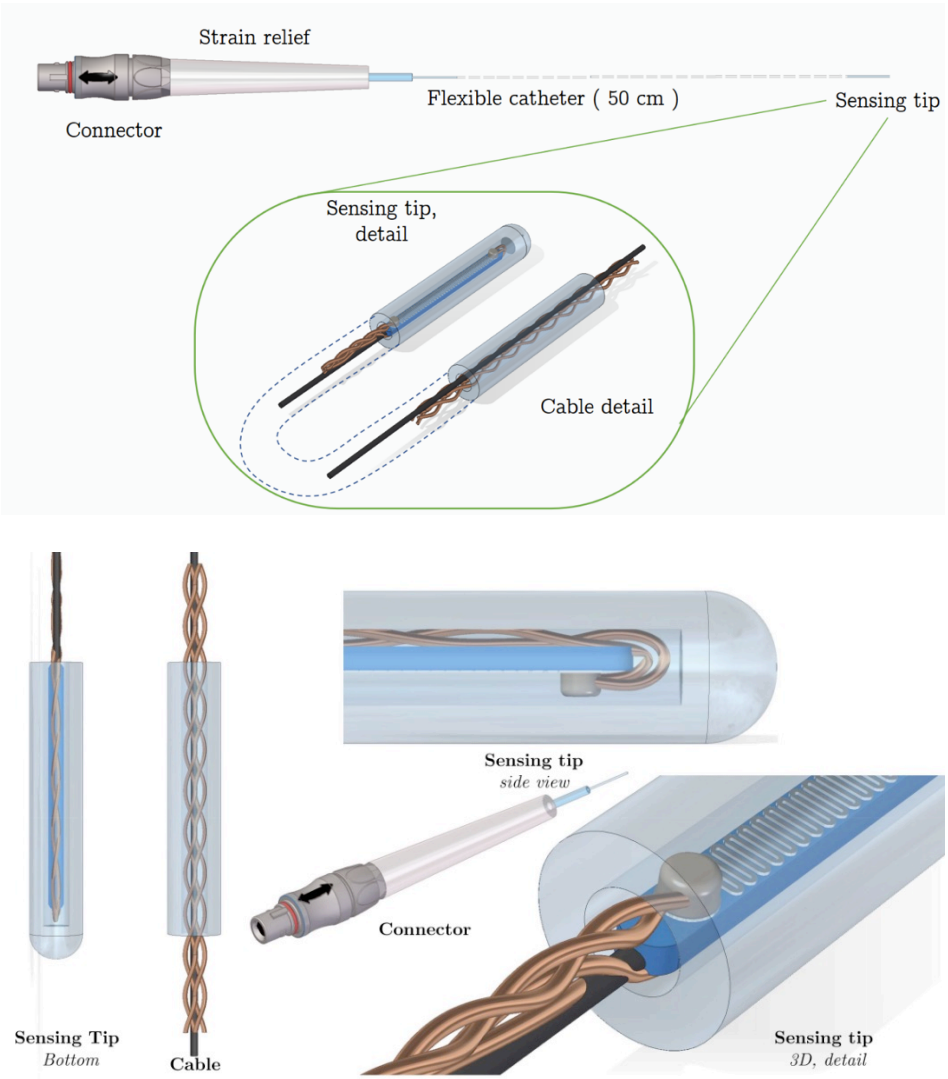


Figure 13: The temperature sensor, cable and connector overview

2.4 **Device traceability**

There is only one HyperTherm/TempoCure® generation 5 manufactured for use in the FIH-study.
TempoCure® shall comply with the requirements of applicable standards regarding labelling.

There will be identification labels on the TempoCure® as well as on the temperature sensors. Example of the labels are inserted in the IB.

For the identification of the investigation devices, the devices are labeled as "Exclusively for clinical investigations".

A Device Disposition Log (for HyperTherm/TempoCure® generation 5) and a Device Accountability Log (For HyperTemp/TempoSensor® sensors) will be used to track receipt, use and return of all investigational devices.

3 Clinical investigation design

3.1 Study design

The study is a first in-human clinical investigation in advanced solid cancer patients or pancreatic adenocarcinoma patients (TxNxM1). The justification of the design is based on evaluation of pre-clinical data and clinical evaluation of clinical data, safety and/or performance of similar devices/therapies. The study is a mono-centric, non-randomized trial in which the safety and preliminary efficacy of whole-body hyperthermia will be evidenced.

After a potential patient has been pre-screened (by review of medical records by site personnel) and patient satisfies the general inclusion/exclusion criteria, the investigator should obtain informed consent prior to initiating any non-standard of care procedure (i.e. study procedure). Further screening will take place with study-specific procedures.

The **screening period** will be limited in time (Day -16).

Whole-body hyperthermia will be applied in a specialized facility at the University Hospital Antwerp. The patients will be subjected to three treatment episodes of hyperthermia, using the TempoCure®, at a patient temperature of 41.5°C. A warming-up and a cool-down period (estimation time of 4 to 6 hours based on test models) needs to be added to the treatment duration.

Patients will be treated under the current protocol and closely monitored during the treatment period. A DSMB will be installed to assess safety of the patients during the study. After each study cohort (Figure 14), a risk assessment will take place by the DSMB before starting the next cohort, using the data up to 7 days after the 2-hour treatment (for cohort B), up to 7 days after the 4-hour treatment (for cohort A2) and up to Follow up visit 1 (for cohort C and D).

The hospitalization starts the day before and ends two days after the whole-body hyperthermia treatment.

Cohort A1

Three patients with advanced solid cancer (cohort A1) will be subjected to repetitive hyperthermia starting with 2 hours (day 1), 4 hours (day 8) and 6 hours (day 15) using the TempoCure® at a patient temperature of 41.5°C.

As this is a first-in-human trial, it is requested that the second patient of the first cohort should only start the first hyperthermia treatment when the first patient completed the three WBHT treatments. If the first patient is on hold due to unforeseen medical or other causes, the multidisciplinary team can decide to treat the next patient. If the delay is caused by safety related concerns rising from treatment of the previous patient, these must be addressed to the satisfaction of the multidisciplinary team first.

If a first patient is fully treated (3 WBHT treatments), the third patient can start up in the same week as the second patient if logistically possible for the site.

As soon as three patients received a 2 hour WBHT treatment in cohort A1, the DSMB will assess the safety of these patients treated in cohort A1. If the safety profile of these patients treated with 2 hours WBHT is acceptable, cohort B (pancreatic cancer patients only) will be opened for enrolment.

As soon as three patients received a 4 hour WBHT treatment in cohort A1, the DSMB will assess the safety of these patients treated in cohort A1. If the safety profile of these patients treated with 2 hours and 4 hours WBHT is acceptable, cohort A2 will be opened for enrolment.

Cohort A2

The first patient(s) included in this cohort will receive three WBHT treatments of 4 hours per treatment. As soon as one patient received three WBHT treatments of 4 hours per treatment, the next patient will receive three WBHT treatments of 6 hours per treatment.

Cohort B

Three pancreatic cancer patients (cohort B) will be subjected to three WBHT treatments (2 hours duration per treatment) alone (1st treatment) and in combination (2nd and 3rd treatment) with standard of care chemotherapy according to the NCCN guidelines. The choice of regimen is at the discretion of the PI. During the hospitalization for the 2nd treatment, the patient will receive 50% of the standard chemotherapy dosage. During the hospitalization for the 3rd treatment, the patient will receive 100% of the standard chemotherapy dosage.

It is requested that the second patient of a cohort should only start the first WBHT treatment when the first patient completed the three WBHT treatments.
If the first patient is on hold due to unforeseen medical or other causes, the multidisciplinary team can decide to treat the next patient. If the delay is caused by safety related concerns rising from treatment of the previous patient, these must be addressed to the satisfaction of the multidisciplinary team first.
If a first patient is fully treated (3 treatments), the third patient can start up in the same week as the second patient if logistically possible for the site.

After completion of cohort B and the positive DSMB assessment of the patient's safety profile of cohort B, cohort C will be opened for enrolment.

Cohort C
The set-up in Cohort C is identical to cohort B. However, the duration of each WBHT treatment will be 4 hours.

After completion of cohort C and the positive DSMB assessment of the patient's safety profile of cohort C, cohort D will be opened for enrolment.

Cohort D

The set-up in Cohort D is identical to cohort B and C. However, the duration of each WBHT treatment will be 6 hours.

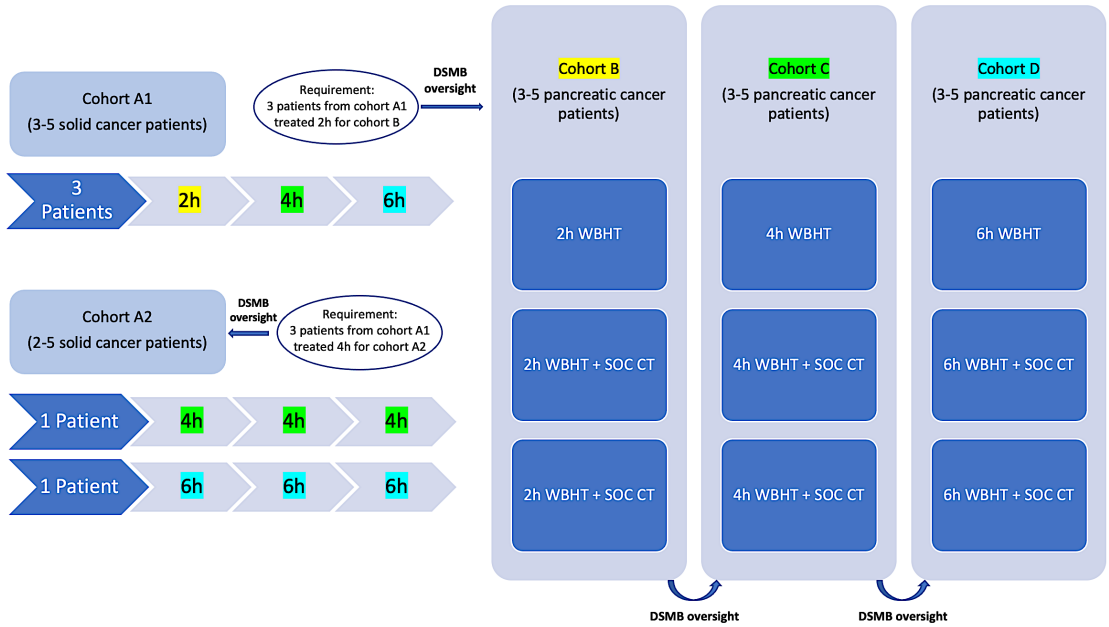


Figure 14: Overview of the different cohorts

The **treatment period** per patient will be 15 days

Patients will be followed for up to 10 weeks after last treatment (**follow up period**) to assess safety and pre-liminary efficacy.
Patients will be followed for up to 10 weeks after last treatment to assess Quality of Life using the EORCT QLQ-C30 (version 3).

3.2 Number of patients

Potential patients (upon pre-screening by site) will be further screened by site upon the completion of the informed consent process. Enrolment is defined as the day the patient signs the informed consent form (ICF). If patient has signed the ICF but is finally not eligible for the study, the patient will be entered on the enrollment log with reason for screen failure. Screen failures will be replaced by eligible patients for treatment.

Approximately 14 (up to 25) patients will be treated. Ideally, only 3 patients will be treated and evaluated in each cohort. The exception is cohort A2, ideally only 2 patients will be treated in this cohort.
In case a subject discontinues the study (see section 3.6), a new subject may be enrolled in the same cohort (up to 5) to obtain at least 3 patients (2 patients in cohort A2) who received 3 whole-body hyperthermia treatments per cohort. The data registered up to discontinuation will be part of the trial. A maximum of 25 patients will be treated.

3.3 Duration of the study

The **screening period** (after informed consent signature) should be limited in time (maximum 16 days (Day -16)).
The **treatment period** per patient is expected to be 15 days, based on 3 full whole-body hyperthermia treatments, and the **follow-up period** after last treatment is 10 weeks.

The total duration of the study for the individual patient is around 12 weeks (3-months) after the screening period or 14 weeks including the screening period. Quality of Life is assessed up to 10 weeks after the last hyperthermia treatment.

The average time to finish a cohort will be 10-14 weeks, including the review by the DSMB.

The goal is to enroll the first patient in Quarter 4 of 2020 (site is authorized for enrollment in October 2020) and to have all patients treated by Quarter 1 of 2023. Study close-out is estimated to take place in 2023.

3.4 Inclusion criteria

Patients must meet ALL of the inclusion criteria to be considered for the clinical investigation.

1. Patients between 18- and 75-years of age at time of signature of informed consent
2. Patients with advanced solid cancer (for cohort A1, A2) or metastatic pancreatic adenocarcinoma confirmed by histology (for cohort B/C/D only)
3. Patients previously treated (for cohort A1, A2) or under treatment with standard of care treatment (cohort B/C/D) or patients without treatment options
4. WHO performance status ≤ 1 (see appendix V)
5. Maximum waist circumference ≤ 150 cm
6. Weight ≤ 100 kg
7. Height ≤ 1,90 m
8. Adequate liver structure (confirmed by CT scan) allowing the placement of the liver sensor

9. No (prostate) pathology that would interfere with the placement of the bladder catheter
10. Adequate bone marrow function defined as
    a. white blood cell count ≥ 2000/µl
    b. neutrophils ≥ 1500 cells/µL
    c. platelets ≥ 100 x 10^9/L
    d. hemoglobin ≥ 10 g/dl documented within 1 week prior to first treatment.
11. Adequate coagulation defined as
    a. PT (%) ≥ 70%
    b. aPTT ≤ ULN
    c. Von Willebrand Factor Antigen ≥ LLN
    d. Von Willebrand Factor Activity ≥ LLN
    e. PFA COL/EPI CT ≤ 1.15 ULN
    f. PFA COL/ADP CT ≤ 1.15 ULN
12. Adequate liver function defined as
    a. Transaminases (AST, ALT) ≤ 2.5 x ULN or ≤ 5.0 in presence of liver metastasis
    b. bilirubin ≤ 2 x ULN documented
13. Adequate renal function defined as
    a. serum creatinine ≤ 1.6 mg/dL (male); ≤ 1.3 mg/dL (female);
    b. albumin ≥ 30g/L
    c. calculated eGFR ≥ 60 mL/min (CKD-EPI equation) documented within 1 week prior to randomization
14. No blood donation 3 months prior to the WBHT treatment
15. No participation in other clinical trial 4 weeks prior to the WBHT treatment
16. No biological therapy 4 weeks prior to the WBHT treatment or during WBHT treatment
17. No surgery 4 weeks prior to the WBHT treatment
18. No radiotherapy 3 weeks prior to the WBHT treatment or during WBHT treatment
19. No chemotherapy 1 week prior to the WBHT treatment (for cohort A and B/C/D) or during WBHT treatment (for cohort A1, A2)
20. No anti-platelet aggregation medication intake from 5 days prior to the first WBHT treatment until 5 days after the last treatment
21. No anticoagulant medication intake between screening and last follow-up visit. However, if deemed necessary by the investigator, the patient may receive prophylactic Low Molecular Weight Heparin on the day prior to the first WBHT treatment until 10 days after the last WBHT treatment.
22. No transdermal patches during participation in the study
23. No piercings (internally or externally) during WBHT treatment
24. Life expectancy of at least 18 weeks
25. Effective contraception for both male and female patients if applicable. Women of childbearing potential must have negative blood pregnancy test at screening visit.
26. Written informed consent must be given according to good clinical practice and national/local regulations.

3.5 **Exclusion criteria**

If ANY of the exclusion criteria are met, the patient is excluded from the clinical investigation and cannot be enrolled in this clinical trial or treated using the investigational device.

1. Pregnant or breastfeeding women (based on HCG levels)
2. Presence of brain metastasis (known or suspected)
3. Other malignant diseases in the medical history during the last 5 years (exceptions: carcinoma in situ of the cervix or adequately treated basal cell carcinoma of the skin)
4. Serious medical risk factors involving any of the major organ systems, including high cardiovascular risk, coronary stenting or myocardial infarction in the last year
5. Clinically significant pulmonary disease which might interfere with mechanical ventilation
6. History of autonomic dysfunction (due to the influence on skin blood flow)
7. History of malignant hyperthermia or a positive diagnostic test (Caffeine-Halothane Contracture test) in case of family history of malignant hyperthermia.
8. History of untreated endocrine pathology (e.g. diabetes type II, hyper - or hypothyroidism).
9. Primary diabetes type I (due to vascular complications)
10. Known allergies to drugs that will be used during the trial (e.g. anesthetic, analgesic, chemotherapy (used in cohort B/C/D))
11. Active infections not controlled by medication
12. Severe, non-healing wounds, ulcers or bone fractures
13. Organ allografts requiring immunosuppressive therapy
14. (History of) clinically significant (investigator decision) psychiatric disorder and/or psychosocial disorder that may interfere with adequate compliance to the protocol or signature of the informed consent
15. Other clinically significant disease which could impair the patient's ability to participate in the clinical trial according to the investigator's opinion
16. Participation in another clinical trial.

3.6 **Patient discontinuation**

Every patient should remain in the study until completion of the required follow up period. However, the patient's participation is voluntary, and the patient has the right to withdraw at any time without penalty or loss of benefit **(patient withdrawal).** In case patient withdraws the consent, the consent to process the health data is also

withdrawn and no new data will be collected by the sponsor. If the biological samples (e.g. blood samples) have already been used or analysed before the withdrawal of the patient's consent, the sponsor still has the right to use the results from those tests.

The investigator and/or anesthesiologist may terminate the patient's participation if the investigator/anesthesiologist estimates it is medically necessary **(investigator termination).**

The hyperthermia procedure will be stopped in the following cases:

- Therapy-resistant hemodynamic lability
- Non-compensable metabolic acidosis or lactate acidosis
- Clinically significant coagulopathy

In case a patient does not complete the scheduled follow up visits but has not officially withdrawn from the clinical investigations, the site should make reasonable effort to contact the patient (i.e. documented phone calls in the source documentation, copy of letter sent to patients home address). In case no contact is made (no response on phones and letter/email), patient should be recorded as **lost-to-follow-up.**

A study completion form must be completed.

- If patient is lost-to-follow-up
- If patient withdraws from the clinical investigation
- If investigator withdraws the patient from the clinical investigation

In addition

- If patient has died
- If patient has completed the clinical investigation.

If a patient misses a follow up visit, it will be considered as missed visit. Patient may then return for subsequent visits.

4 Visits, procedures and assessments

4.1 Study Visit Flow Chart – see appendix I

4.2 Study procedures – see appendix II

4.3 Detailed list of assessments per visit – see appendix III

4.3.1 Screening visit and patient informed consent

Potential patients will be pre-screened (by review of medical records) by principal investigator and study team.

After a potential patient satisfies the general inclusion/exclusion criteria, the investigator should obtain informed consent prior to initiating any non-standard of care procedure (i.e. study procedure). Further screening will take place with study-specific procedures. The **screening period** should be limited in time (maximum 16 days (Day -16)).
Clinical investigation-specific procedures or alteration of patient care cannot be started until a signed informed consent has been obtained. The site should provide insight in standard of care procedures in order to distinct standard of care and study-related procedures.

Screening visit data collection will include demographics, medical history (including history of cancer and available tumor markers CA19.9, CEA and CA125), 12-lead electrocardiogram (ECG), physical examination and vital signs. WHO performance status (see appendix V) will be assessed. Use of medication will be recorded including over the counter (OTC) medication. Potential allergies should be addressed. Current status of advanced solid cancer or pancreatic adenocarcinoma and metastases will be recorded including used/current therapies. Blood sampling will be done to assess different body functionalities (as per appendix III). For women of childbearing potential, a blood pregnancy test should be performed. The performance of these tests is mandatory.

A CT-scan (according to the standard of care protocol for tumor-type) within 28 days before start of treatment (day 1) is acceptable to be used as baseline CT-scan. If last CT-scan is not within this time window, a new CT-scan is requested.

A pretreatment electromyography (EMG) can be performed if deemed necessary by the Investigator. The rationale is to take a baseline measurement of (e.g.) the nervus peroneus and nervus tibialis. If peripheral neuropathy (e.g. drop foot) develops unexpectedly, a second EMG will be performed. This will give the PI an improved dataset to assess the pathogeneses.

For preparation of anesthesia, a preoperative cardiac and anesthetic assessment should be performed before the start of the hyperthermia treatment.
Rx of the thorax, dobutamine stress echocardiography and transthoracic ultrasound/echo (TTE) are mandatory tests to evaluate the lung-heart function.
Upon all screening tests, the patient's case may require discussion by the multidisciplinary team to decide if the patient is eligible. The multidisciplinary team might consist of the PI, co-investigator and the anesthesiologist.

4.3.2 Baseline visit- D-1, D7 and D14

One day before the first treatment, all inclusion and exclusion criteria need to be re-checked and confirmed to ensure the eligibility of the patient. The patient needs to be prepared for the treatment according to the standard hospital protocol.

Physical examination, weight measurement (hospital SOC protocol) and vital signs, WHO performance status, ECG and blood analysis for lab analysis as shown in the detailed list of assessments (appendix III) should be done. **Tumor markers**, specific for the tumor-type should be measured as per hospital protocol. Additionally, the following tumor markers should be analysed: CA19.9, CEA, CA125.

The patient should complete a cognitive test (test-specification to be decided by PI), be on fasten-diet (12h for food and 2h for liquids before the start of the treatment and according to the standard of care hospital protocol) in order to reduce the metabolic activity. In addition, the patient should receive an enema (Fleet or equivalent) in order to reduce the feces amount and minimize the potential local fermentation process. If deemed necessary by the investigator, the patient may receive prophylactic Low Molecular Weight Heparin on the day prior to the first WBHT treatment until 10 days after the last WBHT treatment.

Body lotion (or equivalent) containing aluminium salts should be applied at bedtime and the morning of the treatment to reduce sweating and limit the duration of the warming-up period.

Specific blood sampling should be performed (10 ml serum tube, 3 ml whole blood tempus tube) and aliquoted per 0.5 ml for further analysis by a central laboratory. Baseline urine sample (80ml) will also be collected for further analysis by a central laboratory.

4.3.3 WBHT-treatment on D1, D8 and D15

Appropriate measures should be taken to reduce the risk of decubitus during the procedure.
Anesthesia will be performed according to Standard of Care protocol (as summarized below) and at the discretion of the responsible anesthesiologist.
Note: the actual anesthesia performed may deviate from this protocol.

Two electrodes will be placed over the ulnar nerve at the wrist in order to monitor neuromuscular blockade at the adductor pollicis muscle (TOF Watch, SX NMTM, Organon Ltd, Drynam Road, Dublin, Ireland, or equivalent). The electrodes will be placed on the forehead and the NeuroSense monitor (NeuroWave Systems Inc., Cleveland Heights, OH, USA, or equivalent) will be applied in order to monitor the depth of anesthesia. Two cerebral tissue oximetry sensors will be placed at the forehead (ForeSight Elite, Edwards Lifesciences Corporation, Irvine CA, USA, or equivalent) in order to monitor the cerebral oxygenation.
An intravenous infusion will be sited in a peripheral vein and general anesthesia will be induced using fentanyl, propofol and rocuronium. Following deep neuromuscular blockade (NMB), the trachea will be intubated with a cuffed tube. A nasogastric tube will be inserted. Deep NMB will be maintained throughout the procedure using continuous rocuronium infusion adapted to the need of the patient to remain at PTC 1-2. During preparation for whole body hyperthermia, conventional volume-controlled ventilation (VCV) will be used (FiO2 0.4, PEEP +5 cm H2O, Vt 6-8 mL/kg) and respiratory rate (RF) of 12/min. VCV can be adjusted for normocapnia. Maintenance anesthesia will be provided with sevoflurane ~~(2%),~~* or propofol, fentanyl and rocuronium as needed to maintain WAVes between 40-~~-~~60.

*This study started with sevoflurane as anaesthetic agent for maintenance based on the assumption that this agent would cause the least metabolic activity in the liver and therefore would have the least impact on the overall thermodynamic behaviour of the patient.
However, sevoflurane has a number of drawbacks:
- Airway Irritation
- Potential for Delayed Emergence
- Risk of Malignant Hyperthermia
- Environmental Impact
- Postoperative Nausea and Vomiting (PONV) Incidence

Changes in best practices and recommendations could impact the future use of sevoflurane.

Therefore, after cohort B, the safety and thermodynamic behaviour of propofol as maintenance agent will be tested.
Propofol has a number of advantages over sevoflurane:
- Improved predictable Depth of Anesthesia
- Reduced Respiratory Irritation

- Less Postoperative Nausea and Vomiting (PONV)
- Lower Environmental Impact
- Much Reduced Risk of Malignant Hyperthermia

Each patient will undergo three investigational procedures with the following anesthetic maintenance agents:

Treatment 1: Propofol
Treatment 2: Sevoflurane
Treatment 3: Propofol

This approach allows the investigators to compare the safety of the two agents. Evaluation of safety will take place after each treatment and patient.
In the event of any unexpected safety issues or adverse events, the investigators have the authority to make necessary adjustments to the schedule above (order and frequency of the 2 agents). If required, the test may be discontinued, and patients will receive sevoflurane exclusively.

A radial arterial line will be placed for continuous arterial blood pressure. A FlowTrac arterial system interfaced with the HemoSphere monitor (Edwards Lifesciences, Irvine CA, USA, or equivalent) will be used to continuously monitor cardiac index (CI), cardiac output (CO), stroke volume variation (SVV) and systemic vascular resistance (SVR).

Optional, a pulmonary artery catheter (Swan Ganz, Edwards Lifesciences, Irvine CA, USA, or equivalent) enables the continuous assessment and determination of cardiac output (CO), central venous pressure (CVP), mixed venous oxygen saturation (SvO2), pulmonary artery pressure (PAP), oxygen delivery and oxygen consumption.

Rotational thromboelastometry (ROTEM®; TEM International GmbH, Munich, Germany, or equivalent) will be used for bedside diagnosis of fibrinolysis. Since untreated fibrinolysis can result in consumption of coagulation factors and bleeding, early diagnosis and decision making are desirable. Accordingly, ROTEM variables will be assessed from extrinsically activated assays with (APTEM) and without (EXTEM) addition of aprotinin, clotting time (CT), clot formation time (CFT), α-angle, maximum clot firmness at 10 min (A10), maximum clot firmness at 20 min (A20) for their ability to rapidly identify fibrinolysis during the WBHT treatment.

Bladder catheter and gastric catheter should be introduced after the induction of anesthesia.

All fluids administered to the patient should be pre-heated to ~~a temperature of 39 - 40°C to~~ prevent direct cooling of the patient. The AMPIR-01 fluid and blood warmer will be supplied by the sponsor for a test. The temperature range of this device is an improvement over the SOC device used at UZA. Use of the AMPIR-01 fluid and blood warmer may improve the temperature stability and thus safety of the patient. If the test

is successful, the device will be used in subsequent treatments. Note: the use is not mandatory.

The basic fluid maintenance will be controlled via central venous line at 1.5 ~~ml~~mL/kg/h Plasmalyte. The fluid will be raised with ~~7ml~~7 mL/h for each degree above 37~~-~~°C. The urine production and respiratory compensation will be considered.

Extra fluid administration will be performed via peripheral line ~~10ml~~10 mL/kg/h according to patient need.

Appropriate measures should be taken to reduce transpiration and the associated cooling effect on the patient.

Per-operative data acquisition will be organized by synchronizing the internal clocks of all monitoring devices before starting data collection.
iCollect software (version 5.0 GE Healthcare, WI, USA, or equivalent) will be used to continuously record data online from the Aisys Carestation anesthesia machine. Protocol specific events such as 'start hyperthermia', measurement 30', etc will be annotated in the trace to facilitate post hoc analysis. ECG, arterial pressure, PAP and capnograph waveforms will be sampled with 32-bit resolution at 300Hz, 100Hz, 100 Hz and 25 Hz respectively.
Cardiac index and cerebral saturation will be recorded on the HemoSphere monitor hard drive at 20 second intervals. Data files will be downloaded to a USB stick and opened in Microsoft Excel.
WAVes which denotes depth of anesthesia will be recorded continuously in the internal hard drive of the Neurowave. The mean right and left sided values will be recorded by downloading the data files to Microsoft Excel, via a USB stick.

The laptop time needs to be synchronized to the same time base as used for all other data collected in the same time frame. Similar synchronization procedure has to be used for all equipment, used to record data, printouts or electronic data.

Deep anesthesia is mandatory including the use of neuromuscular nondepolarizing agents, in order to suppress the autonomous reflexes or the contra-breathing process. Propofol-Target Controlled Infusion is not recommended considering the metabolic path through the liver. If possible, steroids should be avoided.
Patient hydration status (clinical and biochemical analysis (82)) should be balanced and well recorded (based on weight, urine production, sweating (observation), hemodynamic parameters and biochemical analysis). The weight of the patient should be measured at least before and after treatment.

Normotension should be maintained by vasopressors, inotropics and crystalloids/colloids. Normocapnia should be maintained by adaptation of the ventilation. Transpiration needs to be evaluated by observation or using automated measures.

As shown in the list of assessments,

- blood analysis (through arterial line), should be performed when the measured temperature in the intrahepatic sensor reaches 37°C, when it reaches 41,5°C, at the end of the plateau phase of 41,5°C and when it reaches 37°C.
- blood gases every 30min (± 5min) from the start of anesthesia till end of anesthesia
- thrombo-elastometry (TEM) when the measured temperature in the intrahepatic sensor reaches 37°C, when it reaches 41,5°C and during the plateau phase ((41,5°C) every 120min (± 15min.)) until the end of the plateau phase (41,5°C).

If necessary, the frequency of these exams can be increased up to the investigators/anesthesiologist's judgement. An example of anesthesia blood sampling is provided in appendix IV.
Urine (80 ml if possible) will be collected at the start of warming up, at start plateau phase and at end of plateau phase (i.e. start of cooling down).

Additional specific blood sampling (5ml of serum) should be performed at the end of the treatment plateau phase of 41.5°C.

The patient's body temperature will be monitored by ElmediX temperature sensors.
Invasive temperature monitoring will be performed in the liver. The intrahepatic sensor will be placed by a surgeon under ultrasound guidance. This sensor is at the most temperature sensitive organ and it is essential for the treatment. In addition, other (oesophageal, rectal and cutaneous) temperatures will be monitored. Optionally, additional sensors can be employed such as ear, gastric, duodenal, urinary bladder and one or more non-invasive, dermal or intradermal sensors.
In order to obtain a correct continuous monitoring of the intrahepatic temperature, a specific semi-flexible temperature probe with 0,6mm diameter should be placed intrahepatic under ultrasound guidance: the angle to puncture the liver capsule must not be too oblique, not under 45 degrees, in order to prevent laceration, tear or hemorrhage of the liver. In order to prevent this, the placement of the sensor will be performed under the guidance of an ultrasound used by a surgeon.
This sensor is at the most hyperthermia sensitive location and essential for the entire treatment process. The cutaneous sensor will be placed at the hypochondriac region. The oesophageal and rectal sensor will be placed under responsibility of the anesthesiologist and according to hospital standards.

In order to detect the catabolic phase (in which heat release occurs rather than heat absorption), a flux meter should be placed on the skin (nearby the anatomic location of the liver). The regulation of the body temperature will be guided by monitoring the intrahepatic sensor targeting a heating rate of 1°C/ 30 min. Slower or faster heating rates can be aimed for, based on the patient's real-time medical status. The catabolic phase will probably be reached at approximately 40.8 °C (cfr. the preclinical data of ElmediX) and measured using the intrahepatic temperature sensor. At reaching 40.8 °C,

the cabin temperature should be reduced (detailed information in the IB) in order to prevent temperature overshoot and to stabilize the intrahepatic temperature at reaching 41.5 °C.

Optional: the surgeon may intermittently perform measurements utilizing a doppler ultrasound to assess the dynamics of blood flow in the patient's liver during treatment.

For patients in Cohort B/C/D, chemotherapy will be used according to the NCCN guidelines and in accordance with the approved Summary of Product Characteristics. Three pancreatic cancer patients (per cohort) will be subjected to three WBHT treatments, alone (1st treatment) and in combination (2nd and 3rd treatment) with standard of care chemotherapy. The choice of regimen is at the discretion of the PI. During the hospitalization for the 2nd treatment, the patient will receive 50% of the standard chemotherapy dosage to evaluate the patient safety profile of WBHT and standard of care chemotherapy. During the hospitalization for the 3rd treatment, the patient will receive 100% of the standard chemotherapy dosage.

After ending the entire whole-body hyperthermia treatment, the patient's temperature will be returned to normothermia. The intrahepatic sensor and other sensors will be removed, and the anesthesia will be gradually terminated when patient reaches normothermia or at the discretion of the responsible anaesthesiologist.

Ongoing monitoring of the patients, during anesthesia, will be done and clinically significant abnormalities should be reported.

The hyperthermia treatment will be stopped in the following cases:

- Therapy-resistant hemodynamic lability
- Non-compensable metabolic acidosis or lactate acidosis
- Clinically significant coagulopathy

4.3.4 Hospitalization after treatment

The patient will be transferred to the intensive care unit (ICU) or the postoperative care unit (PACU). The patient will be followed up according to the PACU protocol and may be transferred to the ward, if stable, the day after the treatment (i.e. day 2, day 9 or day 16).
Before transfer to the ward, the patient should undergo blood analysis (see appendix III)
At the ward, the patient should be weighed on the same scale as during the baseline visit.
In total, the patient is observed for at least 2 days after the treatment (day 2 and 3, day 9 and 10, day 16 and 17). On day 2, 9 and 16, the patient should complete a cognitive test (test-specification to be decided by PI).

Urine collection (80ml) should be performed on each hospitalization day (i.e. day 2 and 3, day 9 and 10, day 16 and 17) for central lab analysis.
The patient can be discharged on day 3, day 10 or day 17 after blood analysis by the local lab and at the discretion of the investigator.
Additionally, blood sampling (10 ml serum tube) for central lab should be collected at day 3, 10 and 17.

4.3.5 Safety follow up before next treatment (D7, D14)

Before each individual patient receives the next hyperthermia treatment, a safety follow up will be done on day 7 and day 14 as listed in appendix III.

However, these timelines may vary when SOC chemotherapy is not administered every week.

As listed in appendix III, blood analysis, Rx of the thorax and transthoracic ultrasound/echo (TTE) are mandatory to evaluate the status of the patient.

If the safety follow-up of the patient is satisfying on day 7 (day 14), the patient will receive the next hyperthermia treatment on day 8 (day 15).

If the safety follow-up of the patient is not satisfying on day 7 (day 14), the multidisciplinary team will consult and decide if the patient may receive the hyperthermia treatment on day 8 (day 15). The multidisciplinary team may also decide to postpone the next hyperthermia treatment of the patient (see 4.3.9), or to stop the hyperthermia treatment(s) (see 4.3.10).

The following information will be collected at each of the time points:
- Any adverse events
- Use and changes in concomitant medication

4.3.6 Mandatory safety follow up (D22, D29, D36, D43)

An additional longer-term mandatory safety follow up will be done on day 22, day 29, day 36 and day 43 (+/- 2 days). Assessments are listed in appendix III and include a 12-lead ECG (if indicative) and local blood analysis. Specific blood sampling (10 ml serum tube, 3 ml whole blood tempus tube) and aliquoted per 0.5ml for further analysis by a central laboratory) should be performed during all the follow up visits. Urine collection (80ml) should be performed at each follow-up visit for central lab analysis.

During follow up visit 2 (D29), imaging is performed to evaluate the efficacy of the treatment. As imaging, CT scan should be performed according to tumor type.
The results of the imaging pre- and post-treatment should be analyzed by the site according to the latest RECIST criteria, to correlate duration of the hyperthermia and regression of the tumor. A separate guideline is drafted in collaboration with the radiologist.
The sponsor might request the CT images. The scans should be anonymised by the site before sending to the sponsor with respect to GDPR rules (e.g. pseudo anonymization (by use of patient number) is acceptable).

The following information will be collected at each of the time points:

- Any adverse events
- Use and changes in concomitant medication

4.3.7 End of study visit on D85

The end of study visit for the patient will take place around 10 weeks upon last treatment (day 85 (+/- 7 days). As imaging, CT scan should be performed according to tumor type (normally, this scan fits in SOC schedule). Last study assessments will be done as per appendix III.

~~Patients will be further followed up by site as per hospital protocol, but~~

4.3.8 Optional data ~~will not~~ collection

Medical information (like tumor markers and CT imaging) performed as standard of care after the End of Study visit, that may be relevant for the interpretation of the study data, may be ~~further~~ collected ~~by sponsor.~~ . This information will only be collected as long as they are relevant, more specific: as long as a new anticancer treatment has not been initiated after the EOS visit of the MATTERS study.

~~4.3.8~~4.3.9 Quality of Life Assessments

Quality of Life, using the EORTC QLQ-C30 (version 3), will be assessed at screening, on the day prior to each treatment, and at each scheduled and safety follow-up visit.

~~4.3.9~~4.3.10 Unscheduled visits

In case patient is seen at non-study visit intervals, the assessments performed are under the discretion of the investigator. Data should be entered in the EDC if relevant (i.e. update of event information).

~~4.3.10~~4.3.11 Patient on hold

If the hyperthermia treatment is postponed due to an unsatisfactory safety follow-up (see section 4.3.5), the patient will receive the next hyperthermia treatment(s) when the safety follow-up is satisfying. The patient will receive mandatory safety follow-up visits on day 7, day 14, day 21 and day 28 (+/- 2 days) after the last hyperthermia treatment. The hyperthermia treatment may take place at any time between day 7 and day 76 after the previous hyperthermia treatment.
The end of study visit will take place on day 70 (+/- 7 days) after the last hyperthermia treatment.

If the hyperthermia treatment is postponed for a patient, another patient may be enrolled to receive 3 consecutive weekly hyperthermia treatments. The patient on-hold may receive the next procedure after or parallel (only for the second and third patient of each cohort) with the other patient.

When the patient receives the postponed hyperthermia treatment, other treatments and assessments are rescheduled as per protocol based on the date of the last hyperthermia treatment.

~~4.3.11~~4.3.12 Early Termination

If the multidisciplinary team decides that the patient will not receive the second and/or third hyperthermia treatment, the patient will receive mandatory safety follow-up visits on day 7, day 14, day 21 and day 28 (+/- 2 days) after the last hyperthermia treatment.
The end of study visit will take place on day 70 (+/- 7 days) after the last hyperthermia treatment.

~~4.3.12~~4.3.13 OPTIONAL: Additional WBHT Treatment

A patient may receive one additional WBHT treatment 8 weeks after the 3rd WBHT treatment at the discretion of the Investigator.
The duration of the additional hyperthermia treatment will be the same as the duration of the 3rd WBHT treatment.
For patients in cohort B, C and D, chemotherapy administration (regimen and timing) will be identical as during the third WBHT treatment.

4 weekly Follow-up visits will be scheduled after the 4th hyperthermia treatment. The End of study visit will be scheduled 9 weeks after the last hyperthermia treatment.

4.4 Central Lab

Well designed and performed early-stage correlative studies have the potential to strongly influence further clinical development of oncology clinical trials, and correlative data obtained from early stage trials has the potential to provide important guidance on the design and ultimate success of later stage trials.

Blood samples will be collected for analysis of:

- Immunological panels (e.g. cytokines, chemokines)
- Exosome research
- RNA expression profiles

Urine will be collected for analysis of:

- Exosome research

The samples should be collected during the following timepoints: (See also appendix III).

	Tubes	Timepoint	Index in table
Immunological panels	5 ml of serum	D-1, D7, D14 D1, D8, D15 (end Plateau phase) D3, D10, D17 FU 1, 2, 3, 4 and End of study	x^I
Exosome research	5 ml of serum	D-1, D7, D14 D3, D10, D17 FU 1, 2, 3, 4 and End of study	x^E
	80 ml of urine (if possible)	D-1, D7, D14 D1, D8, D15 (start warming up, start plateau phase and end plateau phase) D2/3, D9/10, D16/17 FU 1, 2, 3, 4 and End of study	X^U
RNA expression profiles	3 ml of whole blood (tempus tube)	D-1, D7, D14 FU 1, 2, 3, 4 and End of study	x^R

Separate guidelines documents are drafted in collaboration with the Biobank UZA for details about lab kits (tubes), sampling, handling, storage and shipment (when central labs are defined).

Since scientific progress in this area is constant, the remainders of biological (blood) and urine samples might be stored for future research, not described above, if patient agreed in providing (optional) informed consent. The sponsor will use them for future research; to better understand the disease, its treatment and the responses to this treatment.

5 Objectives of the clinical investigation

When combined with drugs, the first step must evidently be to demonstrate safety of the WBHT. The toxicities associated with WBHT may be significant; therefore, careful patient selection and supportive care are essential. Sedation or general anesthesia must be used and continuous monitoring of vital signs, core body temperature, cardiac function (using an electrocardiogram (ECG)) and urine output is necessary.

5.1 Primary objective: Safety

The primary objective of this first in-human study is to assess the safety of different durations (2h, 4h and 6h) and repetitions (3 times, once a week on day 1, 8 and 15 respectively) of whole-body hyperthermia (WBHT) with the TempoCure®, alone or in combination with standard of care chemotherapy in patients with advanced solid cancer or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma.

AEs reported up to 4 weeks after last treatment will be assessed for seriousness, severity and relationship to the device and to WBHT treatment. The relationship will be assessed by the investigator and in addition by the DSMB.
The AEs will include but will not be limited to clinically significant changes in ECG, vital signs, physical examination and laboratory parameters.

Primary endpoint up to 4 weeks after last treatment (Follow up visit 4)

- Incidence of adverse events (ADEs) in relation to the medical device
- Incidence of adverse events (AEs) related to WBHT treatment alone or in combination with standard of care chemotherapy according to the NCCN guidelines
- Incidence of relevant clinically significant abnormalities on electrocardiogram (ECG), vital signs, physical examination and laboratory parameters

5.2 Secondary objective: Pre-liminary efficacy

The secondary objective is to evaluate pre-liminary efficacy parameters of different durations of whole-body hyperthermia with the TempoCure®, alone or in combination with SOC chemotherapy according to the NCCN guidelines, up to 10 weeks after last treatment in patients with advanced solid cancer or stage IV (TxNxM1) metastatic pancreatic adenocarcinoma.

Secondary endpoint up to 10 weeks after last treatment (End of study visit)

- The evolution of clinically significant biological parameters (e.g. tumor specific markers will be measured
- Estimation will be performed of the recommended phase II dose of the combination therapy, guided by safety data. The recommended dose will be based on the anesthesiology procedure during the treatment and reported adverse events.
- Quality of Life assessment

5.3 Clinical endpoint

Up to 10 weeks after last treatment, efficacy parameters measured as per standard of care:

- Tumor measurement – CT scan pre- and post WBHT treatment according to RECIST criteria defined as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) and not assessable (NA)
- Progression free survival
- Overall survival
- Quality of Life assessment

6 Risk and benefit analysis

6.1 Risk assessment of device and FIH investigation

Risk management is an integral part of the product lifecycle, including design, development, manufacturing, clinical evaluation and post market surveillance. Risk management for the TempoCure® is performed in accordance with ElmediX's Product Risk Management procedure conform EN ISO 14971:2012 and the Risk Management Plan. The Risk management process includes the individual device risks, as well as the overall procedure risks related to the combined functioning of the TempoCure® with other medical equipment.

Potential risks were identified, analyzed and evaluated. Risk control measures were implemented as appropriate. The impact of all the identified risks has been significantly reduced after implementation of appropriate risk control measures. For the remaining medium risks, an individual risk benefit analysis was executed on the basis of which it was concluded that the related benefits outweigh the risks. Therefore, no unacceptable risks remained.

In the literature, the following clinical hazards have been identified for whole-body hyperthermia treatment (83) (84)

- Death from cardiovascular causes (e.g. coagulation);
- Stroke;
- Kidney failure;
- Neurological damage

When applied under controlled circumstances, the risk of coagulation, stroke and kidney failure is limited (83). After WBHT in humans, at a maximum temperature between 40 and 43°C for 30 min – 6h, transient central nervous system (CNS) complications were reported. None of the experimental studies available in the literature give evidence of direct heat injury to the CNS after WBHT at uniform temperature. This may mean that the CNS is not an organ at specific risk in WBHT.

Most studies indicate that impairment of the CNS after WBHT was not due to direct heat injury to the brain or spinal cord but was secondary as a result of physiological changes. The limited clinical experience shows that safe hyperthermia treatment of tumors located close to the CNS seems is feasible under appropriate technical conditions with adequate thermometry and taking the sensitivity of the surrounding normal nervous tissue into account.(84)
According to the literature, the side-effects of radiant-heat WBHT, at temperatures above 41.5°C, are skin-burns, peripheral neuropathy, psychogenic disorders and arrhythmias. (85)
However, the hyperthermia treatment used for our clinical trial is based on air flow. Due to the thermal control (using specific sensors), the above addressed side effects are not expected.

In overall, preclinical whole-body hyperthermia for 8 hours in a dog-model has been proven safe (data on file at ElmediX).
In addition, clinical data showed that a therapy of whole-body hyperthermia at 41.8 degrees with gemcitabine and carboplatin was well tolerated leading mainly to the expected hematological side-effects due to chemotherapy. (86)

The main adverse events reported during whole-body hyperthermia of cancer patients was nausea, vomiting and myelosuppression, but these adverse events did not appear above what was already expected from chemotherapy alone. (87)

Tissue heterogeneity, perfusion and the presence of (large) blood vessels can greatly affect local temperatures. Therefore, it is paramount that the temperature is properly monitored.

6.2 Expected patient benefit

Adjunctive hyperthermia is an investigational treatment. Few clinical benefits have yet been established in patients with stage IV metastatic pancreatic carcinoma. The aim is to assess the potential role of whole-body hyperthermia adjunctive to standard of care chemotherapy according to the NCCN guidelines.

Potential patient benefits may be expected based on the following clinical results from literature:

1. Objective tumor responses were achieved in all studies, in often heavily pretreated patients; (88)
2. Tumor regression was observed after hyperthermia alone; (89) (90)
3. Complete responses when combined with cisplatin, in platinum resistant patients; (91)
4. In phase II studies a response rate of 12 to 89%; (48)
5. Colorectal cancer patients, who did not respond to initial chemotherapy, achieved a partial response of 27% after whole-body hyperthermia and chemotherapy. (92)
6. Local hyperthermia improves treatment outcome for pancreatic cancer patients. The overall response rate was 43.9% vs. 35.3% in the control group. (72,93)

In conclusion, the benefits outweigh the potential risks associated with WBHT treatment.

6.3 Anticipated Adverse Device Effects (AADE)

The Anticipated Adverse Device Effects (AADE) according to the ISO 14155 has been identified and are equivalent to the Harm/Effect according to the ISO 14971.
The categories of Adverse Device Effect (ADE) and Serious Adverse Device Effect (SADE) has been translated from the 5 levels of Harm (death, serious injury, moderate injury, minor injury, no injury).
This risk analysis shall also be used as a start for identifying anticipated adverse device effects characterized by their nature, incidence, severity and outcome.
For more details, we refer to Investigator Brochure section 23.5, table 29.

Adverse Events related to the WHBT seen in animal studies, performed by ElmediX are listed in CIP section 13.2. A Serious Adverse Event possibly associated with the sensor placement is laceration, tear or hemorrhage of the liver. In general, the events associated with hyperthermia are transient and not serious.

6.4 Risk and Benefit Conclusions

A risk/benefit analysis following EN ISO 14971, has been performed on all residual risks to control accumulated risks for the TempoCure® device. According to the risk/benefit analysis, it is concluded that the accumulated risk is acceptable.

Having considered all aspects of the design, manufacturing, logistics process and product characteristics, based on the preclinical investigation results (animal testing in mini-pigs and treatment in dogs) and on the basis of the above-mentioned considerations, ElmediX concludes, as the manufacturer of the TempoCure®, that the hyperthermia investigation medical device product is designed and manufactured in such a way that, under the conditions and for the purposes intended, they will not compromise the clinical condition and safety of the patient, and that any risk associated with their use constitutes an acceptable risk when weighed against the benefits to the patient.

7 Statistical considerations

The study is a first in-human exploratory study to establish the safety of whole-body hyperthermia treatment with the TempoCure® alone in patients with advanced solid cancer (cohort A1; A2) or TempoCure® adjunctive to SOC chemotherapy treatment in patients with stage IV (TxNxM1) metastatic pancreatic adenocarcinoma (cohort B/C/D).

The device will be used for the first time in humans, and this justifies an open study in a small number of patients closely monitored to investigate the safety of hyperthermia.

Hyperthermia will be first applied alone (in 3 to 5 patients) for only 2 hours of exposure to 41.5°C and if considered as safe by principal investigator/anesthesiologist/multi-disciplinary team, the patient will be exposed to 4 and 6 hours at 41.5°C (Cohort A1).

After each study cohort (of 2/3 to 5 patients), a risk assessment will take place by the DSMB before starting the next cohort.

In case a subject discontinues the study (see section 3.6), a new subject might be enrolled in the same cohort (up to 5) to obtain at least 3 full whole-body hyperthermia treatments per cohort.

The study in 14 to 25 patients is not powered for any statistical analysis. No formal hypothesis testing is planned. The analysis will be limited to descriptive statistics, taken into account the doses provided and the extra blood sampling taken at different time points. The results will be reported in the Clinical Investigation Report.

8 Data handling and record keeping

For the clinical investigation duration, the investigator will maintain complete and accurate documentation including but not limited to medical records, laboratory reports, signed informed consent forms, device accountability logs, correspondence with EC and sponsor, clinical investigation progress records, adverse events reports, information regarding patient discontinuation or completion of the clinical investigations.

The sponsor may transfer functions as Data Management activities/eCRF setup and monitoring activities to an external organization (CRO). The ultimate responsibility for the quality and integrity of the data shall reside with the sponsor. The sponsor shall be responsible for verifying the existence and adherence to written procedures at the external organization.

8.1 Source Documentation

Source documents shall be created and maintained by the investigation site team throughout the clinical investigation according to the protocol. The principal investigator or institution shall provide direct access to source data (including imaging data) during and after the clinical investigation for clinical investigation-related monitoring, audits, EC review and regulatory authority inspections. Investigator should maintain information in the patient's medical records, which confirm the data collected on the case report forms. At a minimum, the following list of information should be maintained and made available as required by the study monitor (CRA) and/or regulatory authority inspectors:

- Medical history and physical condition of the study patient before involvement in the study to verify investigation plan eligibility criteria;
- Dated and signed notes on the day of entry into the study referencing the sponsor, CIP name, patient number assigned and a statement that informed consent was obtained (including confirmation that informed consent process is followed as per process defined in CIP section 12.2);
- Dated and signed notes from each patient study visit (with reference to the eCRFs for further information if appropriate for specific results of procedures and exams);
- Notations on abnormal laboratory assessment results i.e. Clinically Significant (CS) or Not Clinically Significant (NCS) according to the investigator;
- Adverse events reported and their resolution, including supporting documents such as discharge summaries, lab reports, test results;
- Notes regarding prescription medications taken during the study (including start- and stop dates);
- Study patient's condition upon completion of- or discontinuation from the study.

8.2 Case report form completion

The data will be recorded in an Electronic Data Capture (EDC) system/electronic Case Report Forms (eCRF). The data should be accurately recorded by the principal investigator or any authorized personnel. The persons should be trained on the CIP and on eCRF completion before receipt of the proper authorization and login to the eCRF.

Viedoc is identified as the eCRF system that will be used (set-up and management of data by CRO- QbD Clinical, Lier, Belgium).

8.3 Data Source Data Verification- Monitoring

Monitoring will be performed during the study to ensure that compliance with the CIP and applicable regulations is maintained, that data is collected in a timely, accurate and complete manner and that the investigator continues to have sufficient staff and facilities to conduct the study safely and effectively.

The data will be monitored by ElmediX appointed study monitors/Clinical Research Associate (CRA) during on-site monitoring visits, according to the monitoring plan. Also, remote monitoring might be done by sponsor and/or data management to ensure consistency/clarity of the data.
The sponsor may designate additional monitors at any time during the study.

8.4 Data Management

Before data management activities start, a Data Management Plan will be written and approved by the Sponsor. The data management plan will include

- Procedures used for data review, database cleaning, and issuing and resolving data queries;
- Procedures for verification, validation and securing of electronic clinical data systems, if applicable;
- Procedures for data retention and addressing the regulatory requirement

8.5 Data Archiving

Study records must be kept for the maximum period of time required by the hospital and any relevant national regulations. The documentation must be accessible upon request by regulatory authorities. The Sponsor must approve archiving or transfer of the documentation for relocation purposes, *in writing* and *prior* to actual transfer. The Investigator must notify the Sponsor, in writing of transfer location, duration and the procedure for accessing study documentation. The Investigator must contact the

Sponsor before the destruction of any records and reports pertaining to the study to ensure that they no longer need to be retained.

The sponsor and investigator shall maintain the investigation documents according to the applicable regulatory requirement(s).
They will take the necessary measures to prevent accidental or premature destruction of these documents.

A list of essential clinical investigation documents to be maintained in sponsor and site files is detailed in ISO14155.

8.6 **Source Data Collection**

Anonymized patient medical records related to an event should be provided to the sponsor upon request.

Also, medical imaging recordings might be requested for review by sponsor on ad hoc basis e.g. to check pre-liminary efficacy.

Site should ensure that documents and imaging recordings are properly anonymized before sending to the sponsor or other responsible parties with respect to GDPR rules (e.g. pseudo anonymization by use of patient study ID is acceptable). In case of non-anonymous files, the documents and images will be deleted and the site will be notified.

9 Clinical Investigation Plan deviations

9.1 General

The investigator is not allowed to deviate from the CIP, except under emergency circumstances to protect the rights, safety and well-being of patients.

9.2 Procedures for Recording, Reporting and Analyzing CIP Deviations

CIP deviations will be entered in the eCRF. The classification (e.g. major, minor) will be decided by the investigators and the sponsor according to their reasonable judgment. If the safety of a patient is potentially in danger, the deviation (or violation as per EC website) is considered as major.

9.3 Requirements and Timelines for Notification of CIP Deviations

Major CIP deviations should be reported to the sponsor immediately by entering the data in the eCRF. Minor CIP deviations should also be entered in the eCRF.

Major deviations/violations should be reported to the EC immediately by the investigator/site. Minor deviations (with no impact on safety of the patients) will be reported on a yearly basis – together with the annual progress report to the EC.

9.4 Corrective and Preventive Actions and Principal Investigator Disqualification Criteria

All CIP Deviations will be documented and assessed. If required, corrective and preventive action will be agreed upon and implemented with the relevant site remotely and/or during on-site monitoring visits by study monitor/CRA. These decisions, activities and possible preventive actions will be documented. (e.g. in monitoring reports).

The Principal Investigator can be disqualified if he has repeatedly or deliberately failed to comply with the requirements as specified in the CIP, including compliance with the relevant regulations, or if he has submitted false information in any required report.

10 Clinical Investigation Plan amendments

The CIP and or other clinical investigation documents might be amended as needed throughout the clinical investigation, and a justification statement shall be included with each amended section of the document. Proposed amendments to the CIP shall be agreed upon between the sponsor and principal investigators or the coordinating investigator if applicable.

The amendments to the CIP and the subject's informed consent form (if applicable) or any document already approved by the EC shall be notified to, or approved by, the EC and CA if required. The version number and date of amendments shall be documented.

11 Device accountability

The investigator will maintain adequate records for the receipt and disposition of the investigational device (TempoCure® and sensors). For the identification of the investigational devices, the devices are labeled as "Exclusively for clinical investigations". Use of the device outside of the CIP is strictly forbidden.

The sponsor will instruct the investigation site during the site initiation visit on how to perform and document device accountability. A Device Disposition Log (for HyperTherm/TempoCure® generation 5) and a Device Accountability Log (for HyperTemp/TempoSensor® sensors) will be used to track receipt, use and return of all investigational devices.

Accountability will be checked by the study monitor/CRA or a member of the technical team. The device accountability log should be completed on an ongoing basis, and signed, dated and filed at the end of the study.

The TempoCure® generation 5 is manufactured for use in this FIH-study. After completion of the clinical investigation the TempoCure® generation 5 used in the clinical investigation will have to be returned to the manufacturer ElmediX.

The sensors as part of the TempoCure® will be provided to the sponsor for single-use per patient. There are identification labels on the TempoCure® temperature sensors. All unused as well as used sensors must be returned to ElmediX.

Example of the labels are part of the IB.

An ElmediX team member familiar with the device (e.g. a technical engineer, an TempoCure® operator) will be present during every treatment and will be responsible for the operation of the TempoCure® and for the real-time data capture.
The site visit monitoring log will be completed during each visit by these visitors.

12 Written informed consent procedure

12.1 Overview of written informed consent procedure

Clinical Investigation-specific procedures or alteration of patient care cannot be started until a signed informed consent has been obtained.

The investigator or a person designated by the investigator (as per site signature delegation log) will explain the study and the person will answer questions of the patient.
If the patient agrees to participate, the informed consent must be signed and personally dated by the patient. The investigator (or designee) who conducted the informed consent process must also sign and date the informed consent form (ICF). The original ICF should be filed in the investigator site file (ISF). A copy of the ICF must be provided to the patients.

The ICF used should be approved by the Ethics Committee. The ICF template proposed by the central EC will be used.

12.2 Process for obtaining informed consent

a) The principal investigator or his/her authorized designee (as identified on the site signature delegation log) conducts the informed consent process.
b) All aspects of the clinical investigation that are relevant to the subject's decision to participate throughout the clinical investigation are discussed.
c) Any coercion or undue improper influence on, or inducement of the subject to participate is avoided.
d) The subject's legal rights are not waived or appeared to be waived.
e) Native non-technical language that is understandable to the subject is used.
f) Ample time is provided for the subject to read and understand the informed consent form and to consider participation in the clinical investigation.
g) Personal dated signatures of the subject and the principal investigator or an authorized designee responsible for conducting the informed consent process are included.
h) The subject receives a copy of het signed and dated informed consent form and any other written information.
i) Important new information that can significantly affect a subject's future health and medical care, is provided to new and existing subjects in written form throughout the clinical investigation, if needed. If relevant, all affected subjects shall be asked to confirm their continuing informed consent in writing.

12.3 Vulnerable subjects- Emergency treatment- Subject unable to read or write

For this investigation, no vulnerable subjects will be selected.

The investigation does not involve emergency treatments so consent will be asked to subject itself and not to a legally authorized representative.

In case a subject is unable to read or write, the procedure with an independent witness as defined in ISO14155 should be followed.

13 Adverse events and safety reporting

13.1 Definitions:

Adverse Event (AE)
Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons whether or not related to the investigational medical device.
NOTE 1: This definition includes events related to the investigational device or the comparator.
NOTE 2: This definition includes events related to the procedures involved.
NOTE 3: For users or other persons, this definition is restricted to events related to investigational medical devices.

Serious Adverse Event (SAE)
Adverse event that:
a) led to a death, injury or permanent impairment to a body structure or a body function.
b) led to a serious deterioration in health of the subject, that either resulted in:
- a life-threatening illness or injury, or
- a permanent impairment of a body structure or a body function, or
- in-patient hospitalization or prolongation of existing hospitalization, or
- in medical or surgical intervention to prevent life threatening illness
c) led to foetal distress, foetal death or a congenital abnormality or birth defect.
NOTE 1: Planned hospitalization for pre-existing condition, or a procedure required by the Clinical Investigation Plan, without a serious deterioration in health, is not considered a serious adverse event.

Investigational medical device
Medical device being assessed for safety or performance in a clinical investigation

Adverse Device Effect (ADE)
Adverse event related to the use of an investigational medical device.
NOTE 1- This includes any adverse event resulting from insufficiencies or inadequacies in the instructions for use, the deployment, the implantation, the installation, the operation, or any malfunction of the investigational medical device.
NOTE 2- This includes any event that is a result of a use error or intentional abnormal use of the investigational medical device.

Serious Adverse Device Effect (SADE)
Adverse device effect that has resulted in any of the consequences characteristic of a serious adverse event **(as identified above)**

Device deficiency
Inadequacy of an investigational medical device related to its identity, quality,

durability, reliability, safety or performance. This may include malfunctions, use error, or inadequacy in the information supplied by the manufacturer.

Unanticipated Serious Adverse Device Effect (USADE)
Serious adverse device effect which by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report.
NOTE: Anticipated SADE (ASADE): an effect which by its nature, incidence, severity or outcome has been previously identified in the risk analysis report

13.2 **Anticipated (S)ADE**

Serious adverse events possibly associated with medical device is laceration, tear or hemorrhage of the liver.
In order to obtain a correct continuous monitoring of the intrahepatic temperature, a specific semi-flexible temperature probe with 0,6mm diameter should be placed intrahepatic under ultrasound guidance: the angle to puncture the liver capsule must not be too oblique, not under 45 degrees, in order to prevent laceration, tear or hemorrhage of the liver. In order to prevent this, the placement of the sensor will be performed under the guidance of an ultrasound used by a surgeon.

Adverse Event (AE) related to WBHT (ref. dog patient studies ElmediX)
1. Electrolyte disturbances: decreases in K, Ca and Mg
2. Hematological disturbances: decrease in hematocrit, platelets, fibrinogen, leukocytosis, signs of DIC
3. Total protein reduction
4. Enzyme increase: ALT, AST, ALP, LDH, CK
5. Kidney function disturbances: increase in creatinine and urea, increase urinary output of Na, K, decrease in Mg
6. Cardiovascular: hypotension, cardiac arrythmia

Serious Adverse Event (AE) related to WBHT (83)

1. Nausea
2. Vomiting
3. Diarrhea
4. Problems associated with heart
5. Problems associated with blood vessels
6. Problems associated with other major organs

Improved experience, technology and better skills led to fewer side effects. In general, the events associated with hyperthermia are not serious.

Adverse Events related to chemotherapy (as examples) according to NCCN guidelines:

Adverse Event (AE) of FOLFIRINOX (94)

1. Nausea
2. Vomiting
3. Fatigue
4. Diarrhea
5. Abdominal pain
6. Anemia
7. Thrombocytopenia
8. Neutropenia
9. Neuropathy

Adverse Event (AE) of gemcitabine (95)

1. Nausea
2. Vomiting
3. Fatigue
4. Flu-like symptoms
5. Proteinuria
6. Myelosuppression ± infection/bleeding
7. Edema
8. Musculoskeletal pain
9. Alopecia

Adverse Event (AE) of nab-paclitaxel (96)

1. Anemia
2. Diarrhea
3. Fatigue
4. Neutropenia

13.3 Safety evaluation and reporting procedure by site

The Investigator will monitor the occurrence of adverse events for each patient during the course of the Clinical Investigation. All adverse events (AEs) reported by the patient, observed by the Investigator, or documented in medical records will be listed on the adverse event form whether believed by the Investigator to be related or unrelated to the investigational device (and/or the investigational treatment).

- Related to (investigational) device: AE due to the interaction between the device and patient, where the device behaves outside its described specifications or due to incorrect application of the device.
- Related to (hyperthermia) procedure: AE due to the interaction between the (correct working and applied) device and patient.

As of the point of enrolment (defined as signing the informed consent form), any new event/experience, or worsening of an event present before, is considered an adverse event. This includes also AE that are not related to the investigational device/treatment.

Unchanged, chronic conditions are not adverse events and should not be recorded on the adverse event form in the eCRF.

The site will report adverse events through the EDC in a timely manner upon awareness. All adverse events will be followed up until resolution or until last follow up of the patient whichever occurs first.

The investigator should determine and document in the EDC for each adverse events the seriousness, severity and relationship to the investigational device/treatment (e.g. not related, unlikely, possible, probable, causal relationship). The relationship should be based on assessment of temporal relationships, biological plausibility, association (or lack of association) with underlying disease and presence (of absence) of a more-likely cause.

13.4 **Reportable safety occurrences/ events**

The sponsor will ensure that all SAEs are reported according to the relevant regulations.

As per MDCG 2020-10/1
For the purpose of this guidance and based on the definitions, the following events are considered reportable events in accordance with Medical Device Regulation 2017/745 and MDCG 2020-10/1 Safety reporting in clinical investigations of medical devices under the Regulation (EU) 2017/745.

> a) any serious adverse event that has a causal relationship with the investigational device, the comparator or the investigation procedure or where such causal relationship is reasonably possible;
> b) any device deficiency that might have led to a serious adverse event if appropriate action had not been taken, intervention had not occurred, or circumstances had been less fortunate;
> c) any new findings in relation to any event referred to in points a) and b).

The investigator should report events to his EC according to the EC reporting requirements.

13.5 **Timelines for reporting**

The site will report adverse events through the EDC in a timely manner upon awareness.

Each adverse events or complication meeting the definition of SAE (whether or not considered device-related) should be reported to ElmediX within 48 hours of the investigator's knowledge of event by entering the event into the eCRF.
The completion of an AE/SAE form in the eCRF will trigger an e-mail notification to ElmediX.

13.6 **Safety Monitoring by DSMB**

The sponsor will ensure a Data Safety Monitoring Board (DSMB) is established prior the start of the clinical investigation (before first patient in). The DSMB is an independent committee and does not have any affiliation with ElmediX, the investigators or the clinical investigation site.

Before opening a new study cohort, a risk assessment will take place by the DSMB. Data up to 7 days after the 2-hour procedure in cohort A1 will be used to open cohort B. Data up to 7 days after the 4-hour procedure in cohort A1 will be used to open cohort A2. Data up to follow up visit 1, D22, one week after the third treatment, from cohort B and C respectively, will be used to open cohort C and D. This is to ensure the safety of patients enrolled in this clinical investigation. Therefore, all adverse events will be reported to the DSMB for review.

Similarly, submissions of all adverse event data will continue in a regular schedule throughout the patient follow up period until the end of the clinical investigation.
The DSMB may request additional information as needed. Based on safety data, the DSMB may recommend whether to continue, suspend, modify or stop the clinical investigation. No formal statistical rule for stopping the study will be defined.

The responsibilities of the DSMB as well as the logistics will be detailed in a separate written procedure (DSMB charter). The CRO QbD Clinical (Lier, Belgium) will manage the DSMB activities.

14 Early termination or suspension of the investigation

ElmediX reserves the right to discontinue the clinical investigation at any stage, with suitable written notice to the investigator. The investigator may also discontinue participation in the clinical investigation. The legal terms are defined in the Clinical Study Agreement with the site.

The main objective of the study is to determine whether the WBHT is safe in the intended use population.
Because of ethical and good clinical practice considerations the Sponsor has defined the following stopping/suspension rule(s) for the clinical investigation:

- analysis of specific cohort shows intolerable high clinical risk for the subjects
- on recommendation of the DSMB

Early termination or suspension of the investigation will also be considered if any of the following occur:

- Non-compliance to obtain subject informed consent
- Non-compliance to the inclusion/exclusion criteria
- Failure to follow subjects per scheduled follow ups
- Failure to submit data in a timely manner
- EC approval expiration
- EC suspension of the center
- Interim analysis (if done) shows intolerable high risk for the subjects

Appropriate actions will be taken in case the study is terminated prematurely or suspended including but not limited to communication to sponsor or site, EC and regulatory authorities, to plan to ensure patients are informed and assure appropriate therapy and follow up.

15 Publication policy

The data and results from the clinical investigation are the sole property of ElmediX. ElmediX shall have the right to access and use all data and results generated during the clinical investigation.

The Investigator will not use the clinical investigation related data without the written consent of ElmediX for any other purpose than for Clinical Investigation completion or for generation of publication material, as referenced in the Clinical Site Agreement.

ElmediX must receive any proposed publication and/or presentation materials at least 45 days prior to the proposed date of the presentation or the initial submission of the proposed publication in order for the materials to be reviewed by ElmediX in compliance with the publication policy set forth in the Clinical Site Agreement.

The Sponsor will be responsible for determining whether to register the clinical investigation on www.clinicaltrials.gov or any other clinical investigations, in accordance with the International Committee of Medical Journal Editors guidelines, or any other applicable guidelines. In the event the Sponsor determines that the Trial should be registered, the Sponsor shall be responsible for any registration. Institution and/or Primary Investigator(s) shall not take any action to register the clinical investigation.

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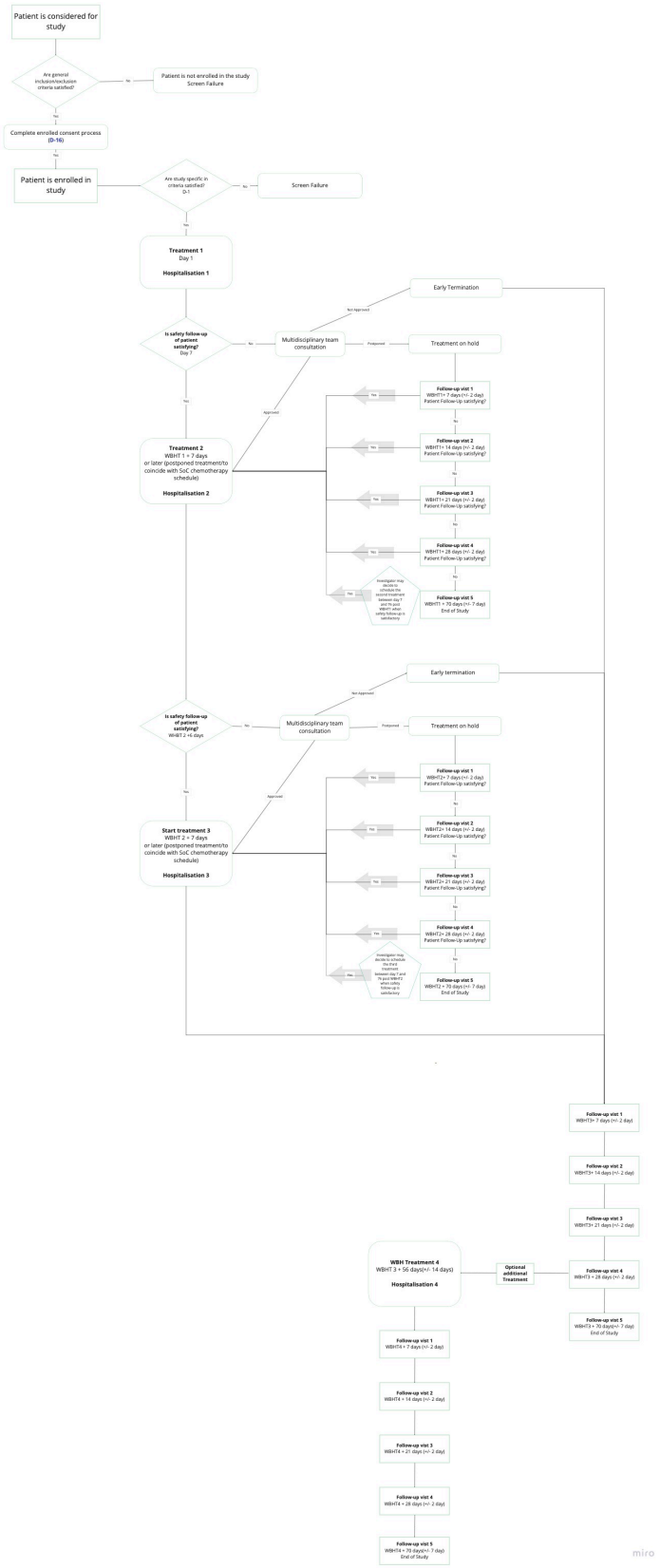
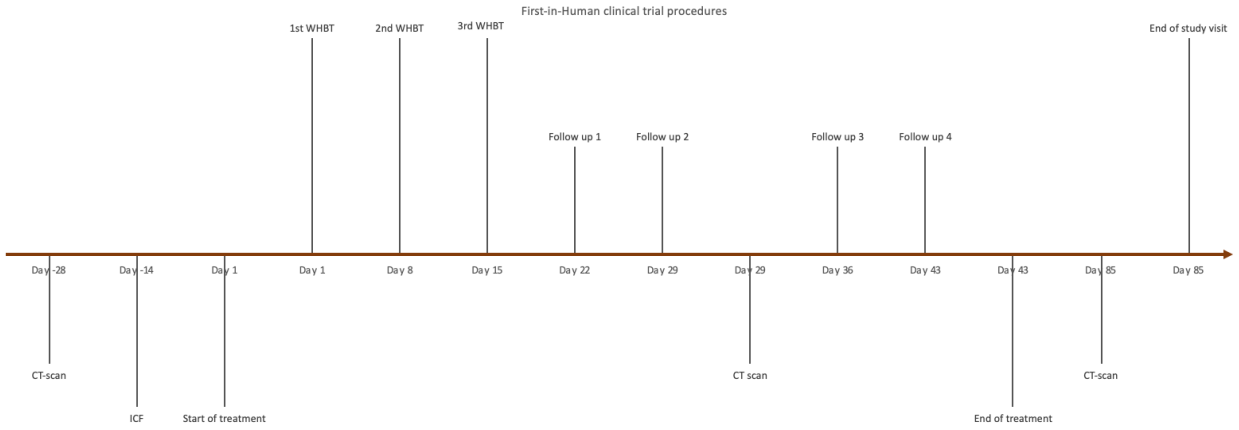
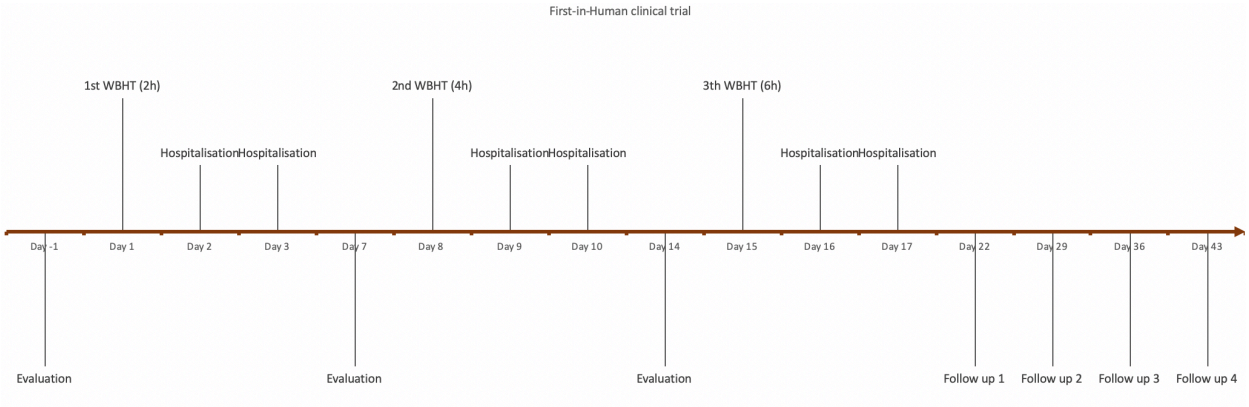
Appendix I: Study Visit Flow Chart



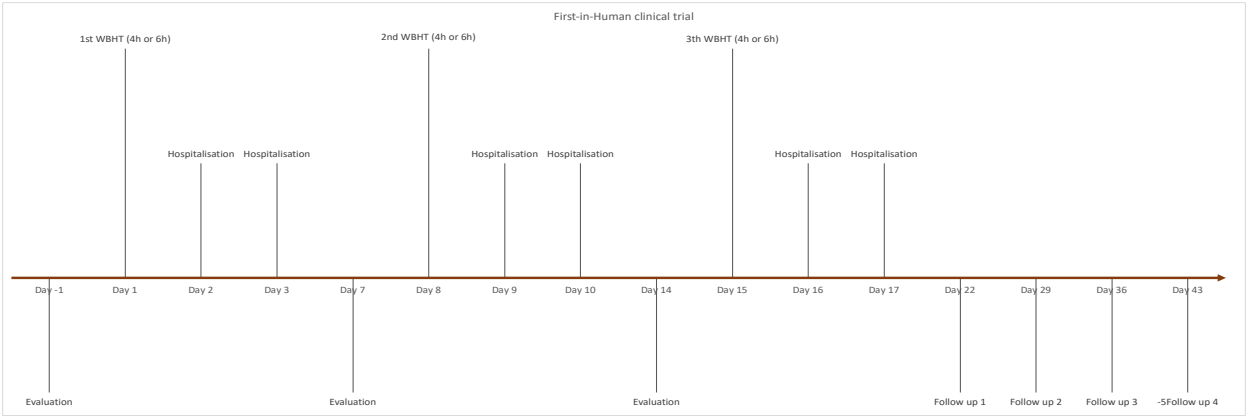
Appendix II: Study procedures



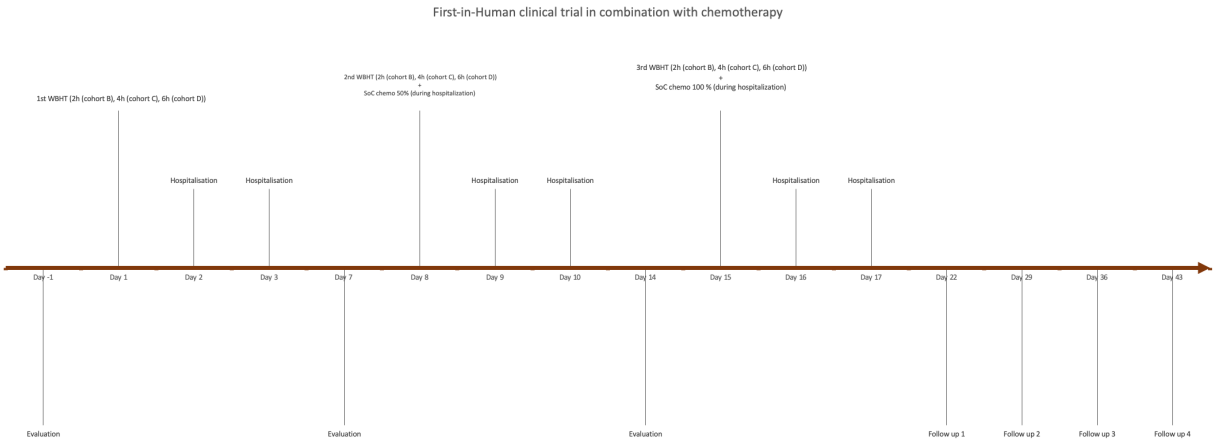
Cohort A1:



Cohort A2:



Cohort B/C/D:


First-in-Human clinical trial in combination with chemotherapy
1st WBHT (2h (cohort B), 4h (cohort C), 6h (cohort D))
2nd WBHT (2h (cohort B), 4h (cohort C), 6h (cohort D)) + SoC chemo 50% (during hospitalization)
3rd WBHT (2h (cohort B), 4h (cohort C), 6h (cohort D)) + SoC chemo 100 % (during hospitalization)
Hospitalisation
Hospitalisation
Hospitalisation
Hospitalisation
Hospitalisation
Hospitalisation
Day -1
Day 1
Day 2
Day 3
Day 7
Day 8
Day 9
Day 10
Day 14
Day 15
Day 16
Day 17
Day 22
Day 29
Day 36
Day 43
Evaluation
Evaluation
Evaluation
Follow up 1
Follow up 2
Follow up 3
Follow up 4

Appendix III: List of assessments per visit

| | | |

Visit name		Screening	Baseline	Treatment 1	Hospitalization 1		Hospitalization 1	Safety FU1	Treatment 2	Hospitalization 2	Hospitalization 2	Safety FU2	Treatment 3	Hospitalization 3	Hospitalization 3	FU 1	FU 2	FU 3	FU 4	End of study visit
Visit Window		D-14	D-1	D1	D2	D3		D7	D8	D9	D10	D14	D15	D16	D17	D22 +/- 2 days	D29 +/- 2 days	D36 +/- 2 days	D43 +/- 2 days	D85 +/- 7 days
	SS = study specific SOC = standard of care (UZA)																		PE	SE
General																				
Inclusion-Exclusion criteria	SS	x	x																	
Informed consent	SS	x																		
Demographics	SOC	x																		
Medical history	SOC	x																		
Medication review	SOC	x																		
Physical Examination	SOC	x	x					x				x				x	x	x	x	x
Vital Signs	SOC	x	x	x	x			x	x	x		x	x	x		x	x	x	x	x
WHO Performance status	SOC	x	x					x				x				x	x	x	x	x
12-lead electrocardiogram (ECG)	SS	x	x					x				x				x^{0}	x^{0}	x^{0}	x	x^{0}
Weight	SS		x		x	x^{1}		x		x	x^{1}	x		x	x^{1}					
Medication review/Change in medication	**SOC**	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Adverse Event assessment	**SOC**		x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x
Blood sampling local lab (see details below)																				
^{1} pregnancy test if applicable	SS	x																		
^{1} bone marrow	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} coagulation	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} liver/pancreas	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} renal function	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} cardiopulmonary function	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} hydration	SS	x	x	x*	x	x		x	x*	x	x	x	x*	x	x	x	x	x	x	x
^{1} Blood gas analysis	SS			x^{A}					x^{A}				x^{A}							
^{1} Thrombo-elastometry analysis (TEM)	SS			x^{TEM}					x^{TEM}				x^{TEM}							
^{1} Tumor markers (specific for tumor-type)	SOC/SS	x (SS); $x^{\dagger}$	x (SOC); $x^{\dagger}$		x (SS); $x^{\dagger}$		x (SS); $x^{\dagger}$			x (SS); $x^{\dagger}$		x (SS); $x^{\dagger}$		x (SS); $x^{\dagger}$		x (SS); $x^{\dagger}$	x (SOC); $x^{\dagger}$	x (SS); $x^{\dagger}$	x (SOC); $x^{\dagger}$	x (SOC); $x^{\dagger}$
Blood sampling central lab	**SS**		$x^{I}/x^{K}/x^{N}$	x^{I}		x^{I}/x^{K}		$x^{I}/x^{K}/x^{N}$	x^{I}		x^{I}/x^{K}	$x^{I}/x^{K}/x^{N}$	x^{I}		x^{I}/x^{K}	$x^{I}/x^{K}/x^{N}$	$x^{I}/x^{K}/x^{N}$	$x^{I}/x^{K}/x^{N}$	$x^{I}/x^{K}/x^{N}$	$x^{I}/x^{K}/x^{N}$
Urine sampling central lab	**SS**		x^{O}	x^{O}		x^{O}		x^{O}	x^{O}		x^{O}	x^{O}	x^{O}		x^{O}	x^{O}	x^{O}	x^{O}	x^{O}	x^{O}
Imaging																				
CT-scan (hospital protocol)	SOC/SS	x^{W}															x			x
RX thorax	SS	x						x				x								
Transthoracic ultrasound	SS	x						x				x								
Dobutamine stress echocardiography	SS	x																		
Electromyography	SS	x^{O}																		
Hyperthermia treatment																				
Fasten-diet (hospital protocol)	SS		x					x				x								
Quality of Life (hospital protocol)	SS		x					x				x				x	x	x	x	x
Cognitive test (hospital protocol)	SS		x		x			x		x		x		x						
Preparation Hyperthermia treatment-Anesthesia protocol (see section 4.3.3; appendix IV)	SS								x				x							
Hyperthermia treatment + monitoring (Cohort A)	SS			x					x				x							
Hyperthermia (SS) and chemotherapy (SOC) treatment + monitoring (Cohort B/C/D)	SS/SOC			x					x				x							

x : x^{*} - CT-scan window is D1- 28 days

x^{I} : blood sampling of 10 ml / 5 ml serum (at the end of treatment plateau phase of 41.5°C)

x^{K}: blood sampling of 10 ml / 5 ml serum

x^{N}: blood sampling of 3 ml whole blood (tempus tube)

x * : multiple blood sampling through arterial line (when the measured temperature in the intrahepatic sensor reaches 37°C, when it reaches 41,5°C, at the end of the plateau phase of 41,5°C and when it reaches 37°C.)

x^{A}: multiple blood sampling of blood gases every 30min (5min.) from the start of anesthesia till end of anesthesia

x^{TEM}: thrombo-elastometry (TEM) when the measured temperature in the intrahepatic sensor reaches 37°C, when it reaches 41,5°C and during the plateau phase (41,5°C) every 120min (± 15min.)) until the end of the plateau phase (41,5°C).

xU: urine collection the day before treatment; during treatment (start treatment, start plateau phase, end plateau phase); during one of the hospitalisation days; during follow up

$x^{\dagger}$: additional tumor marker analysis of CA19-9, CEA, CA125

x^{O}: optional (if indicative)

x ° : optional (if patient do not return to the ward at D2)

Blood sampling local lab

[a] **pregnancy test**:

HCG

[b] **bone marrow function**:

Hemoglobin
Hematocrit
RBC
WBC
(neutrophils)
(eosinophils)
(basophils)
(lymphocytes)
(monocytes)
(macrophages)

Platelets

[c] **coagulation**:

Fibrinogen
APTT
PTT
D-dimers
Protein C
Factor VIII
Factor IX
Von Willebrand Factor Antigen
Von Willebrand Factor Activity

[d] **liver/pancreas function**:

Bilirubin (total)
Aspartate Transaminase (AST)
Alanine Transaminase (ALT)
Gamma-GT
Alkaline phosphatase (ALP)
Lactate dehydrogenase (LDH)
Amylase
Lipase

[e] **renal function:**

Urea
Creatinine
Uric acid

[f] **cardiopulmonary function:**

Creatinine kinase (CK)
CK-MB
Troponin
HDL
LDL
Triglycerides

[g] **hydration status; biochemical analysis (also including weight, urine production and sweating on treatment days):**

NH4
Sodium (Na)
Potassium (K)
Chloride (CI)
Calcium (Ca)
Phosphate (F)
Magnesium (Mg)

[h] **arterial blood gases:**

pH
Partial pressure of oxygen (PaO2)
Partial pressure of carbon dioxide ($PaCO_2$)
Bicarbonate (HCO3)
Oxygen saturation (O2 sat)

[i] **Thrombo-elastometry analysis (TEM/rotem)**

Clotting Time (CT)
Clot Formation Time (CFT)
Maximum Clot Firmness (MCF) at 10 min (A10) beginning from CFT
Maximum Clot Firmness (MCF) at 20 min (A20) beginning from CFT

[j] **tumor markers:**

Specific for tumor type

Specific Blood and Urine sampling– for analysis by central lab (see section 4.4)

Immunology panels (x^I)
Exosomes (x^E)
RNA expression (x^R)
Urine collection (X^U)

Appendix IV: Example anesthesia protocol blood and urine sampling scheme

6u hyperthermie					
		(tijd)	type afname	bloed ml	urine ml
start anesthesia					
start warming up					
opwarmingsfase	37°C	(t0)	urine		80
opwarmingsfase	37°C	(t0)	cyto, coagulation, biochemistry, bloedgas, rotem	15	
		(t30)	bloedgas	1	
		(t60)	bloedgas	1	
		(t90)	bloedgas	1	
plateau phase 41,5°C	41,5°C	(t120)	urine		80
plateau phase 41,5°C	41,5°C	(t120)	cyto, coagulation, biochemistry, bloedgas, rotem	15	
		(t150)	bloedgas	1	
	na 1 uur	(t180)	bloedgas	1	
		(t210)	bloedgas	1	
	na 2 uur	(t240)	bloedgas , rotem	4,5	
		(t270)	bloedgas	1	
	na 3 uur	(t300)	bloedgas	1	
		(t330)	bloedgas	1	
	na 4 uur	(t360)	bloedgas, rotem	4,5	
		(t390)	bloedgas	1	
	na 5 uur	(t420)	bloedgas	1	
		(t450)	bloedgas	1	
	na 6 uur	(t480)	bloedgas, rotem	4,5	
cooling down	end of plateau		urine, immunological	5ml serum	80
	37°C		cyto, coagulation, biochemistry, bloedgas, rotem	15	

Appendix V: WHO performance status classification

The WHO performance status classification categorizes patients as:

0	able to carry out all normal activity without restriction
1	restricted in strenuous activity but ambulatory and able to carry out light work
2	ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	symptomatic and in a chair or in bed for greater than 50% of the day but not bedridden
4	completely disabled; cannot carry out any self-care; totally confined to bed or chair.