Adoptive cell therapy across cancer diagnoses

A phase I/II study

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The study will be conducted as described in this protocol and according to Good Clinical Practice (GCP) guidelines and regulatory requirements. The investigator allows direct access to data sources/documents (including patient charts) for monitoring, audit and/or inspection from the Danish Medicines Agency, GCP-units or other national health authorities.

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Abbreviations

ACT = Adoptive cell therapy
AE = Adverse Event
ALAT = Alanine-Aminotransferase
AR = Adverse Reaction
ASAT = Aspartate-Aminotransferase
BP = Blood pressure
CA-125 = Cancer antigen 125
CCIT = Center for Cancer Immune Therapy
CIMT = Center for IT, Medico og Telefoni
CR = Complete Response
CRP = C-reactive protein
CTC = Common Toxicity Criteria
CTCAE = Common Terminology Criteria for Adverse Events
CTLA-4 = Cytotoxic T-lymphocyte-assosiated antigen 4
DMSO = Dimethyl sulfate oxide
eCRF = Electronic Case Report Form
ECG = Electrocardiogram
ELISA = Enzyme-Linked Immuno-Sorbent Assay
ELISpot = Enzyme-Linked Immuno-Spot
EMA = European Medicines Agency
GM-CSF = Granulocyte-macrophage colony stimulating factor
HCG = Human chorionic gonadotropin
IFN-γ = Interferon-gamma
IMPD = Investigational Medical Product Dossier
IL-2 = Interleukin-2
irAE = Immune related adverse events
irRECIST = Immune related RECIST
KFE = Klinisk forskningsenhed (Clinical Research Unit)
LDH = Lactate dehydrogenase
MR = Magnetic Resonance
NGS = Next Generation Sequencing
P = Pulse
PBMC = Peripheral Blood Mononuclear Cells
PD = Progressive Disease
PD-1 = Programmed cell death protein 1
PD-L1 = Programmed cell death-ligand 1
PET =Positron Emission Tomography
PERCIST = PET Response Criteria in Solid Tumors
PI = Principal investigator
PR = Partial Response
PS = Performance status
PSA = Prostate specific antigen
RECIST = Response Evaluation Criteria in Solid Tumours
REP = Rapid Expansion Protocol
SAE = Serious Adverse Event
SAR = Serious Adverse Reaction
SD = Stable Disease
SUSAR = Suspected Unexpected Serious Adverse Reaction
TAA = Tumor Associated Antigens
TIL = Tumor Infiltrating Lymphocytes
TNF-α = Tumor necrosis factor alpha
Tp = Temperature

Synopsis

Indication and treatment

This protocol describes the application of adoptive cell therapy (ACT) across all cancer diagnoses. Despite many treatment advances in cancer therapy, most metastatic solid cancers are still incurable and fatal diseases and most patients will at some point find themselves without viable treatment options. In Denmark 35.000 patients are diagnosed with cancer every year while over 15.000 patients die of cancer related causes. The majority of deaths are caused by metastatic solid cancers such as lung, colon, breast and prostate cancer. ACT is an established and effective treatment for patients with an increasing number of cancer diagnoses including different metastatic solid cancers. The treatment has a potential in all metastatic solid cancers and we wish to test it in patients who have exhausted conventional treatment options, regardless of the individual solid tumor histology. Hence, patients with metastatic solid cancers in advances stages regardless of cancer diagnoses are eligible to enter this protocol. From included patients, tumor tissue will be removed in order to grow a lymphocyte cell product from the tumor-infiltrating lymphocytes (TILs) in the laboratory. The TILs are then infused back into the patient after a specialized regime of sequential lymphodepletion and immune stimulation that includes a short period of checkpoint inhibition.

Rationale

Adoptive cell therapy (ACT) is a personalized form of immunotherapy, where lymphocytes isolated from the patient's own tumor tissue are expanded 1000-fold ex-vivo and then infused back into the patient. The lymphocytes are then able to recognize and attack remaining cancer cells. This approach has shown remarkable clinical results in several trials conducted worldwide for patients with advanced melanoma – some with durable remissions. Promising clinical results were obtained in smaller trials where patients with disparate solid tumors were treated with TILs. At CCIT we are currently undergoing clinical trials in ovarian and renal cancer, and internationally ACT is being tested in an increasing number of cancer diagnoses, some trials are even recruiting patients across cancer types. Studies have shown that a high intratumoral infiltration with TILs in is correlated to the general clinical outcome of the disease in virtually all solid tumors, thus clinical trials with TIL-based ACT to different cancer diagnoses have been undertaken.

To support the TIL-mediated tumor elimination, in classical ACT protocols patients go through a highly specialized treatment regime before and after TIL infusion. This regime includes lymphodepletion with 7 days non-myeloablative chemotherapy, to provide an *immunological window of opportunity* for the infused TILs, and concomitant immune stimulation with interleukin-2 (IL-2). Checkpoint inhibition to support the antitumor activity of TILs is currently under extensive investigation in several other trials worldwide. Thus, lymphodepletion and IL-2 stimulation are well-established as supportive therapy and already an integrated part of current ACT protocols and while checkpoint inhibition is a new addition at our center; internationally other centers have ongoing comparable trials.

Drug-based immunotherapy in the form of checkpoint inhibitors (anti-PD-1 and anti-CTLA-4) has yielded impressive clinical results across tumor histologies. Recent results indicate that the effect of immunotherapy relies not so much on the cancer diagnoses but rather on the genomic and immunologic features of the individual patient's cancer disease. Both ACT and checkpoint inhibition work by tipping the immunological balance in favor of activation and away from suppression or avoidance by the cancer cells. Scientific evidence now show that administering anti-CTLA-4 and PD-1 could provide a benefit in the ACT setting, and several ongoing clinical trials are testing combations of ACT and checkpoint inhibition. To synergistically maximize the immunological potential, we wish to combine ACT with an anti-CTLA-4 antibody (Ipilimumab) prior to tumor resection and an anti-PD-1 antibody (Nivolumab) in combination with TIL infusion.

At CCIT we have demonstrated that ACT with ex-vivo expanded TILs followed by IL-2 stimulation is a safe and effective treatment of metastatic malignant melanoma. We have also established the feasibility and

safety of its use in ovarian cancer. We have an internationally recognized expertise in TIL production ex-vivo and in treating patients with TILs and the supportive regime. Available evidence indicates that ACT is a safe and feasible treatment option in an increasing number of solid tumors, and as we have the facilities available and in lieu of the widespread success of immunotherapy, we believe that ACT should be tested for treatment of cancer patients regardless of their cancer diagnosis.

Purpose

The primary objective is to evaluate the feasibility and safety of the ACT in combination with checkpoint inhibition across tumor histologies. The secondary objective is to characterize biomarkers of immune responses (immune monitoring, including neo-antigen load and neo-antigen immune responses) as well as to assess the clinical efficacy of the treatment (using RECIST 1.1) in multiple cancer types. Other assessments with PERCIST and irRC are done exploratory. In addition, overall survival (OS) and progression-free survival (PFS) will be described, but not included as primary endpoints.

Study design

The study is an open label phase I/phase II clinical trial. Patients will be included and treated at the Department of Oncology at Herlev Hospital. Patients can also be referred from other oncology centers. The inclusion period is expected to run approximately 2 years, starting in the autumn 2017. We expect all patients to finish treatment and have at least 6 months follow-up within 3 years.

All included patients will be treated in the outpatient clinic with 1 dose of Ipilimumab, 2-6 weeks before surgical removal of tumor tissue for TIL expansion. The TIL infusion, IL-2 injections and the 1st dose of nivolumab will be administered during hospitalization. A period of approximately 4-6 weeks from the tumor tissue resection until the TILs are ready for infusion is expected. If needed, TILs can be cryo-preserved for treatment at a later time point. Hospitalization will span approximately 3 weeks and the TIL product will be administered during that time and only once. Nivolumab treatments will start at the hospital 2 days before TIL infusion and will continue every 2 weeks for a total of 4 treatment series. Patients are expected to be discharged 1-2 weeks after TIL infusion and remaining nivolumab treatments will continue in the outpatient clinic. The total treatment period from initial Ipilimumab to the last nivolumab treatment will span approximately 12-14 weeks.

Blood samples for research purposes will be collected before, during and after hospitalizing. After ended treatment, the patients will be evaluated for up 5 years at the Department of Oncology, Herlev Hospital. Patients will be excluded upon clinical or radiological signs of progression.

The study is monitored by the Good Clinical Practice (GCP) unit and will be reported to the Danish Medicines Agency, the Research Ethics Committee and the Danish Data Protection Agency.

Population

A total of 25 patients will be included and treated in this study. Patients with advanced metastatic or locally advanced solid cancer diagnoses are eligible for inclusion. The tumor must be evaluable by imaging and at least one tumor lesion (>1 cm^3) must be available for safe surgical resection. Other inclusion criteria are performance status (PS) 0-1, acceptable kidney- and liver functions and absence of major co-morbidities. Patients with brain tumors or metastasis are excluded.

Importantly, the treatment will only be completed on patients, where we can manufacture an acceptable lymphocyte product from the resected tumor tissue. For many cancer types, the success rate of expanding TILs to a viable T cell product are not known. In malignant melanoma, we have been able to expand TILs in more than 95% of patients and we have observed similar success rates from head and neck-, sarcoma-,

renal- and ovarian tumors. If clinically safe and feasible tumor resection can be repeated in order to grow a viable cell product.

Toxicity

We have demonstrated the safety of ACT and the supportive regime in several finished and ongoing clinical trials. However, the supportive regimen applied in our previous ACT protocols didn't include checkpoint inhibition with ipilimumab and nivolumab. We currently have another protocol submitted for approval with the same treatment regime in ovarian-, fallopian and peritoneal cancer (GY1721).

Checkpoint inhibitors are approved by major regulatory agencies for the treatment of malignant melanoma, lung-, renal-, bladder-, head and neck- cancer and tumors with mismatch-repair deficiency. Combination therapy is approved for malignant melanoma and their toxicities are well-described. For patients treated with checkpoint inhibitor monotherapy (PD-1 or PD-L1 antibodies) CTC grade 3-4 adverse events (AE) occur at frequencies of about 10% and include rashes, gastrointestinal problems, hepatitis and hypophysisitis and combination therapy is known to cause increased toxicity at rates as high as 55 %. In this study, patients will not receive combination therapy but a sequential administration of 1 series of ipilimumab followed by 4 series of nivolumab with at least 4 weeks interval between Ipilimumab and nivolumab. Sequential administration induces lower toxicity than combination therapy and given the low number of series and 4 weeks interval, no severe toxicity is expected. Management of AE will adhere to national guidelines for checkpoint inhibitory therapy.

In this study, patients receive 7 consecutive days of lymphodepleting chemotherapy. Toxicities include fatigue, rashes, diarrhea, oral mucositis, febrile neutropenia, anemia and thrombocytopenia. Patients are hospitalized and adverse events are closely monitored and treated as described later in this protocol.

During immune stimulation with IL-2, patients experience flu-like symptoms such as fever, chills, diarrhea and general malaise. The patients are hospitalized and vital functions are closely monitored as described in appendix 4. IL-2 will be discontinued if intolerable toxicity occurs. In contrast to most ACT trials, we will use a low-dose IL-2 regime that is known to cause limited toxicity. The low-dose regime is preferred because of the addition of checkpoint inhibitors.

Evaluation of toxicity and clinical response

Toxicity and safety is assessed by Common Terminology Criteria for Adverse Events (CTCAE) at fixed time points. Baseline imaging is performed before TIL infusion according to cancer diagnosis. The follow-up period will continue for up to 5 years with regular evaluation by immune parameters and relevant diagnostic imaging (RECIST 1.1), blood tests and clinical assessment and the specific imaging and blood tests may vary according to cancer diagnosis.

Translational research and immunological response evaluation

To evaluate the ACT and the immunological response, blood samples for experimental analyses are drawn at fixed time points. Blood samples for flow cytometry (immune monitoring) will be collected at: 1) The time of surgery, 2) Before TIL infusion, 3) At discharge and 4) At the following the clinical evaluations. Blood samples for serum analyses will be collected during hospitalization at: 1) Before TIL infusion, 2) Two hours after TIL infusion and 3) Every second day hereafter until discharge. Some immune cells isolated from blood samples will be cryo-preserved for later analysis.

Surplus tissue from the initial tissue sample and the biopsy from the 6 week evaluation will be analyses with flow cytometry and gene analyses. At signs of progression a new tumor biopsy will be perfomed for further analyses.

Introduction and rationale

Metastatic solid cancer

Despite many and continuous advances in oncology, metastatic solid cancer is still regarded an incurable and fatal disease. Treatment options are in end-effect palliative and given with the purpose of prolonging life and ameliorate suffering and disability. In Denmark 35.000 patients are diagnosed with cancer every year and more than 15.000 die from cancer-related causes (1). The 4 most common and most deadly cancers forms are all solid cancers (breast, prostate, lung and colon cancer) that readily metastasize. Combined these 4 diagnoses alone amount to 55% of all new cancer diagnoses and 53% of all cancer-related mortality (2). Standard therapies for these cancers range from hormone suppression to aggressive chemotherapy regimens. In the last couple of years immune therapy has revolutionized many cancer treatments. In solid tumors, malignant melanoma has pioneered the field but it is constantly expanded to other diagnoses. Immune therapy was recently approved for lung cancer (3) and colon cancer might be next (4). The high prevalence and morbidity of metastatic solid cancers leaves room for therapeutic improvements and as current evidence points towards a potential role for personalized immunotherapy (5), and we wish to explore this possibility.

Tumor immunology and TILs

In recent years there has been immense progress in the understanding of the interplay between the immune system and cancer cells. It has become clear that the immune system recognizes and reacts against cancers and that an effective immune response against cancer cells is associated with a better prognosis (6). The presence of an immune response can be clearly illustrated by tumor-infiltrating lymphocytes (TILs) that often exist in an inactivated state or at an immunological equilibrium with the cancer cells in the tumor microenvironment. The inactive state of the TILs is characterized by abnormal intracellular signaling, apoptosis and reduced proliferative capabilities (7). It widely is accepted that cancer cells at some point escape the immunological equilibrium in a process called *immuno-editing* that is an evolution-like process where cancer cells mutate and progressively find ways to avoid immune recognition or activation (8). The escape results in a progressive cancer disease that requires external intervention.

Studies of TILs have been pioneered within malignant melanoma. The sheer numbers of TILs in malignant melanoma are indicative of immune activity and are correlated with the clinical outcome (9). Studies of TILs and the clinical response to interleukin-2 (IL-2) in melanoma patients, led to the to the first successful application of adoptive cell therapy (ACT) in this patient group (10). Similar to malignant melanoma, studies have now shown that the presence of TILs is correlated to clinical outcome in many other cancers such as breast (11), lung (12), gastrointestinal (13), head and neck (14), kidney (15) and ovarian cancer (16).

Adoptive cell therapy

Adoptive cell therapy is a promising personalized therapy that specifically targets individual tumors in individual patients. In this therapy, TILs, primarily T cells, are retrieved from resected tumor tissue, expanded more than 1000-fold ex-vivo and then re-applied to the patient. A high number of the TILs are tumor specific and by expanding them ex-vivo the potential immunosuppression from the cancer cells and the tumor microenvironment is circumvented. The re-applied TILs are so great in number and activation that they are able to tip the immunological balance in favor of cancer killing. ACT has shown remarkable results in melanoma patients with response rates of 40-70% in pretreated patients and 20% with durable ongoing complete responses (17,18). The treatment was developed at the American National Institute of Health and in recent years several studies from international research centers in both USA and Europe have been published with more than 500 patients in total (17,19–22).

Based on the experiences from malignant melanoma and the growing recognition of TILs, ACT is being tested in an increasing number of cancer diagnoses. Ex-vivo expansion of TILs has been performed in many solid tumors and single-diagnosis clinical trials are underway in cervical (NCT03108495), breast, vaginal, ovarian (NCT02482090), renal cell carcinoma (NCT02926053), head and neck cancer (NCT03083873), mesothelioma (NCT02414945). Besides single-diagnosis trials, new multiple diagnose trials similar to this one is emerging. At the National Cancer Institute (NCI), they have undertaken ACT across several solid cancer diagnoses covering breast, gastrointestinal, urothelial, ovarian, endometrial and head and neck cancer (NCT01174121).

The supportive regime

Traditionally, the TIL based cancer killing is assisted by a supporting regime. The most common regime consists of 7 days of lymphodepletive chemotherapy prior to TIL infusion and then 5 days stimulation with interleukin-2 (IL-2) after TIL infusion (23). The lymphodepletion provides an *immunological window* where sufficient stimulatory cytokines and growth factors are readily available for the infused TILs while also depleting regulatory and suppressive cells. IL-2 is a cytokine that signals T-cells to expand and differentiate into effector subtypes. It is used in the ex-vivo expansion of TILs and has in itself been able to induce durable clinical responses in malignant melanoma (24) and renal cell carcinoma (25). Following TIL infusion, patients are given IL-2 to further stimulate and expand the infused cells in-vivo. IL-2 stimulates both the antigen specific and unspecific immunity through specific receptors (26). At CCIT we have provided clinical evidence that IL-2 immune stimulation can alternatively be given in a low-dose regime for 14 days (22) with preserved efficacy and less toxicity. In the current protocol patients will receive the low dose IL-2 regime. The dose regime is chosen to minimize the risk of toxicity in combination with checkpoint inhibition as described below. Following TIL infusion, patients also receive a single dose pegfilgrastim to help reconstitute bone marrow function after the chemotherapy.

Besides the well-established supportive regime of lymphodepletion and IL-2, there is early evidence that the ACT efficacy may be further supported by immune *checkpoint inhibition*. Immune checkpoints are surface receptors on immune cells that serve as regulatory brakes in the immune system to avoid autoimmunity. The checkpoints are exploited by cancer cells to avoid immune recognition and activation. In recent years checkpoint inhibitors (anti-PD-1 and anti-CTLA-4) have yielded impressive results in many cancer forms and have now become standard therapy in malignant melanoma (27), lung (3), renal and bladder cancer(28). Emerging studies show that immunotherapy in contrast to traditional cancer medicines might be more dependent on the mutational load of the individual patient rather than the cancer diagnosis (29,30). This could mean that a more personalized approach to cancer therapy like ACT is needed.

Both ACT and checkpoint inhibition work by tipping the immunological balance in favor of activation and away from suppression or avoidance by the cancer cells. Due to the increasing success of checkpoint inhibitors, it is speculated that combination with ACT would provide a synergistic immunological benefit to cancer treatments - much like combining different checkpoint inhibitors has been (31) - and scientific evidence is aligning with this theory. Studies have shown that blockading the CTLA-4 receptor will increase the numbers of infiltrating lymphocytes in tumors (TILs) (32), which is a prerequisite for producing an effective TIL product ex-vivo. Our own data show that patients who had an earlier time point received anti-CTLA-4 antibody (ipilimumab) have an improved response to ACT (33) and that ipilimumab broadens the repertoire of tumor reactive T cells (34). As a result, the combination of ipilimumab and ACT has been undertaken by a number of centers worldwide (NCT01988077, NCT01701674). Meanwhile, it's been shown repeatedly that tumor-specific TILs frequently express PD-1 (35) and that PD-1 inhibition increase their tumor killing capacities (36,37). This fits the general perception that PD-1 predominantly potentiates the effector functions of T cells at the tumor site (38). The results are so promising that several clinical studies combining PD-1 blockade with ACT are already ongoing (NCT01993719, NCT01174121, NCT02500576, NCT02621021).

Based on the available evidence and in order to optimize the anti-tumor activity of the infused TILs, we wish to combine ACT with both checkpoint inhibitors. To improve the cell product, we administer one dose an anti-CTLA-4 antibody (ipilimumab) in the outpatient clinic two weeks before tumor resection and TIL expansion. To maximize the in-vivo efficacy, we administer an anti-PD-1 antibody (nivolumab) just before and in the weeks following TIL infusion to a total of 4 doses. This same treatment regime is used in another protocol for ovarian- fallopian and peritoneal cancer that we have submitted for approval.

CCIT is a leading center for ACT

Center of Cancer Immune therapy (CCIT) at Herlev Hospital is a leading European center of TIL-based ACT. Despite the promising results of this therapy, widespread application and testing of ACT is limited by the considerable expertise, facilities and resources required for the TIL expansion, infusion and supportive regime. Due to this complexity, only a handful of centers in the world are currently able to perform ACT. At CCIT we have many years of experience, and we have over the years built up the required expertise and effectiveness in all steps of the process. We have demonstrated that ACT with ex-vivo expanded TILs followed by IL-2 stimulation is a safe and effective treatment of malignant melanoma (18,22). We have demonstrated success rates of over 95% in manufacturing viable TIL products from patients with malignant melanoma, renal cell carcinoma and ovarian cancer. We are in the process of establishing the viability and safety of ACT in renal and ovarian cancer (Unpublished data) and clinical trials are currently in process. We have also been able to grow TIL products from sarcoma- and squamous cell tumor tissue ex-vivo (39)(Unpublished data). Other centers have demonstrated TIL production from gastrointestinal cancer (40) and lung cancer (41). For many cancer types, the success rate for extracting and expanding of TILs to a viable T cell product have not been tested, but with our improved TIL expansion protocol (described later), we expect a high success rate within all cancer types. Our TIL production process and ACT capabilities are in general so advanced that we are able to offer this option to all patients with metastatic solid cancers regardless of diagnosis.

TIL production and translational research at CCIT

Besides the successful treatment of patients, further development of ACT is a high priority at CCIT. Our established platform for ACT in malignant melanoma have given us a unique opportunity to study the interactions between the immune system and cancer cells and led to many new discoveries. We have demonstrated that successful ACT is correlated to cell products with long telomeres, short time spent in culture, a favorable cell phenotype (CD27+, CD28+), a high absolute number of T cells and a high number of cytotoxic tumor-reactive T cells in the infusion product (42). Similar with other ACT centers, we have shown that a clinical response correlates to persistence of T cell in the peripheral blood after infusion (17,43). Due to these findings, we have modified the original T cell expansion method from the ”Standard TIL expansion” to an ”Young TIL expansion”, which means a reduced length of cell manufacturing from 4-7 weeks to 2-4 weeks. The shorter time in culture (Young TILs) provides longer telomeres and a more favorable phenotype (CD27+, CD28+). We have shown that these TILs have increased proliferation capabilities, longer persistence in-vivo and higher antitumor activity (44).

The optimized TIL production procedure produces clinically usable TIL infusion products from more than 95% of the patients (18). Furthermore, we have introduced the use of the Wave® bioreactor *(GE healthcare, USA)* in the final phase of the TIL production protocol, which makes it possible to achieve even higher numbers of total cells as well as tumor reactive T cells. Based on the production protocol at CCIT, we have standardized and harmonized TIL production procedures between 3 European cancer research centers and initiated a randomized, multicenter phase III trial with ACT versus standard immunotherapy for malignant melanoma (NCT02278887).

Hypothesis and purpose

Hypothesis

Our hypothesis is that immune therapy with ACT including checkpoint inhibition is a safe and feasible treatment option in metastatic solid cancers across histologies.

Primary endpoint

- To assess tolerability and feasibility of the ACT including checkpoint inhibition in metastatic solid cancers.

Secondary endpoint

- To assess objective responses using RECIST 1.1.
- To characterize biomarkers for immunological response to the treatment
- To describe PERCIST and irRC.
- To describe overall survival (OS) and progression-free survival (PFS).

Study design

Overview

The study is an open label phase I/II clinical trial for patients with metastatic solid cancers. All patients will be included and treated at the Department of Oncology, Herlev Hospital. Patients can be referred to treatment from other centers in Denmark. The study is monitored by the Good Clinical Practice unit (GCP-unit) at the Capital Region (RegionH) and will be reported to the Danish Medicines Agency, the Research Ethics Committee and the Danish Data Protection Agency.

Referral of patients is to be made to the visitation office of the urology-gynecological team (UG team) at the department of oncology, Herlev Hospital.

We aim to include and treat a total of 25 patients. The inclusion period will start in the autumn of 2017 and run for 2 years. Within 3 years we expect to have finished treatment and have at least 6 months follow-up of all patients.

The course of treatment consists of 3 steps followed by clinical examinations, blood tests and radiologic imaging as follow-up:

- **Step 1**: Screening, inclusion, ipilimumab treatment and surgical removal of tumor material followed by ex-vivo expansion of TILs.
- **Step 2**: Treatment during hospitalization with chemotherapy, Nivolumab, TIL infusion and IL-2 administration.
- **Step 3:** Continued treatment with Nivolumab every 2nd week in the outpatient clinic for a total of 4 series.
- **Follow-up:** Regular evaluation of treatment during follow-up for up to 5 years.

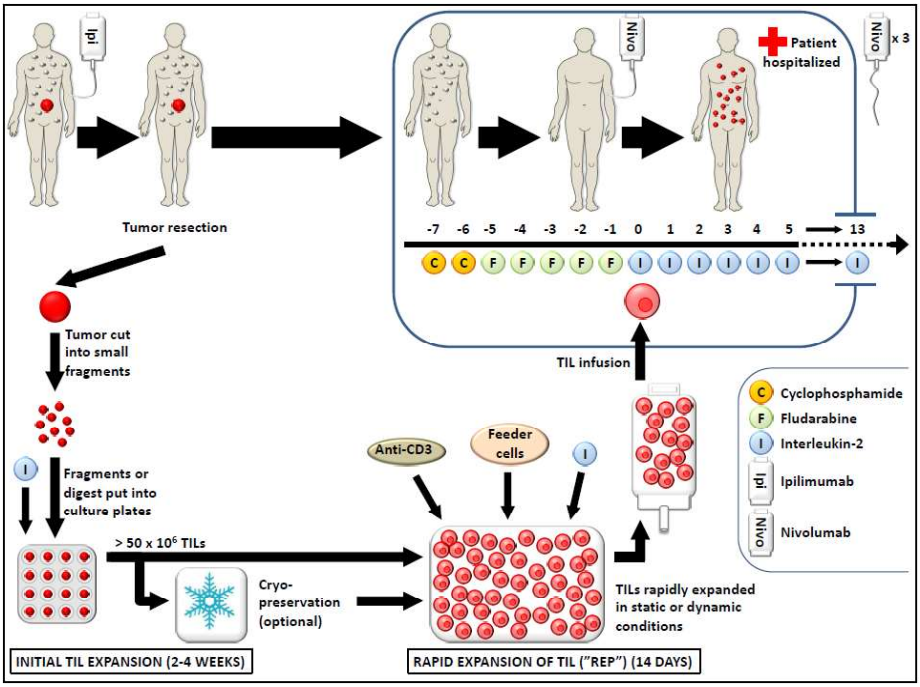

Figure 1: Schematic presentation of the treatment course. One dose of Ipilimumab (Ipi) is administered 2-6 weeks before tumor resection. Resected tumor tissue is transported to the laboratory and placed in growth media. TILs are grown for 2-4 weeks until a cell-number of minimum 50 x 10^6. At this point the cells can be cryo-preserved for later use or

pass on to the Rapid Expansion Protocol (REP) in which the TILs are stimulated for further 2 weeks before they are re-infused back in to the patient. Before TIL infusion, the patient go through 7 days of lymphodepleting chemotherapy with Cyclophosphamide (C) and Fludarabine (F). Nivolumab (Nivo) is administered 2 days before TIL infusion. IL-2 (I) injections is started after TIL infusion and continued for 14 days. Nivolumab treatment is continued every 2nd week in the outpatient clinic to a total of 4 series.

Study population

Patients with metastatic solid cancers in their advanced stages (stage III-IV) are eligible for inclusion. Patients should have exhausted available standard cancer-specific treatment options; an acceptable performance status and no major comorbidity or organ dysfunction. The cancer disease has to be evaluable by radiologic imaging and at least one tumor lesion (>1 cm^3) must be available for safe surgical resection. Patients with brain tumors or brain metastasis are excluded due to the risk of cerebral hemorrhaging during chemotherapy. Importantly, the treatment can only be completed on patients, where we are able to produce an acceptable cell product from the resected tumor tissue.

Inclusion criteria

To be included the participant must meet all the following criteria:

- Histologically verified metastatic or locally advanced cancer diagnosis
- At least one lesion (>1 cm^3) available for surgical resection
- Not candidate for standard treatment options
- Age of 18-70 years
- ECOG performance status of 1 or 0. For information see appendix 2.
- Life expectancy > 6 months
- One or more measurable parameter according to RECIST 1.1.
- No significant toxicity from previous cancer treatments (CTC≤1). Except alopleica (CTC≤2) or neuropathy (CTC≤2)
- Sufficient organ function, including:
    - Absolute neutrophil count (ANC) ≥ 1.500 /µl
    - Leucocyte count ≥ normal limit
    - Platelets ≥ 100.000 /µl and <700.000 /µl
    - Hemoglobin ≥ 6,0 mmol/l (regardless of prior transfusion)
    - S-creatinine < 140
    - S-bilirubin ≤ 1,5 times upper normal limit
    - ASAT/ALAT ≤ 2,5 times upper normal limit
    - Alkaline phosphatase ≤ 5 times upper normal limit
    - Lactate dehydrogenase (LDH) ≤ 5 times upper normal limit
    - Sufficient coagulation: APPT<40 and INR<1,5
- Women in the fertile age must use effective contraception. This applies from inclusion and until 6 months after treatment. Birth control pills, spiral, depot injection with gestagen, subdermal implantation, hormonal vaginal ring and transdermal depot patch are all considered safe contraceptives.
- Signed statement of consent after receiving oral and written study information
- Willingness to participate in the planned treatment and follow-up and capable of handling toxicities.

Exclusion criteria

Participants will be excluded if they meet one or more of the following criteria:

- A history of prior malignancies. Patients treated for another malignancy can only participate if they are without signs of disease for a minimum of 3 years after last treatment.
- Primary brain tumor or verified brain metastases

- Known hypersensitivity to one of the active drugs or excipients.
- Significant medical conditions, including but not limited to severe asthma/COLD, significant cardiac disease, poorly regulated insulin dependent diabetes mellitus.
- Creatinine clearance below 70 ml/min*.
- Acute or chronic infections with HIV, hepatitis, syphilis etc.
- Severe allergies or previous anaphylactic reactions.
- Active autoimmune disease, such as autoimmune neutropenia/thrombocytopenia or hemolytic anemia, systemic lupus erythematosus, Sjögren's syndrome, sclerodermia, myasthenia gravis, goodpasteures disease, addison's disease, hashimoto's thyroiditis, graves' disease etc.
- Pregnant women and women who are breastfeeding.
- Simultaneous treatment with systemic immunosuppressive drugs (including prednisolone† methotrexate etc.)
- Simultaneous treatment with other experimental drugs.
- Simultaneous treatment with other systemic anti-cancer treatments.
- Patients with active or uncontrollable hypercalcemia.

Evaluation before inclusion

The following screening tests need to be available before final inclusion in the protocol.

- Medical history and clinical examination
- Performance status in accordance to the ECOG scale
- Electrocardiogram
- Cr-EDTA clearance
- Urine sample
- Screening blood tests:
  - Hematology: Hemoglobin, leukocytes with diff-count and platelets
  - Liver: ASAT, ALAT, albumin, alkaline phosphatase, bilirubin, LDH, amylase, total protein, INR, APPT.
  - Kidney: Sodium, potassium, creatinine, carbamide, magnesium, bicarbonate, phosphate, Ca-ion
  - Other: Glucose, CRP
  - Chronic infections:, HIV, Hepatitis B surface antigen, Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis C IgG, HTLV IgG, EBV IgG, Trepone,
  - Endocrine: TSH, T4, T3, Cortisol, Estradiol (female), prolactin (female), Testosterone (male)
- Pregnancy test: Women in the fertile age must take a pregnancy test. This includes women who are not surgically sterilized, who are not postmenopausal and who have not used safe contraceptions regularly within the last 6 months.
- Radiological tumor evaluation. CT, MR or PET-CT scans can be used according to cancer diagnosis.
- Reviewing the checklist for inclusion/exclusion for treatment.

Treatment plan

Patients will receive treatment with one dose of ipilimumab 2 weeks prior to tumor tissue removal. If for some reason surgery cannot be performed after 2 weeks, an interval of up to 6 weeks after ipilimumab will be tolerated. When sufficient TILs have been produced after approximately 4-6 weeks, the patients will be hospitalized and treated with 7 days of lymphodepleting chemotherapy followed by treatment with nivolumab.

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* In selected cases it can be decided to include a patient with a GFR < 70 ml/min with the use of a reduced dose of chemotherapy.
† In selected cases a minor daily dose of prednisolone ≤10 mg or an transient treatment that is stopped before protocol treatment can be tolerated

For the chemotherapy and subsequent therapy a central venous catheter will be placed for the entire hospital stay. Two days after the 1st series of nivolumab, the patients will receive the TIL cell product expanded from the resected tumor tissue. The TIL cell infusion is administrated on day 0 at noon. Two hours after the TIL administration, pegfilgrastim is given to help reconstitute bone marrow function. Subcutaneous low-dose IL-2 is begun at 6 pm and continues every day for 14 days. Subsequently patients will be treated with further 3 series of nivolumab every 2nd week in the outpatient clinic to a total of 4 series.

Location		Hospital									Outpatient clinic			
Day	**-48***	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**1-11**	**12**	**13**	**26**	**40**
Ipilimumab (3 mg/kg) IV	x													
Cyclophosphamide (60 mg/kg) IV		x	x											
Fludarabine (25 mg/m^2) IV				x	x	x	x	x						
Nivolumab (3 mg/kg)							x				x		x	x
TIL product IV infusion									x					
Pegfilgrastim (6 mg) s.c.									x					
IL-2 (2 MIU) s.c.									x	x	x	x		

If the initial biopsy proves insufficient for a viable TIL cell product, the procedure can be repeated if deemed clinically acceptable. The pre-treatment with ipilimumab should be repeated if more than 4 weeks have elapsed after the 1st dose.

The course of the treatment can vary between patients depending on the period from surgery to TIL infusion. In most cases the patients will receive the TIL infusion approximately 4-6 weeks after the tumor resection and in these cases the course of treatment from ipilimumab until the first evaluation with diagnostic imaging (6 weeks after TIL infusion) will span across approximately 12-14 weeks.

Blood samples will be collected before, during and after hospitalizing. After ended treatment, the patients will be evaluated for up 5 years at the unit of experimental cancer treatment (EFEK) at the department of oncology, Herlev Hospital.

Overview of blood tests and evaluation during the treatment course

The patients are continuously monitored on several parameters before, during and after TIL infusion. They are also monitored before every treatment with ipilimumab and nivolumab.

* The time from ipilimumab to cell infusion is varies according to time needed to produce TIL cell product

Time point	Screening	Baseline (Before ipilimumab)	Tumor tissue removal	Before admission (14 days)	At admission	Daily during hospitalizing	Before nivolumab 1st of 4	At TIL infusion	At discharge	Before nivolumab 2nd of 4	Before nivolumab 3rd of 4	Before nivolumab 4th of 4
Day	**-57***	**-50***	**-34***	**-22 to -9**	**-8**	**-7 to 11**	**-5**	**0**	**11†**	**12**	**26**	**40**
Performance Status	x	x‡			x	x	x	x	x	x	x	x
Clinical examination	x	x‡			x	x	x	x	x	x	x	x
Weight, BP, P, Tp	x	x‡			x	x	x	x	x	x	x	x
Toxicity assessment	x	x‡			x	x	x	x	x	x	x	x
Screening blood tests	x											
Research blood tests		x‡	x		x	x§		x	x			
Standard blood tests		x‡			x	x	x	x	x	x	x	x
Immunological blood tests	x				x							
Specific cancer markers	x						x		x			
ECG	x				x		x					
Cr-EDTA				x								
Urine sample					x							
Tumor resection			x									
Tumor evaluation	x			x								

Overlapping samples are only taken once.

Screening blood tests: Hemoglobin, platelets, leukocytes with differential-count, creatinine, sodium, potassium, carbamide, bicarbonate, magnesium, phosphate, Ca-ion, albumin, ALAT, ASAT, LDH, alkaline phosphatase, bilirubin, amylase, total protein, INR, APPT, glucose, CRP, TSH, T3, T4, cortisol, prolactin (female), estradiol (female), testosterone (male), HIV, Hepatitis B surface antigen, Hepatitis B surface antibody, Hepatitis B core antibody, Hepatitis C IgG, HTLV IgG, EBV IgG, trepone.

Research blood tests: See “Research blood test” in the section “Translation research”.

Standard blood tests: Hemoglobin, platelets, leukocytes with differential-count, creatinine, sodium, potassium, magnesium, phosphate, ASAT, ALAT, bilirubin, alkaline phosphatase, Ca-ion, CRP, INR, APPT.

Immunological blood tests: Hemoglobin, platelets, leukocytes with differential-count, creatinine, sodium, potassium, carbamide, bicarbonate, albumin, urate, ALAT, ASAT, LDH, alkaline phosphatase, bilirubin, amylase, glucose, TSH, T4, Ca-ion, cortisol, prolactin (female), estradiol (female), testosterone (male).

Specific cancer markers: PSA, CA-125, α-fetoprotein, HCG, etc. according to diagnosis.

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* Depends on time needed to produce TIL product
† Depends on recovery period after TIL infusion
‡ Examination and blood tests will only be repeated if clinically indicated or >2 weeks from screening
§ Every second day

Tumor evaluation: Radiologic imaging with CT, MR, PET-CT or a PET-MR scan according to cancer diagnosis. Before inclusion a recent scan must to be available for review. Before hospital admission baseline imaging will be performed.

Medicinal products used in the protocol

Ipilimumab *(Yervoy®)* is an anti-CTLA-4 antibody that inhibits the suppressive CTLA-4-CD80/CD86 pathway on immune cells. Normally, when CTLA-4 is stimulated it downregulates activation and proliferation of T cells. When CTLA-4 is blocked the immune cells are better at initiating immune responses and recognizing cancer cells. Ipilimumab alone or in combination with a PD-1 inhibitor is approved by the European Medicines Agency (EMA) for treatment of metastatic malignant melanoma (45). Clinical trials are underway for the combination with PD-1/PD-L1 inhibitors in lung, bladder and prostate cancer.

One dose of Ipilimumab will be administered in the outpatient clinic after inclusion and 2-6 weeks before surgical removal of tumor tissue for TIL production in approved dosage of 3 mg/kg.

Cyclophosphamide *(Sendoxan®)* is an alkylating drug that works by creating covalent bindings with biologically important macromolecules. In cancer therapy, it binds and links DNA strings preventing cell division. The effect can be opposed by cell DNA repair systems that are usually impaired in cancer cells. The binding also damages important cellular functions and leads to cell death. Cyclophosphamide is used to treat breast cancer and hematological diseases as myelomatosis (46).

Cyclophosphamide is given as an intravenous infusion for two consecutive days in a dosage of 60 mg/kg body weight. An increased dosage of 60 mg/kg compared to the regular 20-40 mg/kg is used since a lymph depletive effect is wanted and is in accordance to earlier ACT protocols. The treatment takes place during hospital admission and with supplementary hydration and Mesna injections to minimize toxicity.

Fludarabine phosphate *(Fludara®)* is a pro-drug that is converted to the active triphosphate 2-fluoro-ara-ATP. It is an anti-metabolite that inhibits DNA synthesis while simultaneously reducing RNA and protein synthesis. Fludarabine phosphate is used in the treatment hematological diseases as chronic lymphatic leukemia (47).

Fludarabine phosphate is given as an intravenous infusion for 5 consecutive days in a dosage of 25 mg/m^2 body surface (starting the day after the last dosage of cyclophosphamide). The treatment takes place during hospital admission.

Nivolumab *(Opdivo®)* is an anti-PD-1 antibody that inhibits the PD-1/PD-L1 pathway on T cells. PD-1 is surface receptor on activated T cell and when it binds PD-L1 on target cells, it induces the cell death of the activated T cell. Cancer cells use PD-L1 expression to avoid immune recognition. Nivolumab is approved by the EMA for the treatment metastatic malignant melanoma, hodgkins lymphoma, lung and renal cancer (48).

Nivolumab is administered 2 days before TIL infusion and every 2nd week hereafter to a total of 4 series in the approved dosage of 3 mg/kg. The administrations after discharge will be given in the outpatient clinic.

The TIL cell product contains the tumor-specific T cells in an albumin solution after ex-vivo expansion. They are infused intravenously into the patient at noon on day 0. The number of cells in the product depends on the specific TILs and varies between patients. It usually consists of approximately 10^{10} cells. See the Investigational Product Medicinal Dossier (IMPD) for more information.

Interleukin-2 *(Proleukin®)* or IL-2 is an immune modulatory cytokine that physiologically produced by activated T cells. IL-2 is used as a drug in malignant melanoma and renal cancer (49).

In this study, IL-2 is given in a low-dose subcutaneous setting to stimulate TILs after infusion. A subcutaneous injection with IL-2 (2 MIU) is administered starting from 4 hours after TIL infusion at day 0 and

continuing every day until day 13. During hospitalization the injection will be given by a trained nurse. If the patient is well enough to be discharged within these 14 days, the patient or relatives will be taught to perform the remaining injections. The supplied amount of IL-2 injections for self-administration will be registered by batch number, so that the total amount of administered IL-2 can be correctly determined. Moreover, the IL-2 will be labeled in accordance with current guidelines from the Cytostatica Unit, Herlev Hospital, and in accordance with Annex 13 of the GMP rules regarding study drugs. See appendix 6 for the IL-2 labels that will be used.

Pegfilgratim *(Neulasta®)* is an analog of human granulocyte colony stimulatory factor (GM-CSF). It stimulates the bone marrow into producing white blood cells and increasing the peripheral blood count. It is usually given to cancer patients who suffer from low blood counts following chemotherapy (50). Pegfilgrastim helps the patients recover from the lymphodepleting chemotherapy by a single dose of 6 mg s.c. at 2 hours after TIL infusion.

Guidelines for supportive and concomitant therapy

Supportive treatment is given on ordinary medical indications estimated by the clinician responsible for the treatment. Any measures should be specified in the patient chart and flow sheet. The following is meant as a guideline and other medications can be administered if appropriate.

During the lymphodepleting chemotherapy the following are given to protect mucosa of the bladder and relieve dehydration, nausea or vomiting:

- Supplementary fluid therapy during cyclophosphamide treatment.
- Inj. Mesna, (25% of cyclophosphamide dosage) IV x 4 on day -7 and -6.
- Inj. Aloxi 250 µg IV on day -7 and -5.
- Tabl. Emend, 125 mg x 1 on day -7, 80 mg x 1 on day -6 and -5.
- Tabl. Motilium, 20 mg x 3.
- Tabl. Temesta, 1-2 mg if needed, max x 4.
- Tabl. Pantoloc, 40 mg x 1-2 daily.

To prevent opportunistic infections:

- Tabl. Sulfamethizole with Trimethoprime, 400/80 mg x 1 daily for 6 months from day -7.
- Tabl. Aciclovir, 400 mg x 2 for 6 months from day 0.
- Tabl. Diflucan, 100 mg x 1 on day 0 and until the neutrophile count is > 1000/µl.

After TIL infusion:

- Inj. Pegfilgrastim, 6 mg x 1 s.c. on day 0, two hours after TIL infusion.
- Tabl. Pethidin, 25 mg if needed max x 4.
- Oxygen on nasal catheter if needed.

During the low dose IL-2 treatment:

- Supplementary fluid therapy as appropriate
- Tabl. Motilium, 20 mg if needed, max x 3.
- Tabl. Ondansetron, 8 mg if needed, max x 2.
- Tabl. Imolope, 2 mg if needed, max x 8.
- Tabl. Paracetamol 1000 mg if needed*, max x 4.

* Preferably only if patients develop fever > 41°c

- The further toxicities, guidelines for high-dose IL-2 bolus regime described in appendix 4 will be followed

In case of fever during neutropenia

- Patients are treated with antibiotics according to local guidelines for febrile neutropenia described in appendix 5.

In case of anemia or trombocytopenia

- Transfusions with filtered and radiated blood should be administered by hemoglobin ≤6.0 mmol/L or if it is otherwise clinically indicated.
- Transfusion with platelets is indicated if platelets < 20/µl or if it is otherwise clinically indicated.

In case of diarrhea:

- Patients are treated according to local guidelines that include loperamide and fecal samples.
- Loss of fluid and electrolytes are corrected orally or intravenously and evaluated daily.

In case of immune related adverse events (irAEs) to checkpoint inhibitors local guidelines are followed and e.g. corticosteroid can be administered:

- IrAEs are managed according to national guidelines that are available at https://immuntox.dk.

In some cases, e.g. localized bone pain, local radiotherapy can be prescribed.

- Radiotherapy is preferably to be avoided within the 3 weeks period of hospitalization
- Radiated areas cannot be used as parameters in the assessment of treatment response.
- If possible, not all evaluable areas should be included in the radiated area. If all evaluable areas are treated, the patient is withdrawn from the study to ensure correct evaluation.

Follow-up and end of study

Patients who finish the treatment course will be closely followed by a structured evaluation schedule. The first evaluations are at 1.5 and 3 months after TIL infusion – hereafter at every 3 months. After 2 years the evaluation schedule will change to every 6 months until a total of 5 years have elapsed. The schedule is discontinued if patients suffer disease progression in which cases the patients are referred back to the relevant oncologic department for further treatment. If needed, signs of progression will be consulted with an oncologic specialist within the relevant cancer diagnosis group.

A clinical examination, blood tests and a relevant radiologic evaluation according to cancer diagnosis will be performed in connection with every evaluation. At the first three evaluations the blood tests will include immunological parameters and a toxicity assessment. If tumor tissue is accessible, a tumor biopsy will be performed at the first evaluation.

"End of study" is reached when all participants have either had 6 months of follow-up or been discontinued due to e.g. progressive disease. OS and PFS will subsequently be followed for up to 5 years.

Evaluation (months)	**1.5**	**3**	**6**	**9**	**12**	**15**	**18**	**21**	**24**	**30**	**36**	**42**	**48**	**54**	**60**
Clinical examination	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PS, Weight, BP, P	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Subjective complaints	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Toxicity assessment (CTC)	x	x	x												
Research blood tests	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

Evaluation blood tests	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Immunological blood tests	x	x	x												
Cancer markers	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
ECG	x	x	x												
Tumor evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tumor biopsy	x*														

Research blood tests: See below in "Research blood test" in the "Translational research" section.

Evaluation blood test: Hemoglobin, platelets, leukocytes with differential-count, creatinine, sodium, potassium, magnesium, phosphate, Ca-ion, ASAT, ALAT, bilirubin, alkaline phosphatase, albumin, LDH, CRP, INR, APPT, TSH, T3, T4.

Immunological blood tests: Hemoglobin, platelets, leukocytes with differential-count, creatinine, sodium, potassium, carbamide, bicarbonate, albumin, urate, ALAT, ASAT, LDH, alkaline phosphatase, bilirubin, amylase, glucose, TSH, T4, Ca-ion, cortisol, prolactin (female), estradiol (female), testosterone (male).

Specific cancer markers: As according to diagnosis: PSA, CA-125, α-fetoprotein, HCG, etc.

Tumor evaluation: Radiologic evaluation with CT, MR, PET-CT or a PET-MR scan according to cancer diagnosis. Before inclusion a current scan need to be available for review. Before hospital admission a baseline scan has to be performed.

Tumor biopsy: A tumor biopsy is carried out at the 1.5 months evaluation and in case of progressive disease. See more below in "Tumor biopsy" in the "Translational research" section.

Early termination of the treatment

Several reasons can lead to an early termination of the planned treatment course for individual patients:

- If it is deemed impossible to grow an acceptable TIL product from resected tumor tissue.
- The treatment can be stopped at any time at patient's request.
- The treatment can be stopped at any time in case of unexpected medical conditions if the principal investigator finds it to be in the patient's best interest.
- Patients will be excluded if they start treatment with another experimental drug or if other systemic anticancer treatment is initiated after inclusion in this protocol.
- The patient will be excluded if systemic treatment with corticosteroids is initiated unless it is in agreement with the sponsor and principal investigator.
- Treatment is terminated in case of adverse events that make completion of the study impossible.

Patients who stop IL-2 injections or nivolumab infusions prematurely due to toxicity or other reasons will still be followed in accordance with the protocol.

Patients who are for any reason excluded before the TIL infusion will still be offered treatment at Herlev Hospital of any adverse events that occurs in relation to their participation in the protocol.

* Repeated at progression

Manufacturing of the TIL cell product

Acquisition of tumor tissue

Tumor tissue will be removed from the tumor lesion that is most appropriate and safe for removal. The procedure will be performed at relevant department within the RegionH depending on location and cancer diagnosis. In special cases the procedure can be performed outside RegionH in accordance with the patiens wishes. After sufficient tissue material for pathological examination has been removed, remaining tumor tissue will be labeled with date and patient study number and placed in a sterile container for transport to the GMP facilities 54J7 or 54I6 at Herlev Hospital for further processing. The detailed transportation procedure is described in the attached Investigational Medical Product Dossier (IMPD).

Expansion of ”Young TIL” cultures

TILs from the tumor tissues are expanded using the recently established method for ”Young TILs” (42). The tumor mass will be isolated with a scalpel, and cut into small 1-3 mm^3 fragments. Fragments (typically 24-72 fragments in total) are placed separately in the wells of a 24 well/plate. A TIL culture is established from each fragment by passive migration of T cells from the tumor tissue into the IL-2 based media. The T cells are then kept at a density of about $1x10^6$ cells/ml in a growth media containing IL-2. Cell cultures from the different fragments are pooled into a single cell culture. T cell expansion is performed without selecting for specific cancer antigens to produce a polyclonal TIL repertoire that targets multiple epitopes for a more effective tumor cell destruction in-vivo. The establishment of ”Young TIL” cultures usually takes about 2-4 weeks with a rate of success more than 90%. See the IMPD for more details.

Rapid Expansion Protocol (REP)

When the TIL cultures have reached approximately $5x10^7$ cells, they are either frozen for later use or transferred directly for further expansion by the Rapid Expansion Protocol (REP). In the REP T cells are grown with radiated (40 Gy) allogeneic PBMCs (peripheral blood mononuclear cells) that work as “feeder cells” and with IL-2 and anti-CD3 antibody for further stimulation. In this way it is possible to reach a large number of activated tumor specific T cells with a high level of activity against tumor associated antigens (TAA) and tumor in only 14 days. Ultimately, the autologous T cells are concentrated in a 400 ml infusion bag for intravenous infusion. See the IMPD for further details.

Handling and transportation of the TIL product

The infusion bag with the TILs is labeled with patient ID and a patient specific transport schedule is filled out. The infusion bag and transport schedule are placed in a secure hatch for immediate pickup by a trained clinician, who validates the information on the infusion bag and the transport schedule. The transport schedule is signed and the infusion bag is transported directly to the patient. Before administration, the information on the infusion bag is revalidated by the treatment staff and matched to the ID of the patient through patient identification.

Phenotype determination

The prevalence of T cell phenotypes (e.g. CD4+ and CD8+) as well as characterization of T cell stages in both Young TILs and REP TILs will be determined by flow cytometry. See the IMPD for more details.

Adverse events, toxicity and precautions

Adverse events and reactions

Adverse events (AE) are defined as an undesirable event in a participant during a clinical trial – regardless of whether or not the event is considered related to the investigational treatment. All AEs reported spontaneously by the subject or observed by the investigator or staff will be recorded and described in the patient chart and the electronic Case Report Form (eCRF). The severity and consequences will be recorded for each AE and the severity and relation to the study medication will be assessed in accordance with the guidelines described here.

Relation to the treatment will be determined in accordance with the following guideline:

- 0: No relation– no temporal relation, other etiologies very likely the cause
- 1: Possible relation – less clear temporal relation, other etiologies likely the cause
- 2: Probably related – clear temporal relation with recovery at termination of treatment and reasonably explained by the patient's known clinical condition
- 3: Related – clear temporal relation with laboratory confirmation or a positive retreatment test

Grading of Adverse Events refers to the intensity and severity of the reaction. Events are graded using CTCAE version 4.0 - see appendix 3. The following scale can be used if this CTCAE is not applicable:

- 1: light
- 2: moderate
- 3: severe
- 4: life threatening
- 5: lethal

Patients who experience AEs will be monitored by relevant clinical and paraclinical evaluation assessed by the attending clinician. All AEs must be monitored until satisfactory restitution or stabilization. Results of the monitoring must be recorded in the patient chart and eCRF.

Abnormal laboratory tests are not recorded in the eCRF unless they have caused an AE, resulted in termination of the treatment or meet the criteria of a serious adverse event (see the following).

Serious Adverse Events (SAEs) are defined as any medical events or affects that:

- results in death
- is life threatening (at the time of the event)
- requires hospitalization or prolongation of existing patients' hospitalization
- results in persistent or significant disability or incapacity
- leads to a congenital anomaly or birth defect
- is an otherwise significant medical event

Adverse Reactions (ARs) can be either expected if they are described in the IMPD or in the relevant product summary, or unexpected if the grade or severity does not fit the available product information. If an event is assessed to be caused by the investigational treatment, it is classified as an adverse reaction (AR). An AR can be classified as a Serious Adverse Reaction (SAR) by the same criteria as a SAE.

Suspected unexpected serious adverse reaction (SUSAR) is an unexpected adverse reaction that is deemed related to the investigational treatment. The section describing known side effects and their frequencies in the prescribing information (Section 4.8) of the used medicinal products will be used as reference for

classifying an AE as unexpected. Side effects from the TIL product will be classified as unexpected using "Reference Safety information" section in the IMPD.

Reporting of Adverse Events and Adverse Reactions

The investigator reports SAEs, SARs and SUSARs to the sponsor within 24 hours. Sponsor reports SUSARs to the the Danish Medicines Agency within 7 days if fatal or considered life threatening, and otherwise within 15 days. Consequences for the study must be reported. Sponsor submits a yearly list that summarizes any SAEs and SUSARs as well as a report regarding the safety of study participants to the Danish Medicines Agency and the Research Ethics Committee (investigator can report to the Research Ethics Committee as well).

Sponsor submits a final report to the Danish Medicines Agency at the end of the study with a description of all SAEs, SARs and SUSARs.

The following is not to be reported:

- deaths caused by the malignant disease or progression
- hospitalizations or prolongation of current hospitalization caused by the malignant disease including:
    - weight loss
    - fatigue
    - electrolyte derangement
    - pain management
    - anxiety
    - palliative hospitalization
    - stay at hospice or terminal care
    - progression of the underlying disease
- hospitalizations or prolongation of current hospitalization if the sole reason for hospitalization or prolongation is one or more of the following:
    - fluid treatment or treatment of nausea
    - blood transfusion
    - platelet transfusion
    - febrile leucopenia/neutropenia
    - administration of investigational procedures
    - placement of a permanent intravenous catheter

These events are registered in the eCRF.

Known adverse reactions and toxicity

Ipilimumab is a checkpoint inhibitor and can cause toxicities by an *overactivation* of the immune system called immune related adverse events (irAEs). They include skin toxicities (erythema, eczema or exanthema with generalized itching and urticarial, worsening of existing autoimmune skin disorders (such as psoriasis and roseaca), gastrointestinal toxicities (diarrhea/colitis), endocrinopathies (hypophysitis, thyroiditis etc.) hepatotoxicity, pneumonitis, ocular toxicity, myocarditis, neuropathy and immune related nephrotoxicity. The most common side effects of ipilimumab that affect more than 10% of patients are fatigue, loss of appetite, diarrhea, nausea, skin rash, itching, reaction at injection site and fever. Further information is available from the EMA (45).

Cyclophosphamide is a classical chemotherapeutic drug and its usual limiting toxicities are myelosuppression (neutropenia, thrombocytopenia and anemia) and urotoxicity (cystitis, haematuria and hemorrhagic cystitis). Concomitant *Mesna* therapy along with sufficient rehydration markedly reduces the

frequency and severity of the urotoxicity. The most common adverse reactions are alopecia, nausea and vomiting. Further information is available from the Danish Medicines Agency (46).

Fludarabine phosphates most common side effects are myelosuppression (neutropenia, thrombocytopenia and anemia), infections including pneumonia, cough, fatigue, limpness, nausea, vomiting and diarrhea. Other adverse reactions are shivering, edema, malaise, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis and rash. Severe opportunistic infections and death have also been reported. Further information is available from the Danish Medicines Agency (47).

TIL product has, based our and others experience, no immediate risk of serious adverse reactions. The patients might experience transient fever, shivering and mild dyspnea with a few cases of an observed minor decrease in blood oxygen saturation level. There is a theoretical risk of the development of allergic reactions/anaphylactic shock but has not yet been observed according to literature. See the IMPD for more details on previous human exposure and anticipated risks.

Pegfilgastrim. The most common side effects are bone and muscle pain, which are generally mild to moderate in severity and can be treated with standard painkiller.

IL-2 can cause a local inflammatory reaction with reddening and induration at the site of injection. Mild toxicities (WHO grade 1) characterized by flu-like symptoms with muscle soreness, joint pains, malaise and a temperature increase lasting about 12-18 hours have been observed. Fever, nausea and fatigue are the most common side-effects when administering the low-dose IL-2 regime used in this study (22). Most adverse reactions are self-limiting and will disappear within 1-2 days after cessation of treatment. Further toxicity and management will follow the guidelines for high-dose IL-2 bolus regime and are thoroughly described in appendix 4.

Nivolumab is a checkpoint inhibitor and can cause irAEs as described for ipilimumab but generally at lower frequencies. The most common side effects are fatigue, diarrhea, skin rash and itching affecting about 10% of patients. Further information is available from the EMA (48).

The combination of ipilimumab and nivolumab is known to cause greater toxicities than monotherapy with either drug (51). In this protocol a single dose of ipilimumab is administered about 7-9 weeks before nivolumab. Ipilimumab has a plasma half-life of approximately 2 weeks, which means that the toxicities similar to monotherapy are expected. Only one trial is reported where treatment with ipilimumab (four cycles) was followed by sequential administration of nivolumab (up to progression) with a planned switch (52). In this trial, the most frequent toxicities were rash, itching, fatigue and diarrhea and easily manageable with established guidelines. Grade 3-4 toxicities appeared at frequencies around 20% with colitis as the predominant toxicity.

The combination of ACT and checkpoint inhibition is largely unknown but several international clinical trials are underway as described earlier. The desired synergy in tumor killing may also be the cause of augmented autoimmune toxicity. The patients will be treated according to national guidelines for management of toxicities to checkpoint inhibitors available at https://immuntox.dk. As a precaution the patients will receive the low-dose IL-2 stimulatory regime.

Risks and disadvantages regarding invasive procedures

Tumor resection will be assessed radiologically prior to inclusion. The tumor tissue needs to be available for safe removal and the general condition of the patient must allow the minor surgical procedure. An appropriate surgeon according to cancer diagnosis will perform the procedure.

Tumor biopsies have a slight risk of infection and bleeding. Pain and bruising might also occur in the area. Other risks are highly dependent on anatomical location. Biopsies will only be performed if deemed safe by relevant specialist.

Blood tests can cause local pain and bruising. Blood testing will involve frequent hospital visits.

A central venous catheter is associated with minor risks that can be divided into immediate and delayed complications. There is an immediate risk of incorrect placement and bleeding while the delayed risks involve infection, thrombosis and embolization. After the procedure, an x-ray will be performed to ensure correct placement.

Monitoring of organ function

Hematological monitoring of blood counts is indicated for all patients throughout treatment. Leukocyte count, platelet count and hemoglobin values will be checked daily at fixed intervals. Blood cell counts will be performed before start of chemotherapy, IL-2 and afterwards until neutrophil counts is >500/µl and leukocyte count is >1.000/µl. Chemotherapy will not be given to patients with a leukocyte count < 500/µl and/or platelet number <50.000/ µl before the start of chemotherapy.

Kidney- and urine functions are closely monitored. Any obstruction of the urinary tracts, cystitis or infection will be resolved before start of treatment. Patients will be treated with Mesna and fluid therapy to decrease the frequency and severity of bladder toxicity from chemotherapy. The patients' urine will be tested for microscopic hematuria before start of cyclophosphamide and this drug will be discontinued if cystitis associated with micro- or macroscopic hematuria occurs during treatment.

Cardiotoxicity is seen especially when administrating high doses of Cyclophosphamide (>120-240 mg/kg body weight). An electrocardiogram (ECG) will be performed before the start of treatment. Patients with known heart disease will not be included in the study. Necessary investigational procedures will be performed if the patients show signs of cardiac affection (e.g. chest pains, shortness of breath). Myocarditis has been described following high dose Il-2 treatment, if suspected relevant diagnostics (CKMB, TPN, ECG) and cardiologic consultation will be performed.

Risks and precautions

Infertility can occur during chemotherapeutic treatment and there is also a risk of a permanent affection of the fertility.

Vaccination with live vaccines should be avoided prior to- and 6 months after treatment with checkpoint inhibitors.

Depolarizing muscle relaxants that inhibits the cholinesterase activity interact with cyclophosphamide and in concomitant treatment could modulate the effect of drugs such as *suxamethonium*. This can result in prolonged apnea when anesthetized and the combination should be avoided. The anesthesiologist should be informed if the patient has received treatment with cyclophosphamide within 10 days before put into general anesthesia.

Grapefruit or grapefruit juice should be avoided since grapefruit contains a substance that can impair the activation of cyclophosphamide and thereby hinder its effect.

Transfusion related graft versus-host reactions have been observed in patients receiving treatment with fludarabine phosphate after transfusion with non-radiated and non-filtered blood. Participants in need of blood transfusions within 6 months after receiving with fludarabine phosphate should only receive radiated and filtered blood. To this end, the blood bank at Herlev Hospital will be informed for the patients' enrollment in the protocol and the patient will then only receive radiated blood for 6 months after treatment. All blood in the Capital Region is filtered.

Endpoints

Primary endpoints

Feasibility: The treatments feasibility is measured by the number of patients and which cancer diagnoses who are able to successfully complete the treatment course.

Safety and toxicity: Registration of all AEs and unexpected events in relation to the treatment will be done in accordance with the common terminology criteria for adverse events (CTCAE) criteria as described in appendix 3.

Secondary endpoints

Immune biomarker evaluation: Continuous ex-vivo analyses of the specific immune reactivity against tumor antigens to evaluate the immunological effect of treatment. The immunological response against tumor antigens before and after treatment will be compared, as well as between responding and non-responding patients. Particular focus will be given to tumor-antigens deriving from mutations (neo-antigens).These analyses will be done on blood samples and tumor biopsies.

Clinical evaluation: The clinical efficacy of the treatment will be evaluated using the objective response rate in accordance with RECIST 1.1, overall survival (OS) and progression-free survival (PFS). Immune related RECIST (irRECIST) and PERCIST will only be used exploratory.

RECIST 1.1 Guidelines (53):

- Complete response (CR): All lesions disappear.
- Partial response (PR): Defined as a ≥ 30 % reduction in the sum of all measurable parameters longest diameter.
- Stabile disease (SD): Defined as a < 30 % reduction in the sum of all measurable parameters longest diameter or a < 20 % increase in the sum of all measurable parameters longest diameter.
- Progressive disease (PD): Defined as a > 20 % increase in the sum of all measurable parameters longest diameter *or* the appearance of new lesions.

CR and PR are to be verified by a subsequent evaluation after no less than 4 weeks.

Statistical analysis

The study is non-blinded and non-comparative. Descriptive statistics will be used to estimate the immunological- and clinical response rate. Descriptive statistics will also be used to sum up the duration of response and the patient characteristics. The study is designed as a phase I/II study and the primary aim is to determine feasibility, safety and toxicity to the treatment. A power calculation for the required sample size to determine of primary- and secondary endpoints can therefore not be formally calculated. A sample size of 25 participants is chosen because of practical considerations that include a realistic time frame as well as the limited available facilities and trained personal.

All patients receiving T cell therapy will be included in the statistical analyses. Patients can be excluded from the statistical analyses for the following reasons:

- Insufficient TIL expansion or the inability to produce a TIL cell product
- Withdrawal of consent
- Started other cancer treatment

Translational research

Blood samples and tumor tissue are collected for research purposes.

Research blood tests

In total 500 ml of blood will be collected for research purposes in the 12-14 weeks from inclusion to the first evaluation after treatment. These blood samples are collected to assess the effect of treatment on the immune system. The amount of blood taken during the course of the study does not exceed what the body itself is capable of producing between each test. Blood samples for research purposes will not be taken at hemoglobin levels <6 mmol/l.

Immune monitoring is performed on blood samples of 100 mL heparinized blood by flow cytometry. These will be collected at: 1) At baseline 2) Before TIL infusion, 3) At discharge and 4) At the following the clinical evaluations.

Mononuclear cells from the heparinized blood (PBMCs) are isolated using Lymfoprep/Leucosep density gradient technique. The mononuclear cells are washed and resuspended in a freezing media consisting of 90% heat inactivated humane type AB serum and 10% DMSO. The cells are frozen at -150°C until analysis. A panel of relevant immunological assays for testing of antigen specific immune reactivity will be applied, including measurements of cytokine production (multimeric fluorescence coloring, ELISpot and ELISA), proliferative- and cytotoxic potential.

Serum samples for analyses of 8 mL will be collected during hospitalization at: 1) Before TIL infusion, 2) Two hours after TIL infusion and 3) Every second day hereafter until discharge. Serum samples are centrifuged and frozen for later use.

Plasma samples for gene analysis are made from 8 mL EDTA blood drawn at 1) baseline and 2) at the following clinical evaluations. Plasma samples are frozen at for later analysis.

Tumor biopsies and isolation of tumor cells

All tumor tissue will be examined for infiltrating immune cells including their phenotype and specificity. Tumor cells are isolated from the tumor fragments by enzymatic processing or seeding of cells from the tumor fragments. These cells are frozen and will be used to determine the anti-tumor activity of the patients' lymphocytes. This is an exploratory analysis and it will not influence any prior or subsequent surgical decisions or medical treatment.

Biopsies are performed from accessible tumor lesions or involved lymph nodes depending on localization and primary cancer diagnosis. A larger surgical resection of tumor tissue is performed at protocol inclusion for standard pathologic testing and so a TIL product can be produced. Excess tissue is used for research purposes similar to the following biopsies. Tumor biopsies are performed at first evaluation at 1.5 months after treatment and in case of progression. Biopsies will only be performed is deemed safe and without unreasonable risk for the patient.

Cytokine Release Assay

TIL cultures are tested for activity against tumor associated antigens (TAAs) and autologous cancer cells. The activity is measured by the production of the activating cytokines (INF-γ and TNF-α) and is quantified by Elispot and flow cytometric analysis with intracellular staining.

Gene-based arrays

The aim of the gene analyses is to learn about:

- Differences among patients in expression level of a panel of relevant normal genes in the tumor microenvironment which could influence the chance of benefit from treatment.
- Expression of tumor/patient specific mutated genes which could influence the chance of benefit from treatment.
- Specific tumor gene expression signatures (54) and mutations in the tumor cells and patient-specific neo-antigens derived from these mutations (55).

These analyses will contribute to the identification of patients that are most likely to respond to treatment as well as contribute to the optimization of T cell therapy based on the selection of neo-antigen specific T cells.

Tumor tissue gene expression profiles will be analyzed on either cryopreserved or FFPE-preserved tumor tissue. We will retrieve gene expression data on approximately 500 cancer/immunity related genes using *Illumina targeted RNA* sequencing applicable to degraded RNA. Targeted sequencing on a limited number of defined genes will be performed on the tumor tissue to obtain an *immune profile* to determine which genes are expressed in the tumor tissue.

Next Generation Sequencing (NGS) with whole genome sequencing of tumors and normal cells of the individual patient will be used to obtain information on tumor-specific mutations. Gene sequencing will be performed on tumor cells (tumor gene profile) and leukocytes (normal gene profile) and by subtracting the two the tumor specific gene expression (neo-antigens) is found.

Data handling and reporting

The participants are allocated a study number at inclusion to ensure that they can't be directly identified. The PI will create an allocation key with study numbers and social security numbers that will be securely stored in an access-restricted folder created by the Capital Regions IT department "CIMT". Selected collaborators at CCIT will be able to gain access to patient personal information to secure proper treatment and evaluation. All handling of personal data will be reported and approved by the Danish Data Protection Agency "Datatilsynet" through Herlev Hospital and according to Danish law. The PI has access to the patient chart including medical records and medication list before inclusion in the study in order to obtain relevant information and ensure patient safety.

Electronic case report form

All relevant data is registered in the eCRF (electronic Case Report Form) developed in cooperation with the Clinical Research Unit (KFE). The PI is responsible for creating the eCRFs and the subsequent recording and reporting of data. The sponsor and PI are both responsible for data analysis on all included patients.

Patient data and eCRF will be stored for 5 years in accordance with current guidelines for storage of personal information. A final report will be drafted in collaboration between the members of the study group.

The eCRF will include:

- Toxicity (CTC registration)
- Immunological response
- Clinical effect parameters

At the end of the study, personal information and tissue samples will be anonymized.

End of study report

The study sponsor will inform the Danish Medicines Agency and Research Ethics Committee within 90 days of the study completion. The definition of study completion is 6 months following the last treatment or after exclusion of last patient. In addition, patients will be followed for PFS and OS for up to 5 years as described earlier. If the study is prematurely terminated, the Danish Medicines Agency and the Research Ethics Committee will be informed of the reason for the termination.

The sponsor will within a year of study completion submit a final study report to the Danish Medicines Agency and the Research Ethics Committee with the study results including publications based on the study.

Research at partner institutions

Laboratory analyses are performed at CCIT or other locations within RegionH. Other analyses on either tumor tissue or blood samples can be performed at partner institutions but only after establishing a specific written agreement for transfer and handling of materials and data. In such cases, the personal information will be anonymized. Protocol amendment will be made according to guidelines described in "Amendments" in the section "Ethical aspects".

In cases where patient samples are to be sent abroad, they will be handled according to national laws and regulations in the receiving country. All personal information will be anonymized and a data processing agreement will be signed between the sponsor or PI and the data processors abroad. If any gene analysis is performed abroad, the data processing agreement will include the five criteria described later in "Ethic considerations regarding gene-based research" in the section "Ethical aspects". If the data processing is performed in another country or location within Denmark, permission will be applied for at the Danish Data Protection Agency.

Ethical aspects

Recruitment of study patients and informed consent

Eligible patients will be referred from the oncological department at Herlev Hospital and other oncology centers in Denmark. Knowledge of the study will be spread to other relevant clinicians by scientific presentations at the oncologic departments or at conferences. Referred patients will be screened for eligibility and invited to an informative consultation with the project manager or a collaborator at the oncologic department at Herlev Hospital.

The patients will receive oral and written information and signed consent of participation will be obtained before inclusion. The oral information includes information and counseling about the planned genetic analyses to enable patients to decide on whether or not to receive information about unexpected findings. The study will adhere strictly to the Helsinki declaration. The patient information follows the guidelines listed in appendix 1. Any contact to eligible patients will be done in accordance with the Danish Health Act, § 46, paragraph 3.

Insurance

Participants of the study are covered by insurance from the Danish Patient Compensation Association "Patienterstatningen".

General ethical considerations

Despite many treatment advances in cancer therapy, most metastatic solid cancers are still incurable and fatal diseases. Most patients will at some point find themselves without viable treatment options. The purpose of this study is to investigate whether ACT could benefit these patients regardless of the specific cancer diagnosis. As described earlier, ACT is a sizable treatment that contains possible toxicities and it is important that all precautions are followed and inclusion criteria are met. It is also a prerequisite that established and effective treatments options are exhausted before patients enter this protocol. That being said, ACT has shown remarkable results in patients with advanced metastatic malignant melanoma and available clinical evidence clearly points towards a potential in other solid cancer diagnoses.

Based on the current knowledge and when the described precautions are taken, we find that the potential benefit of participating in this study outweighs the potential risks and downsides. At the individual level, eligible patients have advanced disease and suffered progression on traditional treatments. ACT may represent an additional treatment option and that may for many patients outweigh a potential risk of toxicity. Participation is always voluntary and is preceded by oral and written information of the study. The treatment will be stopped at any time in case of unacceptable adverse reactions or at the patient's request. At the collective level, this study will expand our knowledge about ACT in general and about which patients could benefit from it. This knowledge could help countless patients in the future.

Ethical considerations regarding gene-based research

Data regarding potential disease causing genes may be generated as a byproduct of certain modern laboratory techniques. Byproduct data will not be used or explored further since only data regarding tumor specific genes are processed more closely. Therefore, we do not expect to obtain explicit knowledge regarding disease-causing genes. If by chance these analyses will discover known mutations with potential significant impact on patient's health, the case will be discussed in an independent expert workgroup in collaboration with the Clinical Genetics Department at Rigshospitalet, unless the patient has chosen not to be informed as stated in the patient information. The following criteria will determine if further actions are indicated:

- A reasonable degree of possibility that a genetic disposition is present.
- Solid documentation linking the genetic disposition and the development of disease.
- The tests used to determine the genetic disposition are well established.
- The disease in question can be prevented or treated.
- The link between the genetic disposition and the development of disease has considerable importance for the patient.

If indicated, the patient will be contacted for referral to the relevant Genetics Department for additional information and testing. In cases where a patient is deceased, or do not want information regarding significant health issues, a medical assessment using the 5 above mentioned criteria will determine whether relatives to the patient is to be informed in accordance with Danish law (*Sundhedsloven* § 43, stk. 2, nr.2.).

Approvals

The PI is to obtain permission from the Danish Medicines Agency "Lægemiddelstyrelsen", the National Research Ethics Committee "National Videnskabsetisk komité" and the Danish Data Protection Agency "Datastyrelsen" before starting the study.

The law dealing with personal data will be respected. Information concerning study participants is protected according to the law concerning personal data and the Act on Research Ethics Review of Health Research Projects.

Amendments

An application to the Danish Medicines Agency and the Research Ethics Committee will be made if substantial changes to the protocol are to be made. These changes can then be implemented after approval. Changes to the protocol are considered substantial in accordance with guidelines described in "Vejledning om ændringer af et godkendt forskningsprojekt" on www.nvk.dk/forsker/forskervejledning and "Skema om ændringer til kliniske forsøg" on www.sundhedsstyrelsen.dk.

Biobanks

Research biobank

In connection with the current study, blood samples and surplus tumor tissue will be stored at -140 °C in a research biobank at the CCIT in room PA102. They will be stored until all analyses concerning the study are finished. In case of surplus samples after all planned analyses are finished, these samples will be transferred to a biobank for future research.

Biobank for future research

Surplus samples are stored in a biobank for future research at CCIT. The participants will sign a separate consent agreement and the Danish Data Protection Agency will be applied for a separate approval for sample storage for future research. Remaining material will be disposed of according the local guidelines for disposal of biohazardous waste. For research in other areas with material in the biobank, new approvals from the Research Ethics Committee and Danish Data Protection Agency will be needed.

Administrative aspects and publication

Patient identification

Patients are allocated a study number at inclusion. The number is given sequentially at enrollment in the study and is not based on the patients name or birthday. This number will be used to identify the patient and will be used in the eCRFs. Data and patient materials will be treated pseudonymized and confidential.

Publications

The primary project managers are Inge Marie Svane and Anders Kverneland. The members of the project group have joint copyright of the obtained results. Positive as well as negative results will be published in international journals. Manuscripts will be produced in cooperation with the project managers and other members of the study group, but with the project managers as being primarily responsible. The project managers are co-authors on publications made on the basis of this study, given that the Vancouver rules are met. Author succession will be determined based on the individual contributions. Use of study data, oral as well as written, at congresses, teaching etc., will only take place if accepted by the project managers. The project managers are obliged to publish results from the study and are naturally interested in the propagation and implementation of the results in clinic. Publications are expected to be completed in 2020.

The study will be part of the PI Anders Kvernelands Ph.D. thesis.

Economy

The study is initiated by CCIT in cooperation with the Department of Oncology at Herlev Hospital and is partially financed by these two departments. In addition, operational and salary funding is applied for ongoing from public and private foundations. Currently, research grants have been obtained from an internal research grant at Herlev and Gentofte Hospital and from The Danish Cancer Society “Kræftens Bekæmpelse” through their “Knæk Cancer” campaign.

The Research Ethics Committees will be informed in case new funds are obtained. None of the involved parties have any economic interests in the study and there is no potential economic gain for the departments or of personnel in connection with the study. There are no economic ties between the financial supporters and the project managers.

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