T-cell Therapy in Combination with Checkpoint Inhibitors for Patients with Advanced Ovarian-, Fallopian Tube- and Primary Peritoneal Cancer.

A phase I/II study

EudraCT nr.: 2017-002179-24

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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List of Abbreviations

ACT = Adoptive Cell Therapy
AE = Adverse Event
ALAT = Alanine-Aminotransferase
AR = Adverse Reaction
ASAT = Aspartaet-Aminotransferase
BP = Blood Pressure
CA 125 = Cancer Antigen 125
CCIT = Center for Cancer Immune Therapy
CD3 = Cluster of differentiation 3
$CD4^+$ Cells = Helper T cells
$CD8^+$ Cells = Cytotoxic T cells
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
Cy = Cyclophosphamide
DMSO = DiMethyl SulfOxide
eCRF = Elektronic Case Report Form
ELISA = Enzyme-Linked ImmunoSorbent Assay
ELISpot = Enzyme-Linked ImmunoSpot
EMA = European Medicines Agency
GM-CSF = Granulocyte-macrophage colony stimulating factor
HLA = Human Leukocyte Antigen
IFN-γ = Interferon-gamma
IMPD = Investigational Medical Product Dossier
IL-2 = Interleukin-2
irAE = Immune related adverse events
KFE = Klinisk forskningsenhed / Clinical Research Unit
LDH = Lactate dehydrogenase
MM = Malignant melanoma = skin cancer
NSCLC = Non small cell lung cancer
OC = Ovarian cancer
ORR = Overall Response Rate
P = Pulse
PD = Progressive Disease
PD-1 = Programmed cell death protein 1
PD-L1 = Programmed cell death-ligand 1
PD-L2 = Programmed cell death-ligand 2

PET =Positron Emission Tomography
PR = Partial Response
PS = Performance status, ECOG scale 0-4
RECIST = Response Evaluation Criteria In Solid Tumours
REP = Rapid Expansion Protocol
SAE = Serious Adverse Event
SAR = Serious Adverse Reaction
SCLC = Small cell lung cancer
SD = Stable Disease
SUSAR = Suspected Unexpected Serious Adverse Reaction
TAA = Tumor Associated Antigens
TAL = Tumor Associated Lymphocytes
TIL = Tumor Infiltrating Lymphocytes
TNF = Tumor Necrosis Factor
VEK = Videnskabsetisk Komité / Health Research Ethics Committee

Synopsis

Indication and treatment

Patients with advanced ovarian-, fallopian tube-, and primary peritoneal cancer will be treated in this phase I/II study. The treatment involves treatment with the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) antibody Ipilimumab before surgical removal of tumor tissue, administration of lymphodepleting chemotherapy followed by infusion of tumor-infiltrating lymphocytes (TILs) isolated from removed tumor tissue, subsequent treatment with the programmed cell death protein 1 (PD-1) antibody Nivolumab as well as subcutaneous injections with the immune-stimulating cytokine, interleukin-2 (IL-2).

Rationale

T-cell therapy is an experimental personalized immunotherapy where TILs are isolated from the patient's own tumor tissue, expanded *in vitro* to billions of cells and then administered to the individual patient with the purpose of eliminating the remaining cancer cells.
Lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate is administered to the patient before TIL infusion to reduce the number of irrelevant immune cells- as well as regulatory T cells known to inhibit T-cell mediated cancer cell killing. IL-2 is administered after TIL administration to activate and stimulate further proliferation of the infused TILs.
At Center for Cancer Immune Therapy (CCIT), we have shown that adoptive cell therapy (ACT) with TILs and IL-2 stimulation in accordance with the decrescendo regimen for patients with advanced ovarian cancer (OC) is feasible and tolerable (Pedersen M et al., manuscript in preparation).
CTLA-4 is involved in the regulation of the early stages of T-cell activation while PD-1 predominantly potentiates the effector functions of T cells at the tumor site. It is therefore hypothesized that targeting both these two receptors will lead to a positive and synergistic effect on the efficacy of TILs[1].
Subcutaneous IL-2 in a low dosage is given to reduce the risk of toxicity when combining the treatment with a PD1 antibody.

Purpose

The primary objective is to evaluate the tolerability and safety of the treatment. The secondary objective is to characterize antitumor immune responses (immune monitoring) as well as to assess the clinical effect of the treatment by use of the objective response rate (using RECIST 1.1). Also, Cancer antigen 125 (CA-125) meassurements will be done. Assessment with PERCIST and irRC will be done exploratively. In addition, overall survival (OS) and progression-free survival (PFS) will be described, but not included as endpoints.

Study design

The trial is an open label phase I/phase II study.
Patients will be included and treated at the Department of Oncology at Herlev Hospital.
Patients can be referred from other oncology or gynecology centers.

All eligible patients will be treated in the outpatient clinic with one dose of Ipilimumab, 2-6 weeks before surgical removal of tumor tissue for TIL manufacturing. TIL administration, IL-2 injections and the start of Nivolumab treatment will take place during hospital admission. It is anticipated that it will take approximately 4-6 weeks from the tumor tissue is removed for TIL manufacturing to the start of treatment. Hospitalization and treatment will span approximately 3 weeks with Nivolumab treatment continuing after discharge in the outpatient clinic for a total of 4 series. T-cell therapy will be administered only once
In some cases, TILs will be isolated from tumor tissue in advance and cryo-preserved for later treatment. After the end of treatment, the patients will be followed with clinical- and imaging controls for up to 5 years in a specialized immunotherapy unit at the Department of Oncology, Herlev Hospital. Patients will be excluded upon clinical or radiological progression.
The inclusion period is expected to be approximately 2 year, starting from July 2017. We expect all patients to have finished treatment and 6 months follow-up within three years.
The study will be monitored by the Good Clinical Practice (GCP)-unit, and reported to the Danish Medicines Agency, the Research Ethics Committee and the Danish Data Protection Agency.

Population

Patients with histologically verified advanced ovarian-, fallopian tube- and primary peritoneal cancer will be eligible for treatment if they meet the criteria's of inclusion, including an acceptable performance status, acceptable kidney- and liver function, and the absence of major co-morbidities. Initially, 6 patients will be included and treated. The treatment will only be completed in patients with successful manufacturing of TILs. If feasible and tolerable, additional 6 patients, giving a total of 12 patients, will be treated.
Prior clinical trials have shown that the success rate of TIL manufacturing from metastatic melanoma exceeds 90% [2]. To date, we have successfully generated viable TIL cultures from 34 out of 34 samples (100%) from patients with ovarian cancer who have undergone debulking surgery (our internal observation). In our clinical pilot study, we managed to grow TIL cultures from 9 out of 10 ovarian cancer tumor samples (90%) (Pedersen M et al., manuscript in preparation).
The actual success rate of TIL manufacturing in this trial may depend on additional factors e.g. previous chemotherapy regimens that the patients have received before enrolment, but any major difficulties in manufacturing TILs from the majority of patients are not anticipated.

Toxicity

As mentioned above, we have shown that TIL therapy and decrescendo intermediate doses of intravenous IL-2 for patients with advanced OC is feasible and tolerable. We have previously conducted a study treating 6 patients with metastatic MM with TILs and low-dose subcutaneous IL-2 that showed minimal toxicity compared to high-dose IL-2[3]. Therefore, low dose subcutaneous IL-2 is expected to be safe and with minimal toxicity.
The toxicities associated with Ipilimumab are well-described[4]. Two clinical studies by the same group have treated patients with OC with a vaccine and subsequent CTLA-4 blockade. Initially, 2 OC patients were treated with Ipilimumab several years after the vaccine[5] and in the continued study 9 additional OC patients were treated with Ipilimumab between 1-4 months after vaccination[6]. No serious toxicity was seen in the first study, while 2 patients experienced grade 3 gastrointestinal toxicities, which resolved after oral corticosteroid administration, in the second study. A low level of toxicity is anticipated as the patients will only receive 1 dose of Ipilimumab.
The toxicities associated with Nivolumab are also well-described, and in general are milder and observed less frequently than with ipilimumab[7]. A clinical study treating 20 patients with OC with Nivolumab has shown acceptable toxicity in a phase II trial[8].

Evaluation of clinical response

The patients will be clinically evaluated 6 and 12 weeks after treatment with TILs and every 3 months hereafter. Evaluation by diagnostic imaging will take place before the treatment, before discharge following TIL treatment, and in connection with the clinical evaluations starting from 6 weeks after TIL treatment.

Immunological response evaluation

Blood samples of 110 ml will be collected at the time of surgery, before TIL infusion, at discharge and in connection with the clinical evaluations. Serum samples (10 ml blood samples) will be collected during hospitalization at day 0 before TIL infusion, 2 hours after TIL infusion and every 2 days hereafter until discharge. Immune cells will be isolated from the collected blood samples by standard gradient centrifugation and cryo-preserved for later analysis. Flow-cytometric analyses will be used to assess the quantity and function of different immune cell subsets (e.g. $CD4^+$, $CD8^+$) before and after treatment at several time points.

Introduction and rationale

Ovarian cancer

Approximately 1900 women are diagnosed with a gynecological malignancy each year in Denmark. Of these, approximately 450 have ovarian-, fallopian tube- and primary peritoneal cancer. 70-80% have local- or advanced disease at the time of diagnosis and it is the 4th most common cause of cancer death among women in Denmark[9]. Inoperable patients are treated with combinational chemotherapy (carboplatine and a taxan). Despite recent progress in treatment strategies, patients with advanced disease still have dismal survival rates[10].

Tumor immunology

Remarkable progress has been made in the understanding of the reactions of the immune system against cancer in recent years. It has become clear that the immune system reacts against certain tumors *in vivo* and that immunological response against cancer cells are associated with a better prognosis[11,12]. In addition, it has been shown that a small percentage of patients with widely metastatic cancers can be cured with a variety of immune-activating approaches, including transfer of T cells[13].

T-cell therapy in patients with OC has only been investigated scarcely and most of the existing studies were conducted over 15 years ago. Importantly, none of the studies used current protocols of TIL manufacturing or preparative chemotherapy regimens. These studies are described in details below (section "T-cell therapy").

Immune checkpoint inhibition in patients with OC has been investigated more recently and in several trials. The clinical trials with CTLA-4-, PD-1- and PD-L1 antibodies are described in more details below (section "Checkpoint inhibition").

Tumor infiltrating lymphocytes (TILs)

Tumors are often infiltrated by large amounts of T-cells (TILs) that specifically recognize tumor antigens but typically are inactive, or not sufficiently active, in the tumor microenvironment. The inactive state of the T cells in the tumor tissue is characterized by abnormal intracellular signaling, apoptosis and reduced proliferative capability, which are probably caused by various immune inhibiting factors in the tumor environment[14,15]. However, it is possible to amplify and reactivate such TILs for tumor cell killing *in vitro* by use of activating factors like IL-2[16,17].

T-cell therapy

T-cell therapy, which is also called "Adoptive T-cell Therapy" (ATC), is an immunotherapeutic cancer treatment that has shown very promising results, especially in metastatic MM. This treatment, which uses the patient's own T cells for tumor cell killing, was developed at the American National Institute of Health and in recent years several studies from research

centers in other countries in both USA and Europe have been published with more than 500 patients having received the treatment in total[18–23].

The treatment is defined as the infusion of T cells isolated from the patient's own tumor tissue after *ex vivo* activation and several rounds of expansion, and takes advantage of the high number of tumor reactive T cells in tumor tissue compared to peripheral blood[24].

Briefly, tumor-infiltrating T-lymphocytes are harvested from freshly resected tumor material from an individual patient and initially expanded *ex vivo* over a period of 2-4 weeks by growing T cells in high concentrations of the T-cell growth factor IL-2. Upon TIL isolation and initial growth, cells are further expanded to around $5x10^{10}$ cells in a standard 14 days rapid expansion protocol (REP), where TILs are cultured in the presence of allogeneic or autologous irradiated peripheral mononuclear blood cells (PBMCs, "feeder cells"), soluble anti-CD3 antibodies and IL-2. Prior to infusion of the TIL product, patients receive in-hospital lymphodepleting chemotherapy for 7 days as a conditioning treatment. This last step has no direct impact on tumor growth, but is crucial for subsequent in vivo TIL persistence and expansion. Following intravenous administration of the T-cell product, IL-2 is administered to support the growth and survival of the infused T cells. The patients remain in hospital for a total of 14-21 days (see Figure 1 for an overview of the procedure). When an autologous and polyclonal tumor specific T-cell population is infused under these conditions, migration of anticancer T cells to the tumor site leads to a broad and patient-specific recognition of both defined and undefined antigens expressed on tumor cells leading to tumor cell killing and, eventually, tumor regression.

This makes T-cell therapy a highly specialized and individualized form of cancer immune therapy.

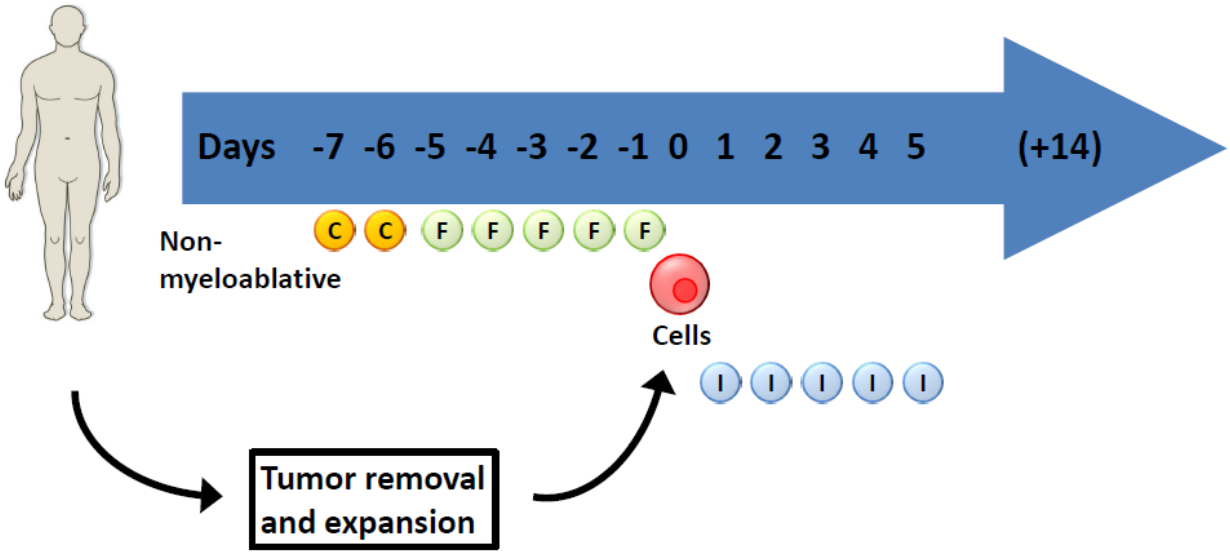

Figure 1: Course of treatment with T-cell therapy
Tumor tissue for manufacturing of the T-cell infusion product is removed immediately after inclusion. Treatment with Cyclophosphamide (C) and fludarabine phosphate (F) is commenced a week before the T cells are ready for infusion and are followed by infusion of the T cells and administration of IL-2. Duration and dosage of IL-2 varies among protocols.

T-cell therapy has shown very promising results in metastatic MM with overall response rates (ORR) around 50%, which has been confirmed in several phase I/II "single institution" studies[19–21,25,26]. Complete response (CR) has been observed in about 20% of the treated patients of which most CR are of long duration and with patients being free of disease more than 7 years after the treatment[18]. Thus, TIL based T-cell therapy seems to have the potential of curing a significant number of the treated patients with metastatic MM.

Previous experiences with more than 500 patients with metastatic MM treated with T-cell therapy has shown that the treatment is safe to administer to patients in a good performance status and adequate organ function, despite considerable - but temporary - toxicity. The observed side effects are reversible and acute and consist of known and expected side effects to lymphodepleting chemotherapy and high dose IL-2.
Of the older studies investigating T-cell therapy for ovarian cancer, one study administered TILs in the peritoneal cavity with a low dosage of IL-2 and found manageable toxicity but no measurable response[27]. Two studies administered TILs without simultaneous IL-2 administration. One of the studies administered 1 series of cyclophosphamide or cisplatine-based chemotherapy followed by administration of TILs and found CR in 14,3% and 70%, respectively, while finding partial response (PR) in 57,1% and 20%, respectively, in very small patient groups (7 and 10 patients)[28]. The other study was performed in an adjuvant setting following primary operation and adjuvant chemotherapy. Thirteen patients were treated with 5-floururacile and cyclophosphamide or cyclophosphamide and Adriamycin

followed by TILs randomized against chemotherapy alone for 11 patients. They found 100% and 67,5% 3 years overall survival rate and 82,1% and 54,5% 3 years disease-free survival rate, both significant results[29]. More recently, a pilot study using gene-modified T cells with PBMCs derived from leukapheresis were tested for treatment of patients with recurrent epithelial OC. The treatmen was shown to be safe, but no clinical responses were observed[30]. A recent study administering T-cell therapy to patients with metastatic ovarian cancer has been performed here at CCIT and is mentioned in details below (see "Clinical trials").

Checkpoint inhibition

Immune checkpoint therapy targets regulatory pathways in T cells with the purpose of enhancing antitumor immune responses. The regulatory pathways of interest to this study are described briefly in the following.

MHC-molecule presentation of tumor antigen in the context of B7 costimulation by professional antigen-presenting cells is necessary for *priming* of tumor-specific T cells. This priming leads to activation, acquisition of effector functions and infiltration of tumor sites. Upon T-cell activation, an induction of CTLA-4 expression leads to an accumulation of CTLA-4 on the surface of the activated T cells that, at a certain level, can block B7 costimulation and thereby subsequently suppress T-cell activation[31–33]. The majority of Activated T cells that infiltrates the tumor microenvironment express PD-1 on their cell surface and many types of cancer express its inhibitory ligands, PD-1 and PD-L1, thus inhibiting local anti-tumor responses in the T cells *effector phase*[31,33,34].

Two clinical studies have investigated CTLA-4 blockade in patients with OC. In one study 9 previously vaccinated cancer patients were treated with the CTLA-4 antibody Ipilimumab (3 mg/kg). Two of these patients had OC. The vaccine consisted of irradiated, autologous tumor cells engineered to secrete GM-CSF. No serious toxicities directly related to the antibody were observed and the two OC patients experienced a brief reduction in CA-125 values followed by stabilization (total 4 months) and a stabilization (1 month), respectively[5]. The other study from the same group continued treating previously vaccinated cancer patients with the difference that CTLA-4 blockade (Ipilimumab 3 mg/kg with subsequent treatments at 2-3 months intervals as clinically indicated, range 1-16) was given between 1 and 4 months after vaccination instead of after several years, as in their earlier study. The same vaccine was used. Nine additional OC patients were treated. Two patients experienced grade 3 gastrointestinal toxicities which resolved after the administration of oral corticosteroids. One of the two also manifested Sweet's syndrome. The remaining 7 patients only experiences minor inflammatory toxicities. Three patients achieved stable disease (SD) (2,4,6+ months) and one patients achieved a partial response (PR) (35+ months)[6].

One clinical study has investigated anti-PD-1 antibodies in patients with OC. They treated 20 patients with platinum-resistant OC with the PD-1 antibody Nivolumab (two 10-patient cohorts, the first treated with 1 mg/kg, the second with 3 mg/kg, treatment every 2 weeks for up to six cycles with four doses per cycle). Eight of 20 patients (40%) experienced grade 3- or 4 treatment-related toxicities. Two patients experienced severe adverse events. Six patients

achived SD (4 in the 1 mg/kg group), one achived PR (in the 1 mg/kg group) and two achieved CR (both in the 3 mg/kg group)[8].
One clinical study investigated anti-PD-L1 antibodies in patients with OC. They treated a total of 207 patients with the anti-PD-L1 antibody BMS-936559 (escalating doses from 0.3 to 10 mg/kg every 14 days in 6-week cycles for up to 16 cycles). Seventeen of these patients had OC. Treatment-related grade 3 or 4 adverse events were observed in 19 of 207 (9%) and serious adverse events in 11 of 207 patients (5%). No information about these events was available based on the different cancer types. Of the 17 OC patients, 1 achieved PR and 3 achieved SD for at least 24 weeks. All were in the 10-mg dose group[35].
A number of other trials are currently enrolling patients, but results are so far unknown (source clinicaltrials.gov).

CCITs experience with T-cell therapy

Clinical trials

The complicated methods of manufacturing TILs has been established at the Center for Cancer Immune Therapy (CCIT), Herlev Hospital, as one of the few places in the world[36]. CCIT and the Department of Oncology at Herlev Hospital have several years of experience in treating cancer patients with T-cell therapy and different regimens of IL-2.
All patients have been treated with classic lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate followed by TIL infusion and subsequent administration of IL-2.
In the original "T-cell regimen" described by Dudley et al[15] very high dosages of IL-2 (720.000 IU/kg i.v.) where given as bolus injection every 8 hours until treatment limiting toxicity. It is unknown how high a dosage of IL-2 is necessary to maintain T-cell expansion, and consequently CCIT has tested the treatment with low and intermediate doses of IL-2 to investigate whether clinical efficacy can be maintained while toxicity is decreased.
Initially, low dosage subcutaneous IL-2 was given to 6 patients in a pilot study initiated in the summer of 2009 and the results are now published[19]. Two of the six treated patients achieved CR and are presently without evidence of disease (NED).
To achieve a higher response rate (RR), the dosage of IL-2 was then increased to an intermediary dosage and administered after the decrescendo-regimen equal to the decrescendo regimen used in Denmark as standard treatment of metastatic MM.
Additionally 25 patients have been treated after the increase in IL-2 dosage. Of the 31 treated patients, five achieved CR (48 (NED), 13 (NED), 38+, 22+ and 11+ months) and 7 patients achieved partial PR (35+ (NED), 12, 18+, 25+ (NED), 11, 8 and 6+), of which 7 are having ongoing responses varying from 6-38 months. Thirteen patients had stable disease (SD) for 4-6 months and 5 patients progressed immediately after treatment. An ORR of 39% has been observed, which is comparable to other studies administering high dosage of IL-2. The lymphodepleting chemotherapy induced, as anticipated, myelosuppression with anemia, leucopenia and thrombocytopenia and all patients received prophylactic antibiotics and blood

transfusions. All patients experienced transient grade III-IV toxicities during the 3 weeks of hospitalization but recovered quickly after the treatment. The above mentioned results have recently been published[37]. Markedly reduced toxicities have been observed with the low- to intermediary dosage of IL-2 and the treatment has shown to be manageable at a regular Department of Oncology with limited need for intervention from an Intensive care unit. Recently, an international phase III study treating patients with metastatic MM with TILs and a high dosage bolus IL-2 regimen (600.000 IU/kg) was initiated at the Department of Oncology, Herlev Hospital. So far, toxicity has been acceptable.
Our recent pilot study with 6 patients has shown that it is feasible and safe to treat patients with metastatic ovarian cancer with T-cell therapy and IL-2 according to the decrescendo regimen. Only expected and manageable toxicities have been observed and all patients' hematopoietic system recovered without the need for stem cell support. Four patients experienced SD for 3 months and the last two patients had SD for 5 months (Pedersen M et al., manuscript in preparation).

Translational research

Further development of T-cell therapy with optimizing and expansion to other cancer forms has a high priority at CCIT. Our already established platform for T-cell therapy for MM gives us a unique opportunity to study the interactions between tumor and immune system and thereby identify possible methods for optimization of T-cell therapy, as well as extension to other tumor histologies.

Several studies has shown that the following T-cell characteristics are important for achieving a clinical response after T-cell therapy: long telomeres, short time spent in culture, a favorable T-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[38] as well as an increased persistence of T-cell in the peripheral blood after infusion[18,36,39]. AT CCIT, we have modified the original T-cell expansion method from "Standard TIL expansion" to "Young TIL expansion" based on these characteristics and leading to a reduction of the length of cell manufacturing from 4-7 weeks to 2-4 weeks. A decreased amount of time in culture (Young TIL) provides the TILs with longer telomere sequences and more favorable phenotypes (CD27+, CD28+) with the ability of increased proliferation, an increased persistence in vivo and a higher antitumor activity, all of which are correlated to an increased clinical response[18,36]. This optimization of TIL production has made it possible to produce clinically usable TIL infusion products from more than 90% of the patients[40–43]. Furthermore, during the final expansion phase, the Rapid Expansion Protocol (REP), we have introduced the use of the Wave® bioreactor[44], which optimizes the conditions of proliferation of T cells and has made it possible to achieve a higher total number of cells as well as tumor reactive T cells in the TIL infusion product. We have standardized and harmonized TIL production methods between 3 European cancer research centers based on these TIL production protocols developed at CCIT and have initiated a randomized, multicenter TIL-based phase III trial with T-cell therapy versus standard

immunotherapy (clinicaltrials.gov identifier: NCT02278887) with the purpose of the approval of T-cell therapy as standard treatment for patients with MM.

Recent studies suggest that TIL based ACT potentially can be used with success in other cancer forms including colon, breast, head and neck, kidney, sarcoma and ovarian cancer[45–50]. It has been shown that a high intratumoral presence of T cells is correlated with longer survival and functional analysis has shown that tumor infiltrating T cells show *in vitro* anti-tumor activity similar to that seen in MM. In a preclinical study, we have successfully manufactured TILs from 34/34 samples obtained with primary debulking surgery from patients with ovarian cancer. Tumor cell lines have been established from 14/33 tumor samples and we have been able to detect an anti-tumor response (either CD8+ or CD4+ T cells) in 19 of 31 patients tested against either tumor cell line or tumor digest (Westergaard MCW et al., ESMO IO 2014). Subsequently, in our aforementioned pilot study with T-cell therapy in OC, we were able to manufacture TILs from 9/10 patients, establish tumor cell lines from 5/10 tumor samples, and detect anti-tumor responses (either CD8+ or CD4+ T cells) in 5/6 of the treated patients tested against either tumor cell line or tumor digest (Pedersen M et al., manuscript in preparation).

The rationale of the drugs used in the study

Lymphocyte depleting chemotherapy

Activating cytokines (the signal molecules IL-2, IL-7, IL-15, IL-21 etc.) need to be available for the tumor specific T cells to sustain a immunological response against tumor tissue. A large number of "irrelevant" T cells will decrease the availability of these cytokines for the relevant T cells through competition. Thus, a high number of tumor specific T cells with a high specificity as well as a reduction of irrelevant T cells and regulatory T cells (Tregs) are needed to create an environment that facilitates the T-cell mediated antitumor response.
This study will use combination chemotherapy with two days treatment with cyclophosphamide and 5 days with fludarabine phosphate to create such an environment. This combination has been chosen based on earlier studies where it was shown safe and effective[51,52].

Cyclophosphamide

Cyclophosphamide is an alkylating drug that works by creating covalent bindings with biologically important macromolecules. Of special interest is the creation of a binding and linkage to DNA. Cell division can be prevented if the linkage is not canceled by the cells repair systems. The binding to important proteins in the cell can damage important cellular functions and lead to cell death. Cyclophosphamide is among others used to treat breast cancer and in the treatment of hematological diseases as myelomatosis[53].

Fludarabine phosphate

Fludarabine phosphate is a pro-drug that is converted to the active triphosphate 2-fluoro-ara-ATP. It is an anti metabolite which inhibits DNA synthesis while simultaneously reducing RNA and protein synthesis. Fludarabine phosphate is used in the treatment hematological diseases as CLL among others[54].

Interleukin 2

IL-2 is physiologically produced by activated T-lymphocytes and stimulates the antigen specific and non-specific immune system through specific receptors[55].
Intermediate-dose interleukin-2 (IL-2) administered intravenously according to the decresendo-regimen[56] is used in Denmark as standard treatment for suitable patients with metastatic MM. Low-dose subcutaneous IL-2 has previously been administered to 6 patients with metastatic MM in a T-cell therapy pilot study with CR in 2 patients and generally low occurrence of toxicity[3]. Moreover, low-dose IL-2 is used for the treatment of metastatic kidney cancer.
In this study, IL-2 will be given in a low-dose subcutaneous setting to reduce the risk of the occurrence of adverse events when given in combination with Nivolumab.

Checkpoint inhibition in combination with T-cell therapy

As mentioned earlier, a high absolute number of T cells and a high number of cytotoxic tumor-reactive T cells in the infusion product is important for achieving a clinical response after T-cell therapy[38]. Furthermore, CTLA-4 is involved in the regulation of the early stages of T-cell activation while PD-1 predominantly regulates T-cell effector functions in the periphery and it is therefore hypothezied that targeting of these two receptors will lead to a positive and synergistic effect on the TILs[1].
One study has shown an increased intratumoral infiltration with CD8+ cells in posttreatment biopsies when treating patients with metastatic malignant melanoma (MM) with a CTLA-4 antibody[57]. Another study investigated 40 patients with metastatic MM treated with the CTLA-4 antibody Ipilimumab and found that clinical response was associated with an increase in absolute lymphocyte count ($p = 0.008$), absolute T-cell count ($p = 0.02$) and the absolute number of activated T cells in peripheral blood ($p = 0.003$) after 1 series of treatment[58]. Other recent studies from our laboratory and others have shown that blockade with a CTLA-4 antibody broadens the intratumor and peripheral T-cell repertoires[59] (Bjørn J et al., Oncotarget 2017 in press). Our laboratory has also shown that *in vitro* stimulation of TIL cultures from OC patients with a CTLA-4 antibody increases the frequency of CD8+ T cells. We were able to generate TIL cultures in 5/5 cultures stimulated with Ipilimumab vs. 3/5 and 4/5 cultures not stimulated with Ipilimumab. The Ipilimumab stimulated cultures contained an increased amount of TILs per fragment in 4/5 cultures and an increased amount of CD8+ TILs in 5/5 cultures compared to the unstimulated ones.

Thus, pretreating the patient with a CTLA-4 antibody before tumor harvest may lead to increased quality of the TIL product and *in vitro* stimulation with a CTLA-4 antibody may increase the quality of the TIL product even further.
Studies investigating the expression of programmed cell death-ligand 1 (PD-L1) in OC has shown an uncertain correlation to prognosis[60,61], but also that a high intratumoral amount of CD3+ T cells is positively correlated to prognosis. It is hypothesized that PD-L1 expression is upregulated in tumor tissue as a compensatory reaction to an immune response against tumor cells and suppresses antitumor CD8+ T cells[60–62].
In addition, we have shown that a significant fraction of tumor-reactive TILs in the infusion product express an increased level of PD-1 and that in vitro addition of anti-PD1 leads to increased tumor killing by these TILs (unpublished data).
Therefore, clinical targeting of the PD-1 receptor could potentially decrease the suppression of the infused tumor reactive CD8+ T cells.

Ipilimumab

Ipilimumab is an anti-CTLA-4 antibody that inhibits a decrease in the activation and proliferation of T cells when presented to tumor antigens by professional antigen presenting cells in the *priming phase*, as mentioned earlier. Ipilimumab is approved by the European Medicines Agency (EMA) for the treatment of metastatic malignant melanoma and is undergoing clinical trials in combination with PD-1/PD-L1 inhibitors in non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer and metastatic hormone-refractory prostate cancer[63].
Ipilimumab will be administered in the approved dosage of 3 mg/kg for 1 dose 2-6 weeks before surgical removal of tumor tissue for TIL production.

Nivolumab

Nivolumab is an anti-PD-1 antibody that inhibits suppression of the activated T cells in performing their effector functions in targeting the tumor cells in the tumor microenvironment in the *effector phase*, as mentioned earlier. Nivolumab is approved by the EMA for the treatment metastatic malignant melanoma, NSCLC, renal cell carcinoma, Hodgkin's lymphoma, head and neck cancer[64].
Nivolumab will be administered in the approved dosage of 3 mg/kg starting 2 days before T-cell therapy and every 2nd week continuing after discharge in the outpatient clinic for a total of 4 series.

The rationale of the treatment regimen used in the study

The ACT regimen has been determined according to our previous study carried out in MM[2] and OC (unpublished data).

Purpose and hypothesis

Primary

1) To assess tolerability and feasibility of the treatment.

Secondary

1) To clarify whether T-cell therapy in combination with checkpoint inhibitors for patients with advanced ovarin-, fallopian tube- and primary peritoneal cancer can induce a measurable immune response against tumor cells.
2) To describe objective responses using RECIST 1.1.
   PERCIST and irRC will be used exploratively.

Furthermore, overall survival (OS) and progression-free survival (PFS) will be described.

Study design

The study is an open label phase I/phase II study for patients with advanced ovarian-, fallopian tube- and primary peritoneal cancer. 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.

We expect to include and treat 6 patients initially. If feasible and tolerable, additional 6 patients will be treated giving a total of 12 treated patients.
We expect to have completed initial patient enrolment and treatment within 2 years and 6 months follow-up within 3 years.

The course of treatment consists of 3 steps followed by clinical controls and evaluation-scans as follow-up.

- Step 1: Screening, inclusion, Ipilimumab treatment and surgical removal of tumor material followed by production and growth of TILs in the laboratory.
- 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: Evaluation of the effect of treatment during follow-up.

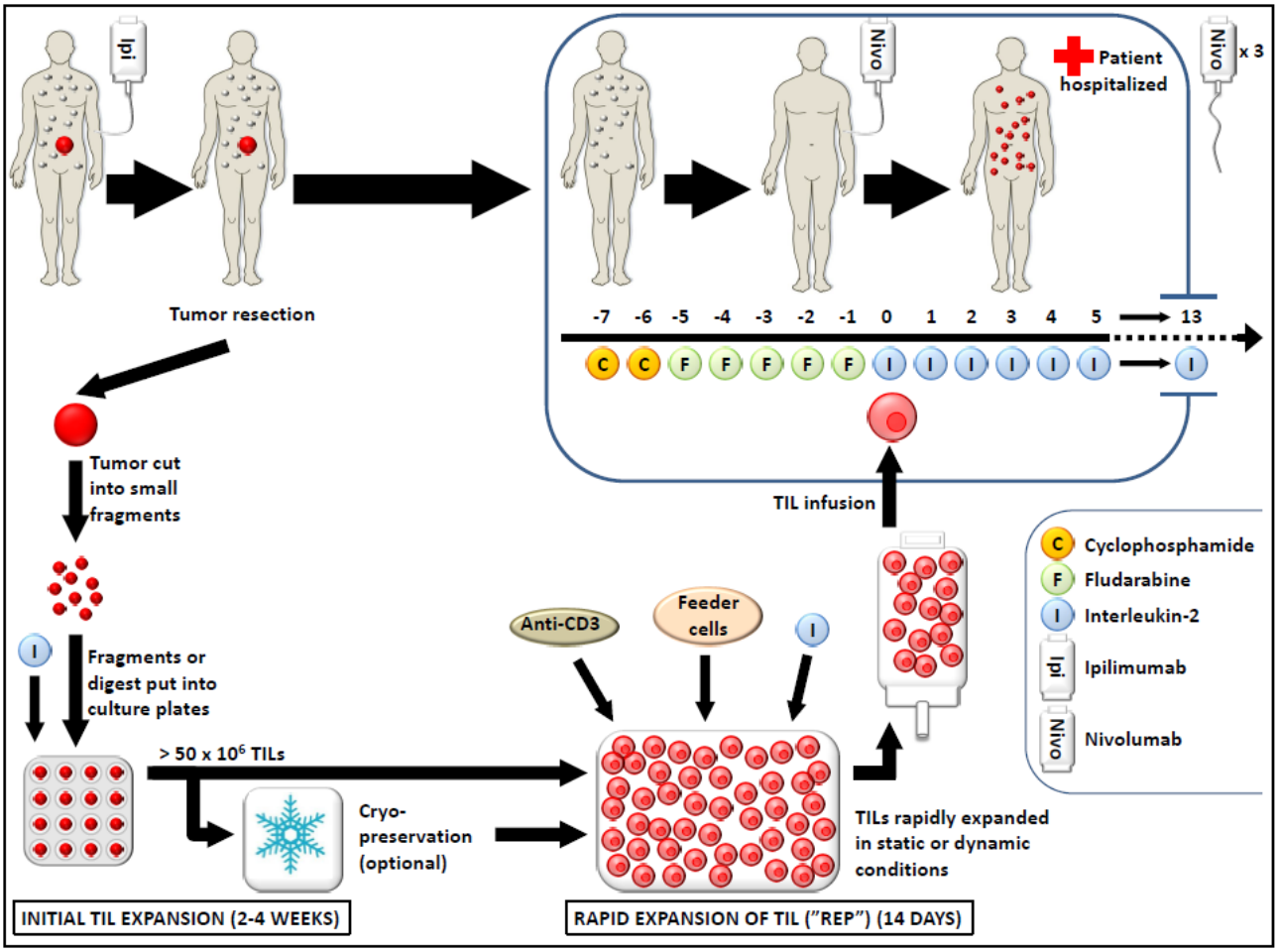

Figure 2: *Schematic presentation of the course of treatment. One dose of Ipilimumab (3 mg/kg) is administered 2-6 weeks before tumor resection. Tumor tissue (metastasis or primary tumor) of minimum 1 cm³ is surgically removed from the patient and transported to the laboratory under sterile conditions where the tumor is separated into suitable fragments of 1-3 mm³ and placed in growth wells with a growth media and IL-2. TILs are then initially grown for 2-4 weeks until a cell-number of minimum 50 x 10⁶. At this point the cells can be cryo-preserved for later use or pass on to the Rapid Expansion Protocol (REP) in which the T cells for 2 weeks are stimulated with anti-CD 3 antibody, allogeneic, radiated PBMC (peripheral mononuclear blood cells), feeder cells and IL-2. After stimulation, the expanded TILs (now billions) are then washed, pooled and re-infused intravenously back in to the patient. Seven days of lymphodepleting chemotherapy is given to the patient before TIL infusion with cyclophosphamide (C) on day -7 to day -6 and Fludarabine (F) on day -5 to day -1 with the purpose of removing all existing lymphocytes in the patient to make room for the infused TILs and remove regulatory T cells (Tregs). Nivolumab (3 mg/kg) is administered 2 days before TIL infusion to boost the activity of the reinfused TILs. Subcutaneous IL-2 (2 MIU) injections is started approximately 6 hours after TIL infusion (day 0 to day 5) in order to further increase activation and to stimulate the infused TILs for further expansion within the patient. Nivolumab treatment is continued in the outpatient clinic for a total of 4 series.*

Study population

Patients with histological verified advanced ovarian-, fallopian tube- or primary peritoneal cancer will be candidates for this study. The patients need to have an acceptable performance status, no major co morbidities and acceptable organ functions.
Only patients in which it is possible to grown T cells from their tumor tissue will be offered treatment with T cells in the study and only patients receiving treatment will be included in the final study population.

Criteria of in- and exclusion

Criteria of inclusion

All of the criteria listed in the following need to be met before patient inclusion.

1. Histological proven advanced ovarian-, fallopian tube or primary peritoneal cancer with the possibility of surgical removal of tumor tissue of > 1 cm^3. All histologies can be included.
2. Progressive or recurrent resistant disease after platin-based chemotherapy (platinum resistant) or progressive or recurrent disease after second line or additional chemotherapy.
3. Age: 18 – 70 years.
4. ECOG performance status of ≤1 (Appendix 2).
5. Life expectancy of > 6 months.
6. At least one measurable parameter in accordance with RECIST 1.1 –criteria's.
7. No significant toxicities or side effects (CTC ≤ 1) from previous treatments, except sensoric- and motoric neuropathy (CTC ≤ 2) and/or alopecia (CTC ≤ 2).
8. 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 ≤ 5 times upper normal limit
    - Sufficient coagulation: APPT<40 and INR<1,5
9. Men and 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.
10. Signed statement of consent after receiving oral and written study information
11. Willingness to participate in the planned controls and capable of handling toxicities.

Criteria of exclusion

Patients will be excluded if they meet one of the criteria's listed below

1. A history of prior malignancies. Patients treated for another malignancy can participate if they are without signs of disease for a minimum of 5 years after treatment.
2. Known hypersensitivity to one of the active drugs or one or more of the excipients.
3. Severe medical conditions, such as severe asthma/COLD, significant cardiac disease, poorly regulated insulin dependent diabetes mellitus among others.
4. Creatinine clearence < 70 ml/min*.
5. Acutte/chronic infection with HIV, hepatitis, syphilis among others.
6. Severe allergies or previous anaphylactic reactions.
7. Active autoimmune disease, such as autoimmune neutropenia/thrombocytopenia or hemolytic anemia, systemic lupus erythematosis, Sjögren's syndrome, sclerodermia, myasthenia gravis, Goodpasteur's disease, Addison's disease, Hashimotos thyroiditis, active Graves disease.
8. Pregnant women and women breastfeeding.
9. Simultaneous treatment with systemic immunosuppressive drugs (including prednisolone, methotrexate among others)**.
10. Simultaneous treatment with other experimental drugs.
11. Simultaneous treatment with other systemic anti-cancer treatments.
12. Patients with active and uncontrollable hypercalcaemia.

* 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 systemic dose of ≤10 mg prednisolone or a transient planned treatment that can be stopped before TIL therapy can be tolerated.

Evaluation before inclusion

The following need to be carried out before the start of treatment:

- 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, 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 antibody, HTLV IgG, EBV, Trepone.
  - Endocrine: TSH, T3, T4.
  - Female specific: Estradiol, prolactin.
  - Ovarian Specific: CA-125
- 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 contraception's regularly within the last 6 months.
- Baseline tumor evaluation: CT, MR or PET-CT scans can be used. A PET/CT scan is preferred.
- Reviewing the checklist for inclusion/exclusion for treatment.

Treatment plan

The course of treatment can vary between patients depending on the time period from surgery to treatment. In most cases (e.g. TILs undergoing massive expansion and infusion without cryopreservation of intermediate products), the patients will receive treatment approximately 4-6 weeks after the operation and in these cases the course of treatment from Ipilimumab treatment before surgery until the first evaluation with diagnostic imaging (6 weeks after treatment) will span across approximately 3-4 months.

Surgical removal of tumor tissue for TIL expansion can be repeated if not possible to grow TIL after initial surgery if additional accesible tumor tissue is present and based on an individual clinical assessment. Presurgical treatment with Ipilumumab is to be administered 2-6 weeks prior to surgery and is to be repeated if more than 6 weeks has passed since the initial Ipilimumab treatment.

Stem cell harvest by leukapheris will not be performed since we and others have observed that the patients' hematopoietic system recovers after the lymphodepleing chemotherapy without the need for stem cell support[65–68] (unpublished data).

Location		Hospital												Outpatient	
Week	*	-2	-1							0-1				3	5
Day	*	-8	-7	-6	-5	-4	-3	-2	-1	0	1-11	12	13	27	41

Ipilimumab (3 mg/kg) iv	x														
Pt. is admitted		x													
Cyclophosphamide (60 mg/kg) iv			x	x											
Fludarabine (25 mg/m^2) iv					x	x	x	x	x						
Nivolumab (3 mg/kg) iv								x				x		x	x
TILs iv										x					
Pegfilgrastime (6 mg) sc										x					
IL-2 (2 MIU) subcut. inj.										x	x	x	x		

* Ipilimumab is administered 2-6 weeks before surgery

Examination plan during the course of treatment

The patients are continuously monitored on several parameters before, during and after TIL infusion.

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. Patients will be excluded upon clinical or radiological progression.

Time point	Screening	Before Ipilimumab	Tumor tissue removal	<14 days to admission	At admission	Daily during hospitalization	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
Week		a	b	-4 → -2	-2	-1→c	-1	0	c	2	3	5
Day		a**	b	-22 → -9	-8	-7→c	-5	0	c	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, Temp.	x	x			x	x	x	x	x	x	x	x
Toxicity assessment (CTC)		x			x	x	x	x	x	x	x	x
Screening blood tests	x											
Research blood tests		x			x	x*		x	x			
Standard blood tests		x			x	x	x	x	x	x	x	x
Immunological blood tests	x				x							
Ovarian specific blood tests	x	x			x		x		x	x	x	x
ECG	x	x			x		x			x		
Cr EDTA				x								

Urine sample					x							
Biopsy			x									
Tumor Evaluation	x			x					x			

[a] At least 14 days before tumor tissue removal (b)

[b] Day of tumor tissue removal.

[c] Depends on recovery period after TIL infusion, approximately at day 11.

* Every second day untill discharge.

** Examination and blood tests will only be repeated if clinically indicated or >2 weeks from screening

Overlapping samples are only taken once.

Screening blood tests: Se previous section "Evaluation before inclusion".

Research blood tests: See section "Blood samples for immunological monitoring" under the section "Immunological Monitoring".

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, Estradiol.

Ovarian specific blood tests: CA-125.

Tumor evaluation: CT, MR, PET-CT or a PET-MR scan can be used. Before inclusion a current scan need to be available for review. A PET/CT scan is preferred. Before hospital admission a baseline scan has to be performed.

Medicinal products used in the study

The medicinal products used in this study are Ipilimumab, Nivolumab, cyclophosphamide, fludarabine phosphate and interleukin-2. Mixing and storage of the products is carried out according to existing standard guidelines at the Department of Oncology.

Ipilimumab

Ipilimumab is an antibody targeting the CTLA-4 receptor on T cells. The antibody functions as a negative regulator by blocking of the suppressive signaling through the receptor and thereby potentiating T-cell activity.

Ipilimumab is administered in the outpatient clinic 2-6 weeks before surgical removal of tumor tissue for TIL production as an intravenous infusion. The dosage is 3 mg/kg body weight and administration takes 90 minutes.

Cyclophosphamide

Cyclophosphamide is given as an intravenous infusion for two consecutive days in a dosage of 60 mg per kg of body weight. The treatment takes place during hospital admission and with supplementary hydration and Mesna injections.

Fludarabine phosphate

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

Nivolumab

Nivolumab is an antibody targeting the PD-1 receptor which is expressed on activated T-lymphocytes. By blocking this receptor, Nivolumab prevents inhibition of T-lymphocyte activity through binding of the receptors ligands, PD-L1 and PD-L2.

Nivolumab is administered 2 days before TIL administration as an intravenous infusion over 60 minuttes in a dosage of 3 mg/kg body weight and every 2 weeks for a total of 4 doses.

TILs

The tumor specific T cells are infused intravenously back in to the patient during hospitalization on the day after the last dosage of fludarabine phosphate (day 0). The number of T cells in the product depends on the possible in vitro degree of expansion and is therefore variable, but will normally consist of approximately 10^{10} cells. See the Investigational Product Medicinal Dossier (IMPD) for more information.

Interleukin-2

A daily subcutaneous injection with IL-2 (2 MIU) will be administered from day 0 to 14. 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. Injections for self-administration will be labeled by the cytostatica unit, Herlev Hospital, and in accordance with Annex 13 of the GMP rules.
The supplied amount of IL-2 injections for selfadministration will be registered by use of batch number, so that the total amount of administed IL-2 can be determined. Moreover, IL-2 will be labeled in accordance with current guidelines regarding study drugs.

Pegfilgratim

Is an analog of human granulocyte colony stimulatory factor (G-CSF). It works by stimulating 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[69].
Pegfilgrastim is administered as a single dose of 6 mg s.c. at 2 hours after TIL infusion to help patients recover from the lymphodepleting chemotherapy by.

Guidelines for concomitant treatment

Supportive treatment is given on ordinary medical indications estimated by the physician responsible for the treatment. Any measures should be specified in the patient chart and flow sheet. The following is meant as guidance and other medications can be administered as it is seen fit. One exception is systemic corticosteroid which can be administered du to immune related adverse events (irAEs) in relation to the treatment with checkpoint inhibitors.

<u>During lymphodepleting chemotherapy:</u>
In order to protect the mucosa of the bladder the following is administered:

- Supplementary fluid therapy during cyclophosphamide treatment.
- Inj. Mesna (25% of the cyclophosphamide dosage i.v. x 4 daily on day -7 and -6).

In order to prevent and relieve nausea during chemotherapy (day -7 to -1) the following is administered:

- Inj. Aloxi 250 μg i.v. on day -7 and -5.
- Tabl. Emend 125 mg on day -7, 80 mg on day -6, 80 mg on day -5.
- Tabl. Motilium 20 mg x 3.
- Tabl. Temesta 1-2 mg max x 4 if needed.
- Tabl. Pantoloc 40 mg x 1-2 daily.

<u>After TIL infusion:</u>

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

<u>During IL-2 treatment:</u>
Low-dose subcutaneous IL-2 is expected to be safe and tolerated with minimal toxicity as previously described[3]. If supportive treatment is indicated, it will be as in our prevous trials with TIL therapy.
In order to prevent deranging of electrolytes as well as low blood pressure during IL-2 treatment the following is administered:

- Fluid therapy in accordance with appendix 4.

In order to prevent and relieve nausea during IL-2 treatment (day 0 to 5) the following is administered:

- Tabl. Motilium 20 mg, if needed max x 3.
- Ondansetron 8 mg x 1 if needed max x 2.
- Imolope 2 mg if needed max x 8.

In case of fever:

- Tabl. Paracetamol 1000 mg if needed*, max x4.

*Only if patients develop fever >41◦c.

To prevent opportunistic infections:

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

In case of fever:

If simultaneous neutropenia and fever occurs it will be treated in accordance with local guidelines described in appendix 5.

In case of diarrhea:

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

Immune related adverse events (irAEs) to checkpoint inhibitors:

- irAEs are to be managed in accordance with local guidelines described in appendix 6.

In case of anemia and thrombocytopenia:

- Transfusions with blood should be administered if hgb ≤ 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.
- Radiated and filtered blood is to be administered from day -7 and 6 months ahead if indicated.

Others:

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.

End of treatment

Normal end of treatment including follow-up

Patients who finish the treatment course will be followed with clinical controls and diagnostic imaging before hospital discharge following TIL treatment, at 6 and 12 weeks after treatment and every 3 months hereafter. After 2 years the controls will change to every 6 months for a total of up to 5 years or until progression.

A clinical examination, blood tests and diagnostic imaging will be performed in connection with every evaluation. At the first three evaluations the blood tests will include immunological parameters and a toxicity assessment (any late onset adverse events will registered throughput the entire follow-up period). If tumor tissue is accessible, a tumor biopsy will be performed at the first evaluation and/or after progression.

End of study is defined as 6 months after the last patients' treatment or after progression. OS and PFS will subsequently be followed for up to 5 years.

Follow-up schedule

Evaluation, month	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
BP, P, PS, Weight	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
ECG	x	x	x												
Toxicity assessment (CTC)	x	x	x												
Immunological blood tests	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
Ovarian specific blood tests	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tumor evaluation	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Tumor biopsy*	x														

*If possible, a biopsy is also perfomed at the time of progression.

Research blood tests: See section "Blood samples for immunological monitoring" under the section "Immunological Monitoring".

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, APTT, 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, Estradiol.

Ovarian specific blood tests: CA-125.

Tumor evaluation: CT, MR, PET-CT or a PET-MR scan can be used. A PET/CT scan is preferred.

Tumor biopsy: A tumor biopsy is carried out at 6 weeks evaluation and in case of progression if it is accessible and safe to perform. This is further explained in the "Tumor biopsies" section.

Early termination of treatment

Several reasons can lead to early termination of the planned treatment course:

Not possible to grow TILs: The patient cannot be offered treatment if it is not possible to grow TILs.

Patients own wish: The treatment can be stopped at any time the patient wishes so.

Medical decision: The treatment can be stopped because of medical conditions at any time the investigator finds it in the patients' best interest.

Other treatment: Patients will be excluded at any time a new treatment with an experimental drug or 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 on vital indication an in agreement with the physician responsible for the protocol.

Adverse events: Treatment is terminated if adverse events of a degree that makes completion of the study impossible do occur.

Patients who stop IL-2 treatment prematurely will still be followed in accordance with the protocol.

If a patient is excluded before infusion of TILs a replacement subject will be found. They will be followed until end of adverse events caused by the treatment, but will not be followed with subsequent controls.

Subsequent treatment

Patients that are excluded from the study can receive other treatment freely. If patients progress they can receive other treatment.

Production of TILs

Acquisition of tumor tissue

The patients will receive oral and written information as described elsewhere and a written consent will be obtained before surgery. A tumor biopsy will be performed after sufficient tissue material for pathological examination has been removed. The biopsy will be labeled with date and patient code, placed in a sterile container and transported to the GMP facilities 54J7 or 54I6 at Herlev Hospital for further processing. See the IMPD for more details.
If present, ascites fluid will collected during surgery, if possible to do so without increased risk to the patient. Ascites fluid will be labeled and transported in the same way as the biopsy, Acquisition and examination of ascites fluid will be for research purposes only. See the section 'Immunological monotoring' and 'Additional research analyses' for more details.

Establishment of "Young TIL" cultures

T cells are expanded using a recently established method for "Young TILs"[36]. The tumor mass will be isolated with a scalpel, and cut into small 1-3 mm^3 fragments. Fragments (typically from 24 to 72 fragments in total) will be 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 tumor tissue in the IL-2 based media. IL-2 belongs to the group of homeostatic cytokines which are characterized by having a positive effect on the activation of tumor specific T-cell and thereby tumor cell killing. T-cell density is kept at about $1x10^6$ cells/ml growth media containing the immune stimulating cytokine IL-2. Cell cultures from the different fragments are pooled to a single cell culture. T-cell expansion is performed unselected to produce a polyclonal TIL repertoire targeted against multiple epitopes to potentially achieve more effective tumor cell destruction *in vivo*. The establishment of "Young TIL" cultures usually takes 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 are expanded to approximately $5x10^7$ cells they are either frozen for later use or transferred directly for further expansion by use of the Rapid Expansion Protocol (REP) in which TIL are grown with radiated (40 Gy) allogeneic PBMCs (peripheral blood

mononuclear cells) that work as “feeder cells”, IL-2 and anti-CD3 antibody that activates the TILs. In this way it is possible to reach a large number of activated tumor specific T-cell with a high level of activity against tumor associated antigens (TAA) and tumor in 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 infusion product

The infusion bag is labeled with patient ID and a patient specific transport schedule is filled out and both are then placed in a secure hatch. A trained physician controls the information on the infusion product and transport schedule, signs the latter and transports the infusion product to the patient. Before administration, the infusion product is controlled again by the treatment staff and matched to the ID of the patient through patient identification.

Phenotype- and clonotype determination

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

Adverse events, potential risks and precautions

Adverse Events

Adverse events (AE) are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational treatment. All AEs reported spontaneously by the subject or observed by the investigator or his 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. The severity and relation to the study medication will be assessed in accordance with the guidelines described in the following.
The investigator must attempt to identify all clinical and objective events from patients receiving treatment and determine their relation to the study medication. The investigator determines the relationship between AEs and treatment using the following guidelines:

Grading of Adverse Events

The severity of an AE refers to the intensity of the reaction.

Events are graded using CTCAE version 4.0 (appendix 3)[70]. The following scale can be used if this grading is not applicable:

1 = light
2 = moderate

3 = severe
4 = life threatening
5 = lethal

Patients experiencing AEs will be monitored with the relevant clinical evaluations and laboratory investigations assessed by the attending physician. 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 to be recorded in the eCRF unless they have caused a clinical event, resulted in termination of the treatment or otherwise meet the criteria of a serious adverse event (see the following).

Serious Adverse Events

A serious adverse event (SAE) is to be reported to sponsor within 24 hours and is defined as any medical occurrence or effect that occurs at any dose:

- 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 a significant medical event

Guidelines for adverse events possible relation to the treatment

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 not reasonably explained by the patient's known clinical condition

3 Related – clear temporal relation with laboratory confirmation or a positive retreatment test

If the event is assessed as being caused by the investigational treatment it is classified as an adverse reaction (AR) or a serious adverse reaction (SAR).

Adverse Reactions

An adverse reaction (AR) can be expected if it is described in the IMPD or the relevant product summary, or unexpected if the grade or severity does not correlate with the product information in the before mentioned documents.
If the AR is unexpected, meets the criteria of a serious adverse reaction (SAR) and is found related to the investigational treatment it is classified as a suspected unexpected serious adverse reaction (SUSAR).

Reporting of Adverse Events and Adverse Reactions

Investigator reports SAEs, SARs and SUSARs to sponsor within 24 hours. Sponsor reports SUSARs to the Danish Medicines Agency within 7 days if considered life threatening or fatal, 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 study patients' safety 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:
    - 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:
    - 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 to be registered in the eCRF.

Known Adverse Reactions

Chemotherapy

The adverse reactions to chemotherapy described in the following are all general adverse reactions seen when the drugs are given as the primary treatment for oncological and hematological diseases.
In these cases the treatment is often given over several series. In this study, the treatment will be given as a single dose, why we expect a milder adverse reactions profile.

Cyclophosphamide

The dose limiting toxicities in patients' receiving treatment with cyclophosphamide are myelosuppression (neutropenia, thrombocytopenia and anemia) and urotoxicity (cystitis, haematuria and hemorrhagic cystitis). Sufficient treatment with Mesna alongside rehydration markedly reduces the frequency and severity of the urotoxicity.
Other common adverse reactions are alopecia, nausea and vomiting.
Patients receiving treatment with Cyclophosphamide can experience the following adverse reactions described in the product resumé[53] found at www.laegemiddelstyrelsen.dk.

Fludarabine phosphate

The adverse reactions are myelosuppression (neutropenia, thrombocytopenia and anemia), infection including pneumonia, coughing, fatigue, limpness, nausea, vomiting and diarrhea. Other common adverse reactions are shivering, edema, malaise, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis and rash. Severe opportunistic infections have occurred in patients receiving treatment with fludarabine phosphate. Deaths have been recorded as a cause of severe adverse reactions.
Patients receiving treatment with fludarabine phosphate can experience the following adverse reactions described in the product resumé[54] found at www.laegemiddelstyrelsen.dk.

Checkpoint inhibition and irAEs

The antibodies used in this study targets activated T cells in the immune system by blocking 2 natural brakes in T-cell activation (CTLA-4 and PD-1) which allows a potential immunological

reaction against tumor cells. Due to these mechanisms of action, immune related adverse events (irAE) can occur, which are often mild, but can aggravate and require treatment. Immune related adverse events include skin toxicities (erythema, exema or exanthema with generalized itching and urticarial, as well as possible worsening of existing autoimmune skin disordes (such as psoriasis and roseaca), gastrointestinal toxicities (diarrhea/colitis), endokrinopathias (hypophycitis, hypothyriosis, hyperthyriosis), hepatotoxicity, pulmonary toxicity (pneumonitis), ocular toxicity, myocarditis, neuropathia and immune related nefrotoxicity.
Patients can experience irAEs from multiple organ systems and irAEs can also occur late during or after the course of treatment.
See appendix 6 for details regarding handling of irAEs.

Ipilimumab

Patients receiving treatment with the CTLA-4 antibody Ipilimumab can experience adverse reactions as described above and in the product resumé[63] found at www.ema.europa.eu.

Nivolumab

Patients receiving treatment with the PD-1 antibody Nivolumab can experience adverse reactions as described above and in the product resumé[64] found at www.ema.europa.eu.

TILs

No SARs are expected due to TIL infusion. The patients might experience transient fever, shivering and mild dyspnoea with a few cases of an observed light decrease in saturation. There is a theoretical risk of the development of allergic reactions/anaphylactic shock. This has not yet been observed according to literature.
See the IMPD for more details on previous human exposure and anticipated risks.

Interleukin-2

Frequency and severity of adverse reactions to IL-2 has generally shown to be related to the route of administration, dose and frequency of treatment. Most adverse reactions are self-limiting and will disappear within 1-2 days after cessation of treatment. Subcutaneous IL-2 administration can cause a local inflammatory reaction with reddening and induration at the site of injection. WHO grade 1 toxicity characterized by flu-like symptoms with muscle soreness, joint pains, malaise and a slight increase in temperature with a duration of 12-18 hours has been observed when administering IL-2 locally. Experience shows that fatigue is the most common adverse event when administering the IL-2 dosage used in this study.
Patients receiving treatment with IL-2 can experience the following adverse reactions described in the product resumé[71] found at www.laegemiddelstyrelsen.dk and adverse reactions will be treated as described in appendix 4.

Risks and disadvantages regarding surgery and test sampling

Risks associated with removing tumor tissue

Prior to inclusion it will be assessed whether it is possible to remove some of the patients own tumor tissue in a minor operative procedure. Surgery will mainly be performed by physicians at the Gynecological Department at Herlev Hospital or by physicians from other specialties if necessary. The patient will not be able to participate in the study if no tumor tissue is available for removal or if removal will put the patient at a too large risk.

Risks associated with biopsies

There is a slight risk of infection and/or bleeding when performing a biopsy. Pain and bruising might also occur in the area.

Risks associated with blood tests

Pain and bruising can occur in the area. Blood testing will involve frequent hospital visits.

Monitoring and precautions

Hematological parameters

Careful hematological monitoring of blood counts is indicated for all patients during treatment. Leukocyte count, platelet count and hemoglobin values will be controlled at fixed intervals. Measurements will be made before start of chemotherapy, IL-2 and daily during treatment until neutrophile counts is > 500/µl and leukocyte count is > 1000/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 infections

Any obstruction of the efferent 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. Treatment will be terminated if cystitis associated with micro- or macroscopic haematuria occurs during treatment with Cyclophosphamide. The patients' urine will be controlled for the presence of microscopic haematuria before start of treatment with Cyclophosphamide.

Cardiotoxicity

Cardiotoxicity is especially seen when administrating high doses of Cyclophosphamide (120-240 mg/kg body weight). An electrocardiogram 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 experiences symptoms from the cardiovascular system (e.g. chest pains, shortness of breath). Myocarditis has been described

following high dose Il-2 treatment, if suspected CKMB, TPN, ECG and cardiology consult should be performed.

Immune-related toxicity

Is monitored and handled as described in appendix 6.

Infertility

Patients receiving chemotherapeutic treatment have a risk of affecting their fertility in the future. It is not known whether T-cell therapy increases the risk of cross reaction from T cells to normal ovarian tissue.

Live vaccines

Vaccination with live vaccines is to be avoided prior to- and immediately after treatment with chemotherapy because of the immunosuppressive effect.

Interactions

Cyclophosphamide inhibits cholinesterase activity which increases the effect of depolarizing muscle relaxants such as Suxamethoniumchloride. This can results in prolonged apnea when anesthetized. The anesthesiologist is to be informed if the patient has received treatment with Cyclophosphamide within 10 days before treatment with Suxamethoniumchloride. The combination should be avoided.

The patient is to avoid eating grapefruit or drinking grapefruit juice since grapefruit contains a substance that can impair the activation of Cyclophosphamide and thereby its effect.

Transfusion related graft-versus-host reactions have been observed in patients receiving treatment with fludarabine phosphate after transfusion with non-radiated/non-filtered blood. Patients requiring blood transfusion within ½ a year from treatment with fludarabine phosphate is therefore to receive only radiated or filtered blood. An agreement with the blood bank at Herlev Hospital has been made so that there will be ordered radiated blood only for these patients for ½ a year after treatment. All blood in the Capital Region is filtered.

Effect evaluation, data processing and monitoring

Effect evaluation

Primary effect parameter

Safety and toxicity:

Registration of all AEs and unexpected events that occur in relation to the treatment will be done in accordance with the CTCAE v.4 criteria (appendix 3).

Secondary effect parameters

Immune monitoring:
Patients will be followed continually with *in vitro* analysis of the specific T-cell reactivity against tumor antigens to evaluate the immunological effect of treatment. The immunological response against tumor antigens before and after treatment will be compared. These analyses will be done on blood samples and tumor biopsies.
Clinical evaluation:
The clinical effect of treatment will be rated using the objective response rate in accordance with RECIST 1.1 and overall survival (OS) and progression-free survival (PFS) will be described. Furthermore, PERCIST[72] and irRC[73] will be used exploratively.

Response Criteria

RECIST

Clinical evaluation will be done in accordance with RECIST 1.1 Guidelines[74]:

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.
Progression (PD): Defined as a > 20 % increase in the sum of all measurable parameters longest diameter *or* the appearance of new lesions.

Complete and partial response is to be verified by examination at a minimum of 4 weeks after documentation of the response at the earliest.

Immunological monitoring

Blood samples for immunological monitoring

Blood samples with 100 ml heparinized blood for immunological monitoring and 10 ml blood in a dry glass for freezing of serum are taken: before treatment with ipilimumab, before start of treatment (at ”baseline”), at hospital discharge (approximately 1 week after T-cell infusion) and 6 and 12 weeks after T-cell infusion (see the examination plan and follow-up schedule). Blood samples will be taken for immunological monitoring every 3rd month at evaluation hereafter until the patient is withdrawn from the study. In addition, serum samples (10 ml blood sample) will be taken at day 0 before TIL infusion, 2 hours after TIL infusion and every 2nd day until hospital discharge.
A total of 500 ml of blood will be collected for research purposes in the period from time of surgery until the patient meet for the first evaluation after treatment. These blood samples

are taken to assess the effect of treatment on the immune system for research purposes. 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 if the blood count is not acceptable (> 6 mmol/l).

Mononuclear cells from the peripheral 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 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.

Tumor biopsies

Biopsies are sought taken from accessible tumor lesions or involved lymph nodes depending on localization and accessibility. Biopsies are preferred at evaluation 6 weeks after treatment and at progression if possible (see the examination plan and follow-up schedule).
Biopsies will be performed guided by ultrasound and under sterile conditions by the Ultrasound Department, Herlev Hospital, if the involved areas are not directly accessible. The procedure will be performed in the outpatient clinic and the size will be approximately 5 $mm^{3.}$

Biopsies will be examined for the concentration of immune cells. Furthermore, TILs will be isolated from the lesions and analyzed for clonotype and specificity.

Specimens for Future Biomedical Research

Only leftovers from analyses specifically described in the protocol will be transferred to the biobank for future biomedical research, as described on page 48 “Research biobank including Future Biomedical Research Biobank”. There will be no extra samples to be obtained during this study. These specimens may be used to study various causes for how subjects may respond to the immunotherapy. These speciemens will be stored to provide a resource for future trials conducted by CCIT focused on the study of biomarkers responsible for how immunotherapy works, other pathways immunotherapy may interact with, or other aspects of disease.

Additional research analyses

Isolation of tumor cells

Tumor cells are isolated from the tumor fragments or ascites fluid by the use of enzymatic processing or seeding of cells from the tumor fragments or ascites fluid and are then frozen for later use in determining T-cell anti tumor activity. This exploratory analysis will not influence any prior or subsequent surgical decisions or medical treatment of the patients enrolled.

Tumor-associated lymphocytes (TALs) and chemokines

Lymphocytes found in ascites will be isolated with gradient centrifugation. A portion will be frozen and stored for additional immunological analyses, while a portion will be cultured immediately by using the exact methods used with TILs. Remaining ascites fluid will be centrifuged, and supernatants will be collected and frozen to study which cytokines present in the fluids can influence the activity of TALs.

Cytokine Release Assay

TIL cultures is screened for activity against TAA and autologous tumor by determining their production of the activating cytokines (INF-γ + TNF-α). The production of the activating cytokines is quantified by use of ELISpot technique and intracellular flow cytometric analysis.

Gene-based arrays

The aim of the planned gene analyses is to learn more about;

1. 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

2. Expression of tumor/patient specific mutated genes which could influence the chance of benefit from treatment

Analysis for identifying specific tumor gene expression signatures[75] and mutations in the tumor cells, leading to patient-specific neo-antigens derived from these mutations[76], will be performed.

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. These analyses will not benefit the patients included in this study, but might benefit future patients.

Methods:
Tumor tissue gene expression profiles will be analysed on FFPE tumor tissue. Using Illumina targeted RNA sequencing applicable to degraded RNA we will retrieve gene expression data on approximately 500 cancer/immunity related genes.

Next Generation Sequencing (NGS) 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). Tumor specific gene expression (neo antigens) will be performed by subtracting the two gene profiles from each other. Data regarding potential disease causing genes will be generated as a byproduct of this analysis, but this data will not be used or explored further upon, since only data regarding tumor specific genes will be processed more closely. Therefore, we do not expect to obtain

explicit knowledge regarding disease causing genes. Furthermore, 'Targeted sequencing' on a limitied number of defined genes will be performed on the tumor tissue to obtain a 'immune profile' to determine which genes- and consequently which proteins- are expressed in the tumor tissue. Data will be handled according to national laws.

Patients will have the option of being refered to comprehensive genetic counseling prior to giving their consent. If by chance these analyses will discover known mutations with potential significant impact on patient's health, the case will be discussed with the Clinical Genetics Department, Rigshospitalet, unles the patient has chosen not to be informed as stated in the patient information. The following criteria will determine if further actions are indicated.

- there is a reasonable degree of possibility that a genetic disposition is present,
- there is solid documentation of a link between 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, and
- the link between the genetic disposition and the development of disease has considerable importance for the patient.

If indicated, the patient will be contacted and asked for permission to referral to such Department for additional information and testing.

In case that a patient dies/is dead, 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 (sundhedslovens § 43, stk. 2, nr.2.).

Statistics

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 patient characteristics. The study is designed as a phase I/ phase II study and the primary aim is to determine safety and toxicity to the treatment. A required sample size that allows determination of primary- as well as secondary- and tertiary end points can therefore not formally be calculated.

Data registration and -analysis

The patients are given a patient number at inclusion in the study to secure patient anonymity. Clinical personnel and selected persons in the laboratory will have access to patient information to secure proper treatment.

The principal investigator has access to patient charts to obtain information regarding the cancer disease to be able to compare this information with the project specific analysis performed on cancer tissue and blood tests.

All relevant data is registered in the eCRF (electronic Case Report Form) developed in cooperation with the clinical research unit, KFE. The principal investigator has the responsibility of manufacturing the eCRF and subsequent recording of data when the investigational treatment is finished and eCRFs are reported to sponsor. Sponsor and principal investigator are 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. Drafting of a final report will be conducted in collaboration between the members of the study group.

Analyses will include:

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

Personal data and remaining tests will be coded at the end of the study. All patients receiving T-cell therapy will be included in the statistical analyses. Patients excluded for one of the following reasons will not be included in the statistical analyses:

- Not enough tissue to produce tumor TIL
- Unable to produce TILs in the laboratory
- Withdrawal of consent
- Started other treatment

End of study report

Sponsor will inform the Danish Medicines Agency and Research Ethics Committee within 90 days of study completion. The definition of study completion is 6 months after the last patients' treatment or after exclusion due to progression. In addition, patients will be followed for PFS and OS for up to 5 years. If the study is prematurely terminated, the Danish Medicines Agency and the Research Ethics Committee will be informed of the reason(s) for the termination. Sponsor will 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 within a year of study completion.

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 can be implemented when approved. Changes to the protocol are considered substantial in accordance with 'Vejledning

om anmeldelse, indberetningspligt m.v. (sundhedsvidenskabelige forskningsprojekter)', paragraph 6.0. on www.dnvk.dk and the schedule 'Skema om ændringer (ammendmendts) til kliniske forsøg' on sundhedsstyrelsen.dk.

Ethical aspects

Recruitment of study patients and informed consent

Eligible patients with advanced ovarian cancer will be referred from the Oncological Department at Herlev Hospital or other Oncological centers in Denmark treating patients with these cancers. Information about the study will be given at scientific meetings for physicians at the relevant departments.
Referral of patients is to be made to the uro-gynecological (UG team) visitation office, Department of Oncology, Herlev Hospital.
Contact to eligible patients will be done in accordance with the Danish Health Act, § 46, paragraph 3.

All patients will be informed about the study according to appendix 1.

Insurance

Patients' participation in the study will be covered by "Patienterstatningen".

Ethical aspects

Despite advances in the chemotherapeutic treatment of patients with relapse of ovarian cancer the prognosis is still poor and many of the women are still young and in a good general condition, why the need for new effective treatments is great[10]. The purpose of this study is to improve survival for patients with relapse of ovarian cancer. Based on the current knowledge and the lack of treatment options, the risks and downsides associated with this study are assessed to be acceptable.
Participation is voluntary and is preceded by oral and written information and the treatment will be stopped in case of unacceptable adverse reactions or if the patient wishes so at any time.
The patient will receive treatment after the current guidelines at the department if she does not wish treatment according to the protocol. The study is therefore assessed as ethical proper.

The study follows the Helsinki agreement and the principal investigator is to obtain permission from the Danish Medicines Agency and the Research Ethics Committee.

For future biomedical research, risks to the subject have been minimized. Risks include those associated with venipuncture to obtain the whole blood specimen. This specimen will be obtained at the time of routine blood specimens drawn in the main trial.

Research biobank, including Future Biomedical Research Biobank

In connection with the current study, blood samples (110 ml/blood sample) and tumor biopsies will be stored in coded form at -150 °C in a research biobank at the CCIT in room PA102 until all analysis concerning the study is performed. Samples that are not used in the study will be transferred to a biobank at CCIT for future biomedical research as described below (p. 49).

Analyses will be primarily performed at CCIT. However, some special analyses on tumor tissue or blood test samples may be performed at a partner institution after establishing a specific written agreement. The Research Ethics Committee will be informed if any such agreement is to be established. All patient relevant information will be sent in a codedway. In case the patient's cells will be sent to partner institutions located abroad, these will be handled according to national laws and regulations of the specific nation where these have been sent. In such a case, all patient information will be communicated in coded form. A written data processing agreement will be signed between the data controllers and the data processors abroad. If any gene analysis are to be performed abroad, the data processing agreement will include the 5 criteria (p. 45) regarding the discovery of known mutations with potential significant impact on patient's health, as well as the requirement, that the partner abroad reports back to the primary project managers in Denmark so that relevant actions can be taken as described on p. 43. If data processing is to be performed in a third-country, permission wil be applied for at the Danish Data Protection Agency, or one of the agencies standard contracts will be used.

Samples that are not used in the study will be transferred to a biobank at CCIT for future biomedical research for up to 15 years and is accepted by the Danish Data Protection Agency. If additional studies in other research areas are to be performed on any samples obtained during the conduct of this study/samples that are transferred to a new biobank, a request to do so will be submitted to the Research Ethics Committee as per the 'Act of Processing of Personal Data' §§ 5 and § 10, paragraph 2 and 3. After 15 years, any remaining tissue samples will be disposed of according to the local guidelines for destruction of biohazardous waste. If a patient withdraws his/her informed consent, all biological material is to be destroyed if the patient wishes so.

Reporting to the Research Ethics Committee

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

Administrative aspects and publication

Patient identification

Patients will be given a number after enrollment in the study. This number will be used to identify the patient and will be used in the Case Report Forms (eCRFs). Data and patient materials will be treated in code and confidentially. The number is given sequentially after enrollment in the study and is not based on the patients' initials or birthday.

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, given that the Vancouver rules are met. Positive results, inconclusive results 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, with the project managers as primarily responsible for the preparation. The project managers are co-authors on publications made on the basis of this study. Author succession will be determined based on the individual contributions. Use of study data, oral as well as written, at congresses, teaching or the likes, is only to 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.

Economy

The study is initiated by Center for Cancer Immune Therapy in cooperation with the Department of Oncology, Herlev Hospital, and is partially financed by these two departments. In addition, operational- and salary funding is applied for ongoing. At the current stage, research grants have been obtained from 'The Danish Cancer Society' and 'OvaCure'.

The Research Ethics Committees will be informed if/when new funds support is obtained. None of the physicians involved in the study 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 attachments between the financial supporters and the project managers.
The study is part of the principal investigator Anders Kverneland's PhD project.

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