T cell therapy in combination with peginterferon for patients with metastatic malignant melanoma

A pilot study

EudraCT no.: 2014-001420-29

The clinical trial will be performed according to the present protocol, current Good Clinical Practice (GCP) guidelines and requirements from the authorities. The investigator allows direct access to source data/documents (including patient records) in the event of monitoring, auditing, and/or inspection from the Danish Medicines Agency, the GCP units, or from the health authorities of other countries, respectively.

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Abbreviations

AE = Adverse Event
ALAT = Alanine Aminotransferase
AR = Adverse Reaction
ASAT = Aspartate Aminotransferase
CCIT-DK = National Center for Cancer Immune Therapy
$CD4^+$ Cells = Helper T cells
$CD8^+$ Cells = Cytotoxic T cells
CR = complete response
CTC = Common Toxicity Criteria
CTCAE = Common Terminology Criteria for Adverse Events
CTL = Cytotoxic T lymphocytes
Cy = Cyclophosphamide
DMSO = Dimethyl Sulfoxide
eCRF = electronic Case Report Form
Flu = Fludarabine phosphate
GM-CSF = granulocyte-macrophage colony-stimulating factor
HLA = Human Leukocyte Antigen
IFN-γ = Interferon-gamma
IFN-α = Interferon alpha
IL-2 = Interleukin-2
IMPD = Investigational Medical Product Dossier
KFE = Klinisk Forsknings Enhed (Clinical Research Unit)
LDH = Lactate dehydrogenase
MHC = Major Histocompatibility Complex
MM = malignant melanoma
ORR = Overall Response rate
PD = Progressive Disease
Peginterferon = pegylated interferon alpha 2b
PET = Positron Emission Tomography
PR = Partial Response
PS = Performance Status, ECOG scale 0-4
RECIST = Response Evaluation Criteria In Solid Tumors
REP = Rapid Expansion Protocol
SAE = Serious Adverse Event
SAR = Serious Adverse Reaction
SD = Stable Disease
SUSAR = Suspected Unexpected Serious Adverse Reaction
TAA = Tumor Associated Antigens
TIL = Tumor Infiltrating Lymphocytes
Treg = Regulatory T cells

VEK = Videnskabsetisk Komité (The National Committee on Health Research Ethics)

Synopsis

Indication and treatment

In this pilot study patients with metastatic malignant melanoma (MM) are treated with T cell therapy in combination with pegylated interferon alpha 2b (peginterferon). The treatment includes treatment with the immune modulating cytokine peginterferon, high-dose chemotherapy, infusion of tumor-infiltrating lymphocytes (TILs) isolated from the patient's own tumor tissue and administration of the immune-stimulating cytokine interleukin-2 (IL-2).

Rationale

T cell therapy is an experimental immuno-therapeutic treatment, where TILs are isolated from the patient's own tumor tissue, expanded *ex vivo* into billions of cells and infused into the patient with the intention to eliminate the cancer cells. Before TIL infusion a lympho-depleting chemotherapy regime consisting of cyclophosphamide and fludarabine is administered with the intention to reduce irrelevant T cells and eliminate the regulatory T cells, which are known to be able to inhibit T-cell mediated killing of tumor cells. After TIL infusion IL-2 is administered to activate and stimulate further proliferation of the infused T cells. In this clinical trial the T cell therapy regime described above is combined with peginterferon, which is administered as an injection two days before TIL infusion and 7 and 14 days, respectively, after TIL infusion, with the specific purpose to increase the tumor cell sensitivity to T cell killing and thereby increase the clinical effect of the T cell therapy.

Purpose

The primary purpose is to evaluate tolerability and safety of the treatment. The secondary goals are to characterize the immune response against tumor cells in the tumor as well as in the blood and to evaluate the clinical effect of the treatment, determined by the objective response rate (assessed according to RECIST 1.1). In addition, survival and progression-free survival is described.

Study design

The clinical trial is a pilot study. All patients are included and treated at the Department of Oncology, Herlev Hospital. The patients are referred from oncology departments at Copenhagen University Hospital Herlev, Aarhus University Hospital, and Odense University Hospital. All suitable patients will be treated during hospitalization, with one treatment series stretching over approximately three weeks. The last dose of peginterferon may be given on an outpatient basis. From the removal of tissue from the patient for TIL production until the start-up of the treatment it

will typically take 4-6 weeks. In some cases, TILs will be cryopreserved in preparation for treatment of the patient at a later time point. After the treatment patients will be followed by an immunotherapeutic team with outpatient control visits for up to five years. In the case of progression, patients will be excluded from the study. The inclusion period for the clinical trial is expected to last approximately two years. The inclusion period is expected to last from June 2014 until December 2016. The clinical trial will be reported to the GCP unit, the Danish Medicines Agency, the National Committee on Health Research Ethics, and the Danish Data Protection Agency.

Population

Patients with histologically verified malignant melanoma (MM), which has metastasized, will be candidates for the treatment. The patients, who are included in this clinical trial, must have an acceptable functional status and acceptable kidney and liver function and be free of competing serious conditions. The clinical trial will include 20 patients in total. Only the patients, for whom it is possible to grow T cells from the tumor tissue (approximately 90 % of the patients), will be offered treatment.

Toxicity

We have already in an ongoing phase II study shown that T-cell based immunotherapy with lymphodepleting chemotherapy, TIL infusion, and intermediary dose IL-2 has acceptable toxicity and can be administered safely in an oncological setting. Peginterferon is approved as adjuvant treatment for patients with metastatic melanoma and the side effects are known and manageable.

Clinical response evaluation

Patients are evaluated clinically through outpatient attendance six and 12 weeks after treatment with T cells and thereafter approximately every 3rd month. In addition, imaging-based evaluation (CT, MR, PET-CT, or PET-MR) will be performed before treatment and thereafter in connection with clinical evaluations starting from six weeks after the T cell treatment. Before starting treatment CT/MR scans of the cerebrum will be performed to rule out CNS metastases.

Immunological response evaluation

Project blood samples of 110 ml will be taken at surgery, before TIL infusion, at discharge (approximately 10 days after TIL infusion), and thereafter in connection with clinical evaluation after the treatment. During hospitalization blood serum samples (10 ml blood sample) are taken on day 0 before TIL infusion, two hours after TIL infusion, and thereafter every second day until discharge. Immune cells are isolated from the collected blood samples using the LymphoPrep^{TM} technique and are stored frozen until analysis. Flow cytometry analyses are performed to assess

different immune cells (e.g. $CD4^+$ and $CD8^+$ T cells) before treatment start and during the course of treatment.

Introduction and rationale

Malignant melanoma

In Denmark, approximately 2,100 new cases of malignant melanoma (MM) are diagnosed annually (2011)[1], and the incidence is increasing with approximately 5 % per year. Approximately 5 – 10 % of patients have metastases at the time of diagnosis, and an additional approximately 10 – 15 % develop metastases later in the course of illness[2]. Thus, approximately 350-400 new cases of metastasizing malignant melanoma can be expected per year in Denmark. MM is a very aggressive cancer, and once the disease has metastasized the five-year survival has so far been below 10 %[3].

Adjuvant treatment of malignant melanoma

Interferon alpha 2b (IFN-α) was the first drug, which in a randomized controlled clinical trial showed significant effect on the disease-free survival in patients with malignant melanoma. Based on the ECOG 1684 study, in 1996 high-dose interferon was approved by the FDA as adjuvant treatment for high-risk patients with malignant melanoma[4], and in 2004 high-dose IFN-α was approved by the European Medicines Agency (EMA). In 2011, peginterferon (the pegylated form of IFN-α) was approved by the FDA as adjuvant treatment for intermediary and high-risk malignant melanoma patients[5], even though the ECOG 18991 study, which led to FDA approval, exclusively showed increased disease-free survival without effect on the actual survival[6].

Pegylation of IFN-α, through a covalent bond to polyethylene glycol, increases the compound's biological half-life from 3-8 hours to 22-60 hours without changing its tertiary structure or biological activity. The advantage of this is that peginterferon can be injected only once weekly, whereas standard IFN-α must be injected 3 times weekly[7]. It is still not clarified, which form, dosage, and duration of IFN-α treatment gives the optimal antineoplastic effect with the least possible toxicity[8,9].

Treatment of disseminated malignant melanoma

In Denmark, four treatments for metastatic MM are currently approved: temodal, interleukin-2 (IL-2) + peginterferon, ipilimumab, and vemurafenib. Temodal is a chemotherapeutic, which has been shown to have an objective response rate (ORR) of 10-15 % but no effect on survival[10]. IL-2 is an immune-stimulating cytokine, and IFN-α is immune modulating. The combination of IL-2 and IFN-α is used for patients in good general condition, younger than 70 years of age, and has shown an ORR of approximately 15 % with complete response (CR) in less than 5 %[11] but there seems to be a positive effect on survival. Ipilimumab is one of the new compounds, which in 2012 was approved in Denmark for use after first line treatment of metastatic MM. It is a human monoclonal antibody, which works by blocking the T-cell surface receptor CTLA4 (Cytotoxic T-Lymphocyte Antigen 4),

which works like a natural break in the immune system. In a large phase III study with ipilimumab an ORR of approximately 10 % was found, as well as a CR in 0.6 %, but approximately 20 % achieve stable disease (SD) with significant effect on survival[12,13]. In 2012, Vemurafenib was also approved in Denmark for the approximately 50 % of MM patients, which have an activating mutation in the BRAF-gene. Approximately 50 % of the treated patients have an objective response to Vemurafenib. In addition, up to 30 % have stable disease (stable disease: SD) but the disease recurs among most patients within a median of seven months and the effect on long-term survival and recovery is unknown, as long-term follow-up has not been completed yet. The effect of Vemurafenib is typically fast-acting but most patients will unfortunately only experience short-lasting effects of the treatment[14].
There is therefore still a large need for the development of new and more effective treatments for metastatic MM.

Tumor immunology

In the recent years remarkable progress in the understanding of the immune system reaction against cancer has been made. Thus, it is today clarified that the immune system reacts against certain tumors *in vivo,* and that an immunological response against cancer cells in some cases is associated with a better prognosis[15,16]. Likewise it has been shown that a patient's immune response against tumor cells can be reinforced by treatment with different immune-stimulating therapies, which in some cases appear to be curative[17].

Tumor-infiltrating lymphocytes (TILs)

Tumors are often infiltrated by large numbers of T cells (TILs), which specifically recognizes tumor antigens but typically are inactive. The T cells include both cytotoxic T cells ($CD8^+$ T cells), helper T cells ($CD4^+$ T cells), and regulatory T cells (Tregs), where the latter inhibit tumor cell killing. The T cells consist of different clones with varying degrees of specificity towards tumor antigens, where only few clones have a particularly high specificity. The inactive state of T cells in the tumor tissue is characterized by abnormal intracellular signaling, apoptosis, and a decreased proliferative ability, presumably caused by different immune-suppressing factors in the tumor environment[18,19].
However, it is possible *in vitro* to multiply and reactivate such TILs for tumor cell killing using activating factors such as IL-2[20,21].

T cell therapy

T cell therapy, also called "Adoptive T cell Therapy" is an immunotherapeutic cancer treatment, which has shown very promising results in particular for malignant melanoma. This type of treatment, which uses the patient's own T cells for tumor cell killing, was developed by the American National Institutes of Health (NIH), and in the more recent years several studies have

been published from other cancer research centers in the US and Europe, where more than 500 patients have received the treatment so far[22–27].

TIL based T cell therapy uses the fact that there is a high number of tumor reactive T cells in the tumor tissue compared to peripheral blood[28]. TILs are isolated from the patient's own tumor tissue (metastasis or primary tumor) and expanded *in vitro* for 4-6 weeks into billions of T cells, which are given back into the patient with the purpose to eliminate the cancer cells. This makes T cell therapy a highly specialized and individualized type of cancer immunotherapy.

The actual treatment consists of an initial week of lymphodepleting chemotherapy (cyclophosphamide and fludarabine phosphate) with the purpose of eliminating existing Tregs in the patient and reducing irrelevant T cells. After a week of chemotherapy T cells (TILs) are infused intravenously and shortly thereafter, the patient receives high-dose IL-2 to increase activation and expansion of the infused T cells.

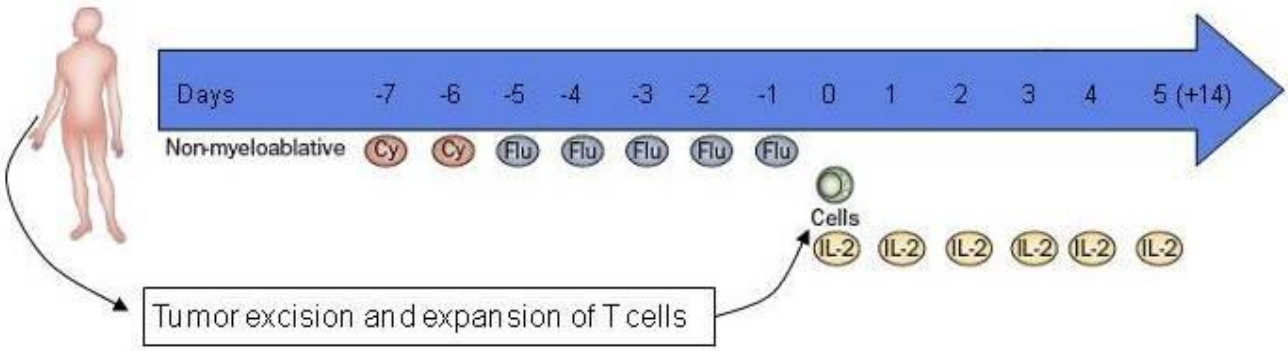

Figure 1: Treatment course for T cell therapy
Shortly after inclusion of the patient, tumor tissue for production of the T cell infusion product is removed. The week before the T cells are ready for infusion, treatment with cyclophosphamide (Cy) and fludarabine phosphate (Flu) is started, followed by T cell infusion and treatment with IL-2. The duration and dosing of the IL-2 treatment varies in different protocols.

T cell therapy has shown very promising results for metastatic MM, where an ORR of approximately 50 % has been observed, which has been confirmed in several phase I/II "single-institution" studies[24,25,29–31]. In addition, CR has been observed in 20 % of the treated patients, of which most are lasting, and the patients remain disease-free for more than seven years after the treatment[22]. TIL-based T cell therapy thereby appears to have the potential to cure a significant proportion of patients with metastatic MM.

Experiences from previous clinical trials with more than 300 treated patients have shown that T cell therapy despite considerable toxicity is safe for use in patients in good general condition with

sufficient organ function. The described side effects are reversible, acute side effects consisting of known and expected side effects of lymphodepleting chemotherapy and high-dose interleukin-2.

CCIT-DK experiences with T cell therapy

Clinical trial

As one of the few places in the world the complicated methods for producing TILs are established at the National Center for Cancer Immune Therapy (CCIT-DK), Herlev Hospital[32] and has been translated into a clinical trial (clinicaltrials.gov identifier: NCT00937625), where we so far have treated 26 patients with metastatic MM. All patients were treated with classical lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate followed by TIL infusion with approximately $100x10^9$ cells and subsequent administration of IL-2 (see appendix 2).

In the original "T-cell regime" described by Dudley *et al.*[15] very high doses of IL-2 (720,000 IU/kg IV) are given as bolus injections every eighth hours until treatment-limiting toxicity. Since it is unknown, whether these very high doses of IL-2 are necessary to maintain T-cell expansion, we have at CCIT-DK, Herlev Hospital tested treatment with low to intermediary doses of IL-2 to determine, whether the response rates can be maintained, while the toxicity is reduced.

A pilot study where six patients were treated with low-dose IL-2 SC was initiated in the summer of 2009, and the results of this are now published[23]. Of the six treated patients, two patients achieved CR.

To achieve a higher RR, the IL-2 dose was thereafter increased to an intermediary dose, where IL-2 is administered after a decrescendo regime[33], which is equal to the IL-2 decrescendo regime, which in Denmark is used as standard treatment of metastatic MM.

After increasing the dose of IL-2 we have treated an additional 20 patients, of which 18 have currently been evaluated with imaging. In total (n = 26), we have seen an ORR of 46 %, which is comparable with what has been achieved in other studies with high-dose IL-2. Three patients achieved complete response (CR; 49, 13, 27+ months), and eight patients achieved a partial response (PR), of which six are still followed with responses lasting from 2-25 months. Five patients had stable disease (SD) for 2-4 months, and three patients progressed shortly after the treatment.

Further, clearly reduced toxicity has been observed at low to intermediary dose IL-2, and the treatment has shown to be manageable in an ordinary oncology department without the need for intervention from an intensive care unit.

Translational research

Further development of the T cell therapy in the form of optimization and expansion to other kinds of cancer is a highly prioritized research area at CCIT-DK. Our already established platform for T cell therapy for MM has given us a unique opportunity to study interactions between tumors and the immune system, and thereby to clarify possible methods for optimization of the T cell therapy.

Several studies have shown that the following characteristics for T cells are important in order to achieve a clinical response from T cell therapy: long telomeric length, short expansion time, a favorable phenotype of T cells ($CD27^+$, $CD28^+$), a high absolute number of T cells and a high number of cytotoxic tumor-reactive T cells in the infusion product[34] and increased persistence of the T cells in the peripheral blood after infusion[22,32,35]. On the basis of this we have at CCIT-DK changed the primary expansion method from "traditional expansion" to "Young TIL" expansion, whereby the first expansion period is shortened from 4-7 weeks to 2-4 weeks. Shorter time in culture (Young TIL) gives TILs with a longer telomeric length and more favorable phenotypes ($CD27^+$, $CD28^+$) with characteristics such as increased proliferation, increased persistence *in vivo* and higher antitumor activity, which as mentioned is correlated with an increased clinical response[22,32]. By decreasing the time-span during which the T cells are grown in culture, a simpler and faster method for production of the TIL infusion product has been achieved and testing of the TIL specificity is no longer necessary. This optimization of the TIL production has made it possible to produce clinically applicable TIL infusion products from more than 90% of the patients[36–39]. Further, we have during the final expansion phase, the Rapid Expansion Protocol (REP), introduced the use of the Wave® bioreactor[40], which optimizes the proliferation conditions and has made it possible to achieve a higher absolute cell number, as well as a higher number of tumor-reactive T cells in the TIL infusion product. Based on these TIL production protocols, developed at CCIT-DK, we have standardized and harmonized TIL production methods among three European cancer research centers, and a randomized, multicenter TIL based T cell therapy phase III clinical trial aiming at approval of T cell therapy as standard treatment for patients with malignant melanoma is planned.

Rationale behind the substances used in the clinical trial

Lymphocyte-depleting chemotherapy

To maintain an immunological response against the tumor, activating cytokines (the signaling molecules IL-2, IL-7, IL-15, IL-21 etc.) must be available for the tumor-specific T cells. Many "irrelevant" T cells will competitively reduce the availability of these cytokines for the relevant T cells. To create an environment, which facilitates a T-cell mediated anti-tumor response, there must in addition to the presence of many tumor-specific T cells with high specificity be made a reduction of irrelevant T cells, as well as an elimination of Tregs.

To create such an environment, a combination of two days of cyclophosphamide and five days of fludarabine phosphate will be used in the present study. A combination chosen on the basis of experiences from previous studies[41,42].

Cyclophosphamide

Cyclophosphamide is an alkylating compound, which works by forming covalent bonds with biologically important macromolecules. In particular, bonds are formed with the DNA and in some cases cross-binding of the DNA takes place. If the cross-binding is not removed by the cell repair systems, the cell division can be prevented. The binding to important proteins in the cell may harm important functions and lead to cell death. Cyclophosphamide is used in oncology for e.g. breast cancer and in malignant hematological diseases such as myelomatosis[43].

Fludarabine phosphate

Fludarabine phosphate is a prodrug, which is changed into the active triphosphate 2-fluoro-ara-ATP. The compound is part of the group of anti-metabolites, which inhibits the DNA synthesis, and at the same time leads to a reduction of the RNA and protein synthesis. Fludarabine phosphate is used for the treatment of malignant hematological diseases e.g. CLL[44].

Interleukin-2

Interleukin-2 (IL-2) after the decrescendo-regime[33] is used in Denmark as a standard first-line treatment for suitable patients with metastatic malignant melanoma. Low-dose IL-2 is also used for treatment of metastatic kidney cancer. IL-2 is normally produced by activated T-lymphocytes and stimulates through specific receptors both the antigen specific and the unspecific immune defense[45]. IL-2 will in this experiment be given according to the decrescendo-regime, which is used for standard IL-2 treatment of disseminated malignant melanoma.

Interferon alpha

Interferons, which are pleiotropic cytokines, were first described in 1957 by Isaacs and Lindenmann[46]. They work by binding to surface receptors of both normal and neoplastic cells, whereby a complex sequence of intracellular processes is initiated, including induction of different proteins, such as the Major Histocompatibility Complex (MHC) molecules. Human interferons are classified as α, β, and γ. Apart from an upregulation of MHC, interferons have been shown to have growth-inhibiting effects, when they are added to tumor cells *in vitro*, and several other indirect immune-modulating effects have recently been described: an increase in tumor-infiltrating cells; development of auto-antibodies and manifestation of autoimmunity; inhibition of the angiogenesis; reduction of circulating Treg; modulation of the STAT1/STAT3 balance in tumor cells and

lymphocytes; changes in cytokine concentrations and normalization of T-cell STAT1 signaling defects in peripheral circulating lymphocytes[47–49].

Interferon alpha 2b (IFN-α) is a potent immune modulating cytokine, which in several studies has been shown to increase the MHC-dependent antigen presentation in a number of different cell types, including melanoma cells[50]. Through this upregulation of the MHC expression, interferons are believed to make malignant cells more immunogenic[47]. Tumor-associated antigens (TAA), which are expressed by malignant cells, can be presented on the surface of the malignant cell by MHC class I or II molecules and can thereafter be recognized by cytotoxic $CD8^+$ and $CD4^+$ T cells. A specific T-cell response against cancer cells thereby requires that these cells express MHC class I or II. Tumor cells can evade a specific immune cell killing by downregulating MHC expression[51], and the MHC expression is also found downregulated in the majority of malignant melanomas[52,53]. Changes in MHC class I expression on malignant cells are correlated with a poor survival, disease progression and limited response to immunotherapy, and generally metastases are characterized by a higher prevalence of loss of MHC class I molecules than primary tumors.

Improvement of T cell therapy with pegylated interferon alpha 2b

At CCIT-DK we have recently characterized the response of a number of TIL products against autologous tumors from patients with MM. We have shown that the tumor reactivity of both the $CD8^+$ and $CD4^+$ T cells can be improved in most TIL products by pretreatment of the autologous tumor cells with IFN-α and/or IFN-γ, respectively (see appendix 4).

We have also previously shown that IFN-γ pretreatment can induce or upregulate Human Leukocyte Antigen (HLA) molecules on the tumor cells and thereby restore or increase the anti-tumor reactivity[34]. Previous studies have as indicated shown that IFN-α can increase the MHC-dependent antigen presentation in several different cell types, including melanoma cells[50].

IFN-α has as described been used safely for many years as an adjuvant treatment for patients with malignant melanoma in different forms, doses, and regimes and is also used in combination with IL-2 for treatment of patients with metastatic melanoma.

Recently, a Dutch research group combined adoptive T cell therapy with interferon alpha[54]. In this phase I/II clinical trial 10 melanoma patients were treated with T cells isolated from peripheral blood in combination with subcutaneous injections of IFN-α 3 MIU (million units) daily for 12 weeks. Degree 2 (CTC) side effects were observed in 5/10 patients and no ≥ degree 2 side effects, and one CR, one PR, and three patients with SD were observed.

Our experiences and those of others indicate that the combination of T cell therapy with IFN-α potentially can increase the clinical effect of T cell therapy by increasing the tumor reactivity of the

infused TILs, which we have previously shown is a critical factor in achieving clinical response[34] (see appendix 3).

Rationale behind the treatment regime used in the clinical trial

The T cell therapy regime is structured according to guidelines described by Dudley et al[15]. A few approaches, however, differ from this regime, and these deviations are described below.

As it is unknown whether high-dose IL-2 is necessary to maintain the T-cell expansion after infusion, we have chosen to treat the patients with intermediary dose IL-2 (decrescendo regime) instead of high-dose bolus infusions, which have been used in earlier studies, and which are considerably more toxic[22]. Our results indicate that it is possible to achieve a response rate of approximately 50 % with intermediary dose IL-2, which is comparable with earlier studies, which have used high-dose bolus IL-2 (see appendix 2).

By combining the T cell therapy with IFN-α, we hope to increase the tumor cell sensitivity to the cytotoxic T cells in the TIL infusion product, whereby the clinical effects of the treatment can be further increased. Our experiences with T cell therapy indicate that the effects of the treatment begin within a few days to weeks after the actual T-cell infusion.

We have among responding patients observed the largest reduction in the tumor size within the first couple of months after the treatment. We will therefore administer IFN-α shortly before (day -2) and in the period immediately after the T-cell infusion (day 7 and 14) to give the T cells the best possible conditions for tumor cell killing. We have chosen to use pegylated interferon, since this formulation only needs to be given 1x weekly. Peginterferon will be given in a dosage of 3 μg/kg 1x weekly, in a total of three doses. The weekly dose of peginterferon thereby corresponds to the dose, which is used in adjuvant treatment of malignant melanoma, where the treatment is given once weekly for two years.

Purpose and hypotheses

Primary

1) To evaluate tolerability and feasibility of the treatment

Secondary

1) To determine whether T cell therapy in combination with peginterferon for patients with MM can induce a measurable immune response against tumor cells

2) To describe an objective response using RECIST 1.1

3) To describe survival and progression-free survival

Study design

The clinical trial is a pilot study for patients with metastatic (inoperable stage III or stage IV) malignant melanoma. All patients are included and treated at the Department of Oncology, Herlev Hospital. The patients are referred for treatment from oncology departments at Copenhagen University Hospital Herlev, Aarhus University Hospital, and Odense University Hospital.

We will include and treat 20 patients and expect that this can be done within two years. We expect that the patients can finish the treatment and the first six months of follow-up within approximately three years. The patients will be evaluated for objective response six and 12 weeks after infusion of T cells, and thereafter every third month.

The inclusion period is expected to begin in June 2014, and the clinical trial is expected to end in December 2016. The actual treatment course can vary from patient to patient depending on the time-span between surgery and treatment. In most cases the patient may receive treatment approximately 4-6 weeks after surgery, and in this case the treatment course from surgery until the first imaging-based evaluation (six weeks after treatment) will last approximately 4-5 months.

Study population

Patients with histopathologically verified metastatic or locally advanced malignant melanoma will be candidates for this clinical trial, except for patients whom have previously been treated with T cell therapy. As is evident from the exclusion criteria below, the patients must be in good general condition, be free of serious competing conditions, and have acceptable organ function. Only the patients, where it is possible to grow T cells from the tumor tissue (more than 90 % of the patients), will be offered treatment with T cells.

Criteria for inclusion and exclusion

It typically takes 4-6 weeks from the patients have tumor tissue excised until the T cells are ready for treatment. If there is long waiting time for the treatment, the T cells will be cryopreserved after the initial expansion ("Young TIL), and the cells can at a later time point be thawed and prepared for treatment in two weeks in the final expansion (REP). Therefore, in some cases it takes longer time between the time of surgery and the actual treatment, and the patients are therefore included in two steps:

Step 1: Inclusion and exclusion criteria for surgery
Step 2: Inclusion and exclusion criteria for treatment

This two-step inclusion also makes it possible to include patients for surgery (Step 1), even though they do not progress after previous treatment for malignant melanoma and therefore do not fulfill the criteria for treatment. In these cases, the T cells will be cryopreserved, until the patient progresses and fulfills criteria for treatment (Step 2).

If the patient is included for surgery but later do not fulfill the requirements for treatment the removed tumor tissue and the blood samples will be used for research.

Inclusion criteria for surgery (Step 1)

All criteria listed below must be fulfilled in order for patients to be included for surgery.

1. Histologically verified metastatic or locally advanced malignant melanoma, where surgical removal of tumor tissue is feasible.
2. Age: 18 - 70 years
3. ECOG Performance Status of ≤1 (see appendix 5)
4. Life expectancy > 3 months
5. At least one measurable parameter according to RECIST 1.1 criteria
6. No significant toxicity or side effects (CTC ≤ 1) from any previous treatment
7. Sufficient organ function, including:

System	Laboratory values
Hematology	
ANC (Absolute Neutrophil Count)	≥ 1,500/µl
Leukocytes	≥ normal range
Thrombocytes	≥ 100,000/µl and < 700,000/µl
Hemoglobin	≥ 6.0 mmol/l (e.g. after blood transfusion)
Kidney	
S-creatinine	< 140 µmol/l
Liver	
Total serum bilirubin	≤ 1.5 times the upper normal limit
ASAT/ALAT	≤ 2.5 times the upper normal limit
Alkaline phosphatase	≤ 5 times the upper normal limit
LDH	≤ 5 times the upper normal limit
Coagulation	
PP	> 40, unless the patient receives therapeutic anticoagulation

INR	< 1.5, unless the patient receives therapeutic anticoagulation

8. Women of child-bearing potential must use safe means of birth control. Likewise, men who participate in the clinical trial or female partners of men, who participate in the clinical trial must use safe anticonception. This goes from the inclusion until six months after the last dose of study drug. Oral contraceptive pills, intrauterine device (IUD), depot injection of progestogen, sub-dermal implantation, hormonal vaginal rings, and transdermal patches are considered safe contraceptive methods.
9. Signed informed consent form after oral as well as written information.
10. Prepared to meet up at the planned control visits and capable of handling toxicity.

Exclusion criteria for surgery (Step 1)

Patients should be excluded from surgery, if they meet just one of the criteria listed below:

1. Other malignant tumors in the anamnesis with the exception of basal cell carcinoma and adequately treated carcinoma in situ colli uteri. Patients treated for other malignant conditions can participate, if the patient is without signs of illness for at least five years after ended treatment.
2. Patients with cerebral metastases. Patients with solitary cerebral metastases can be included after radical surgery or stereotaxic radiotherapy, if the patient at least 1 month later is without signs of cerebral disease activity, clinically as well as by MR scanning. Patients with non-previously treated asymptomatic cerebral metastases can be included according to the investigator's discretion.
3. Patients with ocular malignant melanoma.
4. In the case of confirmed sensitivity to one of the active compounds or one or more of the adjuvant substances.
5. Serious medical condition e.g. serious asthma/COLD, badly regulated coronary disease, Morbus cordis, badly regulated insulin-demanding diabetes mellitus
6. Acute/chronic infection with e.g. HIV, hepatitis, tuberculosis.
7. Serious allergy or previous anaphylactic reactions.
8. Active autoimmune disease e.g. autoimmune neutropenia/thrombocytopenia or hemolytic anemia, systemic lupus erythematosus, Sjögren's syndrome, scleroderma, myasthenia gravis, Goodpasture syndrome, Addison's disease, Hashimoto's thyroiditis, active Grave's disease.
9. Pregnant and breast-feeding women.
10. Concurrent treatment with systemic immunosuppressive medication (including prednisolone, methotrexate etc.) excluding planned shorter-lasting treatment, which according to the study responsible physician's assessment can be discontinued before planned treatment with T cell therapy.

11. Concurrent treatment with other experimental drugs.
12. Patients with active uncontrolled hypercalcemia.

Inclusion criteria for treatment (Step 2)

All criteria listed below must be completed in order for the patients to be included for treatment. Changes with respect to step 1 are written in bold:

1. Histologically verified metastatic or locally advanced **progressing** malignant melanoma.
2. Age: 18 – 70 years
3. ECOG Performance Status of ≤ 1 (see appendix 5)
4. Life expectancy > 3 months
5. At least one measurable parameter according to RECIST 1.1 criteria
6. No toxicity or side effects (CTC ≤ 1) from any previous treatment
7. Sufficient organ function, including:

System	Laboratory values
Hematology	
ANC (Absolute Neutrophil Count)	≥ 1,500/µl
Leukocytes	≥ normal range
Thrombocytes	≥ 100,000/µl and < 700,000/µl
Hemoglobin	≥ 6.0 mmol/l (e.g. after blood transfusion)
Kidney	
S-creatinine	< 140 µmol/l
Liver	
Total serum bilirubin	≤ 1.5 times the upper normal limit
ASAT/ALAT	≤ 2.5 times the upper normal limit
Alkaline phosphatase	≤ 5 times the upper normal limit
LDH	≤ 5 times the upper normal limit
Coagulation	
PP	> 40, unless the patient receives therapeutic anticoagulation
INR	< 1.5, unless the patient receives therapeutic anticoagulation

8. Women of child-bearing potential must use safe means of birth control. Likewise, men who participate in the clinical trial or female partners of men, who participate in the clinical trial must use safe anticonception. This goes from the inclusion until six months after the last dose of study drug. Oral contraceptive pills, intrauterine device (IUD), depot injection of progestogen, sub-dermal implantation, hormonal vaginal rings, and transdermal patches are considered safe contraceptive methods.

9. Signed informed consent form after verbal as well as written information
10. Prepared to meet up at the planned controls and capable of handling toxicity.

Exclusion criteria for treatment (Step 2)

Patients should be excluded, if they meet just one of the criteria listed below:
Changes with respect to step 1 are written in bold:

1. Other malignant tumors in the anamnesis with exception of basal cell carcinoma and adequately treated carcinoma in situ colli uteri. Patients treated for other malignant conditions can participate, if the patient is without signs of illness for at least five years after ended treatment.
2. Patients with cerebral metastases. Patients with solitary cerebral metastases can be included after radical surgery or stereotaxic radiotherapy, if the patient at least 1 month later is without signs of cerebral disease activity, clinical as well as MR scanning. Patients with non-previously treated asymptomatic cerebral metastases can be included according to the investigator's opinion.
3. Patients with ocular malignant melanoma.
4. In the case of confirmed sensitivity to one of the active compounds or one or more of the adjuvant substances.
5. Serious medical condition e.g. serious asthma/COLD, badly regulated coronary disease, Morbus cordis, badly regulated insulin-demanding diabetes mellitus.
6. S-creatinine clearence < 70 ml/min*.
7. Acute/chronic infection with e.g. HIV, hepatitis, and/or tuberculosis.
8. Serious allergy after previous anaphylactic reactions.
9. Active autoimmune disease, e.g. autoimmune neutropenia/thrombocytopenia or hemolytic anemia, systemic lupus erythematosus, Sjögren's syndrome, scleroderma, myasthenia gravis, Goodpasture syndrome, Addison's disease, Hashimoto's thyroiditis, active Graves' disease.
10. Pregnant and breast-feeding women.
11. **Concurrent treatment with systemic immunosuppressant medication (including prednisolone, methotrexate etc.)**
12. Concurrent treatment with other experimental substances.
13. **Other concurrent systemic anti-cancer treatment.**
14. Patients with active uncontrolled hypercalcemia.

*In special cases it can be decided to include the patient despite a GFR < 70 ml/min using a reduced dosage of chemotherapy.

Evaluation before inclusion in the clinical trial

The following tests must be performed within one month prior to treatment start (laboratory tests, however, within 1 week):

- Anamnesis and objective examination
- Performance Status according to the ECOG Scale
- Electrocardiogram
- Cr-EDTA clearance
- Urine analysis test
- Laboratory tests:
  A) Hematology: hemoglobin, leukocytes, granulocytes, and thrombocytes.
  B) Blood chemistry tests: Sodium, potassium, creatinine, LDH, alkaline phosphatase, ASAT, ALAT, bilirubin, ionized calcium, CRP, TSH, Calcium ion, INR, PP
  C) Infections: Hepatitis B, Hepatitis C (IgG), HIV, HTLV-I (IgG), EBV
- Pregnancy test: Women of child-bearing potential must do a pregnancy test. This means women, who are not surgically sterilized, post-menopausal, or have used safe birth-control for more than 6 months.
- Baseline tumor evaluation: CT, MRI, PET-CT, or PET-MRI scans can be used.
- Revision of check-list for inclusion/exclusion for treatment.

Examination plan associated with the treatment

	Inclusion Step 1	**Surgery**	**Inclusion Step 2 (before chemotherapy)**	**Before peginterferon**	**Before T-cell infusion**	**Before peginterferon**	**Before discharge**	**Before peginterferon**
Day			**-8**	**-2**	**0**	**7**	**App. 10**	**14**
Performance Status	x		x	x	x	x	x	x
Objective examination	x		x	x			x	x
Weight	x		x	x	x	x	x	x
Adverse events (CTC)			x	x	x	x	x	x
Screening blood samples[a]	x							
Immunological blood samples[b]		x	x				x	
Miscellaneous blood samples[c]			x	x	x	x	x	x
EKG	x		x					

Cr-EDTA[d]			x					
Urine tests			x					
Biopsy[e]			x					
Tumor evaluation[f]	x		x					

Examination plan

a: Screening blood samples: T-cell screening (Labka blood sample package): hemoglobin, thrombocytes, leukocytes, differential counts, sodium, potassium, creatinine, ALAT, ASAT, alkaline phosphatase, bilirubin, LDH, INR, APTT, PP, ionized calcium, CRP, TSH, calcium-ion free, Hepatitis B virus s antigen (HBVSAG), Hepatitis C virus antibody (HCVAB), Human immunodeficiency virus type 1 and 2 antibody and antigen (HIVABAG), HTLV type I antibody + HTLV type II antibody (IgG) (HTLVIGG), Ebstein-Barr virus antibody (EBV), P-Treponema pallidum antibody (TREPONE)

b: Immunological blood samples: HEREKS11. See the section on "Immunological monitoring, blood samples".

c: Miscellaneous blood samples: Hemoglobin, leukocytes, differential counts, thrombocytes, creatinine, sodium, potassium, LDH, ASAT, ALAT, bilirubin, alkaline phosphatase, albumin, ionized calcium, TSH, CRP, INR, PP.

d: Chrome-EDTA clearance is performed in the week before admission.

e: If possible, and depending on localization and availability, excision-, punch- ("stanse"), crude needle- or fine needle biopsy is taken.

f: CT, MRI, PET-CT, or PET-MRI scans can be used. Use of PET/CT scan is advised. Scans before chemotherapy should be performed in the week before admission.

Treatment strategy

The treatment course consists of two treatment steps and a subsequent control course.

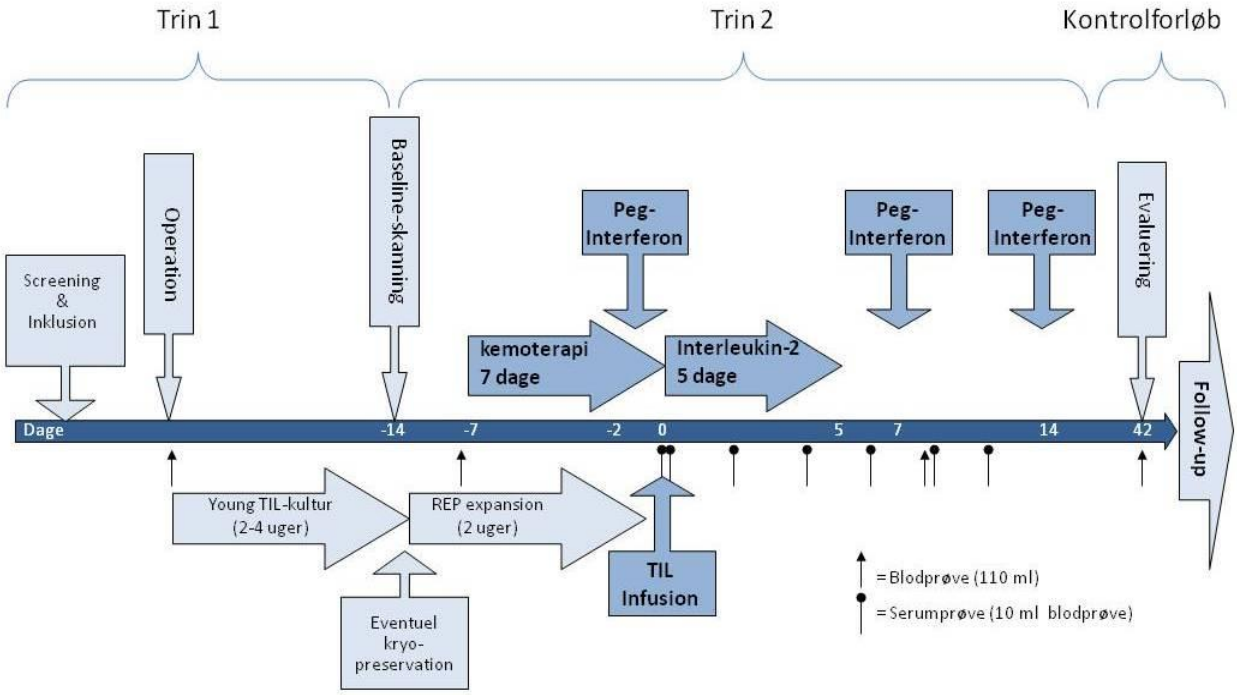

***Figure 2**: Overview of the treatment course*

Step 1: Screening and inclusion and surgical removal of tumor material with subsequent preparation and expansion of TIL cultures in the laboratory.

Step 2: Treatment during admission with chemotherapy, peginterferon, TIL infusion, and IL-2. The last dosage of peginterferon is given as outpatient care, or the patient receives a syringe for self-injection at home.

Control course: Evaluation of treatment effect and follow-up.

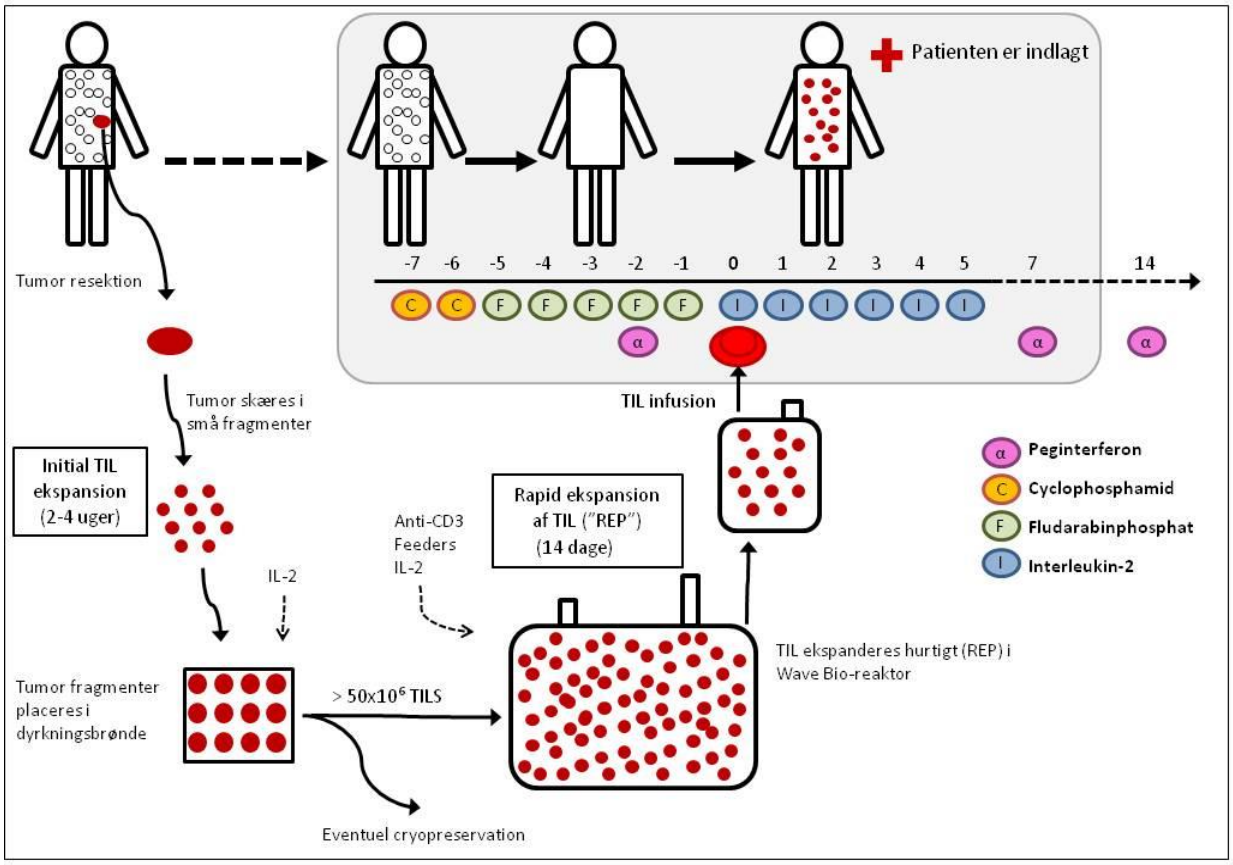

Figure 3: *The figure shows a schematic representation of TIL isolation from tumor tissue and expansion, as well as T cell therapy in combination with peginterferon.*
Tumor tissue (metastasis or primary tumor) of a minimum size of 2 cm^3 is removed from the patient, and the tumor tissue is brought to the laboratory under sterile conditions, where it is cut into suitable fragments of 1-3 mm^3 and placed in cultivation wells with growth medium and IL-2. TILs are initially grown for 2-4 weeks until an appropriate cell number of a minimum of 50 x 10^6 is reached. At this time the cells can be cryopreserved for later use or further expanded in the Rapid Expansion Protocol (REP), where T cells for 2 weeks are stimulated with anti-CD3 antibody, allogenic irradiated PBMCs (peripheral blood mononuclear cells) feeder cells, and IL-2. The expanded TILs (now billions of cells) are washed, pooled, and re-infused intravenously into the patient.
Before TIL infusion 1 week of lymphodepleting chemotherapy is given in the form of cyclo-phosphamide (C) day -7 until day -6 and fludarabine (F) day -5 until day -1 in order to remove all existing lymphocytes in the patient to make room for the infused TILs and remove regulatory T cells (Treg). Two days before TIL infusion a subcutaneous injection of peginterferon (day -2) is given, and after TIL infusion another two doses of peginterferon (day 7 and day 14) is given with the purpose of increasing T cell tumor reactivity. Approximately 6 hours after TIL infusion, continuous IL-2 infusion according to the decrescendo regime (day 0 until day 5) is started to activate and stimulate the infused T cells into further expansion in the patient.

Substances in the clinical trial

The substances, which are included in the clinical trial, are cyclophosphamide, fludarabine phosphate, interleukin-2, and pegylated interferon alpha 2b. Mixing and storage of the substances is performed according to existing standard guidelines in the department.

Cyclophosphamide

Cyclophosphamide is given as an intravenous infusion for two consecutive days in a dose corresponding to 60 mg per kg body weight. The treatment will take place during hospitalization and with supplemental hydration and mesna injections.

Fludarabine phosphate

Fludarabine phosphate is given as an intravenous infusion in a dose corresponding to 25 mg per m^2 body surface given daily for five consecutive days (the day after the last dose of cyclophosphamide). The treatment will take place during hospitalization.

TILs

The tumor-specific T cells are infused intravenously the day after the last dose of fludarabine phosphate (day 0). The number of T cells in the product depends on the feasible degree of expansion *in vitro* and is therefore variable, but an infusion will normally consist of approximately 10^{10} cells. The treatment will take place during hospitalization.

Interleukin-2

After a week of chemotherapy and infusion of T cells on day 0, treatment with interleukin-2 as a continuous intravenous infusion on day 0-4 is started: 18 MU/m^2 over 6 hours, 18 MU/m^2 over 12 hours, 18 MU/m^2 over 24 hours, followed by 4.5 MU/m^2 over 24 hours for 3 days.

Pegylated interferon alpha 2b

Pegylated interferon alpha 2b (peginterferon) is administered as a subcutaneous injection at 3 μg/kg (maximal dose of 300 μg) on day -2 (two days before T cell infusion), day 7 and 14. The two first injections are given during hospitalization. If the patient is discharged before the peginterferon injection on day 14, this injection can be administered on an outpatient basis, or the patient is given a syringe and detailed oral and written instructions in how to self-administer the injection at home. Peginterferon therapy is labeled according to the current Annex 13 in the GMP rules.

Treatment plan

Year: Date:																			
Week	-2		-1							0								1	
Treatment day										0				1	2	3	4		
Time (indicative timepoints)	-12	-8	-7	-6	-5	-4	-3	-2	-1	12:00	14:00	18:00	24:00	12:00	12:00	12:00	12:00	7	14
Peginterferon 3 µg/kg SC								x										x	x
Patient is admitted [1)]		x																	
Cyclophosphamide 60 mg/kg IV			x	x															
Fludarabine phosphate 25 mg/m^2 IV					x	x	x	x	x										
TIL IV										x									
Pegfilgrastim 6 mg SC*											x								
IL-2 18 MIU/m^2 IV continuously for 6, 12, and 24 hours												x	x	x					
IL-2 4.5 MIU/m^2 IV continuously for 24 hours															x	x	x		

Treatment plan

** See the section "supportive treatment"*

Simultaneous treatment

Guidelines for supportive care and treatment

Is given on standard medical indication according to the assessment of the treatment responsible physician and must be specified in the medical record and flow sheets. The following are guidelines and other drugs may be used at your discretion. However, the patient may not receive concurrent systemic adrenocortical hormone.

Prophylactic treatment

Prophylactic treatment includes fluid therapy during cyclophosphamide treatment as well as supportive treatment with mesna (25 % of cyclophosphamide dose IV 4 times daily on day -7 and -6) in order to protect the bladder mucosa. During IL-2 treatment fluid therapy is also administered in order to prevent electrolyte derangement as well as too low blood pressure, cf. appendix 7.

In order to prevent opportunistic infections, the following drugs are given:

- Tabl. Sulfamethizole with trimethoprim, 400/80 mg, 1 tabl. daily, day -7 and 6 mo. on.
- Tabl. Aciclovir, 400 mg x 2 daily. Day 0 and 6 mo. on.
- Tabl. Diflucan, 100 mg daily. Day 0 and until neutrophilocyte number > 1000/µl.

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

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

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

- Tabl. Motilium 20 mg x 4
- Ondansetron 8 mg x 1 as needed max x 2
- Imolope 2 mg as needed max x 8

In order to reduce the duration of neutropenia, Pegfilgrastim, which is a human granulocyte-stimulating factor (G-CSF), is given, 6 mg SC on day 0 after TIL infusion (at 14:00).

Supportive treatment

During infusion with T cells, the patient may experience chills, which can be relieved with injection of Petidin 25 mg subcutaneously as needed max x 4.
Similarly, light difficulties breathing, possibly with a drop in the oxygen saturation, may be seen, which is treated with oxygen through a nasal catheter.

Neutropenia:
In the case of simultaneous neutropenia and fever will be treated according to the instruction (see appendix 8) for “Febrile neutropenia during T cell therapy”.

Diarrhea:
Diarrhea will immediately be treated with appropriate supportive care and treatment, including Loperamide. Loperamide must be discontinued, if there is blood or slime in the feces related to the diarrhea. In these circumstances appropriate diagnostic microbiological samples should be collected in order to exclude an infectious etiology. The patients should also be instructed in drinking plenty of clear fluids to prevent dehydration due to the diarrhea.

Anemia:
Blood transfusion is given if Hgb ≤ 6,0 mmol/L, or if clinically indicated. Irradiated and filtered

blood from day -7 and 6 mo. on will be used.

Thrombocytopenia:
Thrombocyte transfusion is given to patients with thrombocyte numbers < 20/µl, or if clinically indicated.

Local radiotherapy

Local radiotherapy of metastases can be prescribed against bone pain, wounds, or other indication. Out of consideration for the patient radiotherapy should be avoided during the 3-week period, where the actual treatment will take place. Irradiated areas cannot be used as a parameter for assessment of response. If possible, not all evaluable lesions should be included in the irradiated area. If this cannot be completed, the patient is not evaluable for response and will leave the study.

Treatment discontinuation

Normal discontinuation

The patients will only receive one treatment series consisting of one week of chemotherapy, one single infusion of T cells (day 0), 3 subcutaneous injections of peginterferon (on day -2, 7, and 14, respectively), and 5 days of IL-2 according to the decrescendo regimen. The patients will be followed by the immune team for up to 5 years after treatment or until progression.

Follow-up

After the treatment the patients will be followed with outpatient control visits at 6 and 12 weeks after the treatment and thereafter every 3. month. After 2 years the patients will be followed every 6. month. The patients will be followed by an immunotherapeutic team for up to 5 years or until progression.

At each outpatient control visit an objective examination, toxicity assessment, blood samples including immunological blood samples, as well as PET/CT scan will be performed. If the patients have available remaining tumor tissue, tumor biopsies will also be taken at the first outpatient attendance.

Follow-up schedule

Year: Date:																				
Week no.	6		12		24															
Month no.	1, 5		3		6		9		12		15		18		21		24		27		30
Objective examinations and subjective complaints	0		0		0		0		0		0		0		0		0		0		0
Toxicity assessment	0		0		0															
Weight	0		0		0															
PS	0		0		0		0		0		0		0		0		0		0		0
Blood samples: T cell follow-up[1)]	0		0		0		0		0		0		0		0		0		0		0
Biopsy [2)]	0																			
Tumor evaluation [3)]	0		0		0		0		0		0		0		0		0		0		0

1) Blood samples (T cell follow-up): hemoglobin, thrombocytes, leukocytes, differential count, sodium, potassium, creatinine, ALAT, ASAT, alkaline phosphatase, bilirubin, LDH, INR, APTT, PP, ionized calcium, CRP, TSH, calcium-ion free, immunological blood samples – HEREKS11 (See the section on "Immunological monitoring, blood samples")
2) See the section on "Immunological monitoring, tumor biopsy".
3) CT, MR, PET-CT, or PET-MR scan can be used. Use of PET/CT is recommended.

Early discontinuation of treatment

Not possible to grow TIL: If it is not possible to grow TILs, the patient cannot be offered treatment.

The patient's preference: The treatment can be stopped at any time, if the patient wants it.

Medical decision: The treatment can at any time be stopped, if the investigator, for medical reasons, finds it to be in the patient's best interest.

Other treatment: The patients will leave the study, if treatment with other experimental drugs or other systemic anticancer treatment is started after the patient has been included for T cell therapy. The patient will leave the study, if treatment with adrenocortical hormone is started, unless it is based on a vital indication as agreed with the protocol responsible physician.

Side effects: If side effects arise in a patient in relation to the treatment to such a degree that the study cannot be completed, the treatment is aborted.

Patients, who have stopped with IL-2 treatment early, will still be followed according to the protocol.

Patients, who have left the protocol before infusion of tumor-specific T cells, will be replaced by new study subjects. They will be followed until cessation of side effects of the received treatment but will not be followed with further controls after that.

Subsequent treatment

If the patients leave the study, they can freely receive other treatment. If the patient develops progressive disease, they can freely receive other treatment.

Production of TILs

Requisition of tumor tissue

Before the patients are operated, they will be informed verbally, and written consent will be collected according to procedures described elsewhere. When sufficient tissue has been obtained for a possible pathological examination, a biopsy of at least 2 cm^3 will be taken from the tumor tissue. The biopsy is labelled with date and patient code number, placed in a sterile container, and transported to the GMP laboratory 54J7, Herlev Hospital, where the further processing takes place.

Establishing "Young TIL" cultures

The T cells are expanded through our newly established method for "Young TILs"[32]. The tumor tissue is cut into fragments of 1-3 mm^3 and placed in a culture plate with 24 wells. From each fragment a TIL culture is established by either migration of T cells from the tumor tissue or by enzymatic processing. The cell density is maintained at approximately $1x10^6$ cells/ml growth medium, where the immunostimulatory cytokine IL-2 has been added. IL-2 belongs to the group of homeostatic cytokines, characterized by having a beneficial effect on the activation of tumor-specific T cells and thereby on tumor cell killing. The cell cultures from the different fragments are pooled into a combined cell culture. The T cell expansion proceeds in an unselected manner, as a polyclonal TIL repertoire directed against multiple epitopes is desired in order to potentially obtain a more efficient destruction of the tumor cells *in vivo*. The establishment of "Young TIL" cultures typically takes 2-4 weeks with a success rate of more than 90 %. For any further inquiries, please refer to the Investigational Medicinal Product Dossier (IMPD).

Rapid Expansion Protocol (REP)

When the TIL cultures have been expanded to approximately 5 x 10^7 cells, they are transferred to further expansion through the Rapid Expansion Protocol (REP), where TILs are grown together with irradiated (40 Gy) allogenic PBMCs (peripheral blood mononuclear cells), which serve as "feeder cells", and IL-2 and anti-CD3 antibody, which activate the TILs. A very high number of activated tumor-specific T cells with a high activity towards tumor associated antigens (TAA) and tumor can be obtained in the course of 14 days. In the end the autologous T cells are concentrated in a 400 ml infusion bag for the purpose of intravenous infusion. For any further inquiries, please refer to the IMPD.

Isolation of tumor cells

Tumor cells are isolated from the tumor fragments by enzymatic processing or seeding from the tumor fragments and frozen for the purpose of later use for determination of anti-tumor T cell activity.

Phenotype and clonotype determination

In both Young TIL and REP TIL cultures the prevalence of T cell types (e.g. CD4+ and CD8+) and T cell stages are characterized (naïve and activated T cells) through flow cytometry.

Cytokine Release Assay

The TIL cultures are screened for activity towards TAA and autologous tumor by determining the production of activating cytokines (INF-γ + TNF-α). The production of activating cytokines is quantified using the ELISPOT method, flow cytometry, and combinatorial coding flow cytometry.

Gene-based arrays

Simultaneous analyses will be performed in order to identify specific tumor gene expression signatures[55] and mutations in the tumor cells, which lead to the identification of patient-specific neo-antigens derived from these mutations[56]. These analyses can contribute to the identification of patients, whom are more likely to respond to the treatment, and further it may contribute to optimization of the T cell therapy based on the selection of neo-antigen specific T cells.

Adverse Events, potential risks, and precautions

Adverse events (Adverse Event = AE)

Adverse events are unwanted signs, symptoms, or events, which occur during participation in the study, regardless of whether they are causally linked to the treatment or not. All adverse events must be described in the patient's medical record and in the electronic Case Report Form (eCRF). The severity and effects after the treatment should be recorded for each adverse event. The severity of the adverse event and relationship with the administered treatment must be evaluated in accordance with the guidelines described below.
The medical investigator should try to find all clinical and objective reactions from patients in treatment and determine their relationship with the experimental drugs. The investigator evaluates the relationship between adverse event and treatment using the following guidelines:

Graduation of adverse events

Severity refers to the intensity of a reaction.

Events are graded according to CTCAE version 4.0 (see appendix 6)[57]. If this cannot be used, use the following scale:

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

Patients, who experience adverse events, will be monitored with relevant clinical assessments and laboratory testing according to the recommendations by the treatment responsible physician. All adverse events must be followed until satisfactory restitution or stabilization. Results from the follow-up must be recorded in the patient's medical record and in the eCRF.
Abnormal laboratory test results should not be recorded in the eCRF, unless these events either caused a clinical event, led to the discontinuation of the treatment, or fulfill the criteria for an SAE (see below).

Serious adverse event (Serious Adverse Event = SAE)

A serious adverse event must be reported to the sponsor within 24 hours and is defined as an event, which indicates a significant risk, contraindication, side effect, or precaution, and includes events, which:

- result in death or are life-threatening
- lead to hospitalization or prolong existing hospitalization

- result in considerable or continuing disability or incapacity for work
- lead to an inborn anomaly or malformation
- is a significant medical event.

Guideline for possible relationships between adverse events and treatment

0 Not related – no temporal relationship, other etiologies very likely to be the cause.

1 Possibly related – less evident temporal relationship, other etiologies are also possible.

2 Probably related – clear temporal relationship with improvement upon discontinuation of treatment, and not reasonably explained by the patient's known clinical condition.

3 Related – clear temporal relationship with laboratory confirmation or a positive re-treatment test.

If the event is determined to be causally related to the experimental treatment, it is classified as a side effect (Adverse Reaction = AR) or as a serious side effect (Serious Adverse Reaction – SAR).

Side effects

A side effect may be expected, if it is described in the IMPD (appendix 12) or relevant "Summary of Product Characteristics" (SPC), or unexpected, if character or seriousness does not agree with the product information in these documents.
If the side effect is unexpected, fulfill the criteria for an SAE, and is determined to be related to the experimental treatment, it is classified as a serious unexpected side effect (Suspected Unexpected Serious Adverse Reaction = SUSAR).

Reporting of events and side effects

The investigator reports SAEs, SARs, and SUSARs to the sponsor within 24 hours.
The sponsor reports SUSARs to the Danish Medicines Agency within 7 days, if they are deemed life-threatening or are fatal, and otherwise within 15 days. Consequences for the study must be reported.
The sponsor submits a list annually, which sums up possible SAEs and SUSARs, as well as a report of the safety of the study subjects to the Danish Medicines Agency and the National Committee on Health Research Ethics (NVEK) (reporting to the NVEK can also be done by the investigator).

At the conclusion of the study, the sponsor submits a final report to the Danish Medicines Agency, where all events and side effects (SAEs, SARs, SUSARs) will be described.

The following should not be reported:

- deaths where the death is due to progression of the malignant disease
- hospitalizations or prolongation of existing hospitalization, which are caused by the cancer:
    - weight loss
    - fatigue
    - electrolyte disturbances
    - pain management
    - anxiety
    - admission for palliative care
    - hospice stay or terminal care
    - progression of the basic disease
- hospitalizations or prolongation of existing hospitalizations, if the only reason for the hospitalization or the prolongation is the following:
    - fluid or nausea treatment
    - blood transfusion
    - thrombocyte transfusion
    - hospitalization due to febrile leukopenia/neutropenia
    - administration of examination procedure
    - insertion of a permanent intravenous catheter.

These events should be recorded in the eCRF.

Known side effects

Chemotherapy

The side effects of chemotherapy described below are all general side effects of the two drugs, which are seen, when the drugs are given as primary treatment of oncological and hematological diseases. For such indications the treatments will often be given over several treatment series. In this study, the treatment will only be given in one session, and a milder side effect profile is therefore expected.

Cyclophosphamide

In patients, who receive cyclophosphamide, the dose-limiting toxicities consist of myelosuppression (neutropenia, thrombocytopenia, and anemia) and urotoxicity (cystitis, hematuria, and hemorrhagic cystitis). Sufficient treatment with mesna and hydration can markedly reduce the frequency and severity of bladder toxicity.
Other commonly presenting side effects are alopecia, nausea, and vomiting.

Patients, who receive cyclophosphamide treatment, can experience the following side effects:

Infections and parasitic diseases Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100) Very rare (<1/10,000)	 Infections Pneumonia, sepsis Septic shock
Benign, malignant, and unspecified tumors (incl. cysts and polyps) Rare (≥ 1/10,000 to <1/1,000) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	 Secondary tumors, bladder cancer, myelodysplastic changes, urinary tract cancer, acute leukemia. Tumor lysis syndrome. Lymphoma, sarcoma, kidney carcinoma, renal pelvic cancer, thyroid cancer, carcinogenic effect in offspring, progression of underlying malignancy.
Blood and lymphatic system Very common (≥ 1/10) Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	 Myelosuppression, leukopenia, neutropenia. Neutropenic fever. Thrombocytopenia, anemia. Hemolytic uremic syndrome, disseminated intravascular coagulation. Pancytopenia, agranulocytosis, granulocytopenia, lymphopenia, reduced hemoglobin.
Immune system Very common (≥ 1/10) Uncommon (≥ 1/1,000 to <1/100) Very rare (<1/10,000)	 Immunosuppression. Anaphylactic reactions, hypersensitivity reactions. Anaphylactic shock.
The endocrine system Very rare (<1/10,000)	 Vasopressin hypersecretion (SIADH/Schwartz-Bartter syndrome).

Unknown (cannot be estimated based on available data)	Water intoxication.
Metabolism and nutrition Uncommon (≥ 1/1,000 to <1/100) Rare (≥ 1/10,000 to <1/1,000) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	 Anorexia. Dehydration. Water retention, hyponatremia. Increased blood glucose, decreased blood glucose.
Mental disturbances Very rare (<1/10,000)	 Confusion.
Nervous system Uncommon (≥ 1/1,000 to <1/100) Rare (≥ 1/10,000 to <1/1,000) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	 Peripheral neuropathy, polyneuropathy, neuralgia. Dizziness. Cramps, paresthesia, taste disturbances, hepatic encephalopathy. Encephalopathy, posterior reversible leukoencephalopathy syndrome, myelopathy, dysesthesia, hypoesthesia, tremor, hypogeusia, parosmia.
Eyes Rare (≥ 1/10,000 to <1/1,000) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	 Blurred vision. Visual disturbances, conjunctivitis and *eye edema in connection with hypersensitivity. Increased lacrimation.
Ear and labyrinth Uncommon (≥ 1/1,000 to <1/100) Unknown (cannot be estimated based on available data)	 Deafness. Hearing impairment, tinnitus.
Heart Uncommon (≥ 1/1,000 to <1/100)	 Cardiomyopathy, **heart failure, tachycardia, myocarditis.

Rare (≥ 1/10,000 to <1/1,000)	Arrhythmia (e.g. ventricular arrhythmia, supraventricular arrhythmia).
Very rare (<1/10,000)	Atrial fibrillation, ventricular fibrillation, angina pectoris, myocardial infarction, **cardiac arrest, pericarditis.
Unknown (cannot be estimated based on available data)	Ventricular tachycardia, cardiogenic shock, pericardial effusion, hemorrhagic myocardium, left-sided ventricular failure, bradycardia, palpitation, EKG QT prolongation, reduced ejection fraction.
Vascular diseases Rare (≥ 1/10,000 to <1/1,000)	Hemorrhage.
Very rare (<1/10,000)	Thromboembolia, hypertension, hypotension.
Unknown (cannot be estimated based on available data)	Pulmonary embolism, venous thrombosis, vasculitis, peripheral ischemia, flushing.
Airways, thorax, and mediastinum Very rare (<1/10,000)	Bronchospasm, dyspnea, cough, interstitial pneumonia, chronic interstitial pulmonary fibrosis, pulmonary edema, pleural exudate, shock lung (ARDS), hypoxia.
Unknown (cannot be estimated based on available data)	Pulmonary veno-occlusive disease, obliterative bronchiolitis, interstitial pneumonia, allergic alveolar diseases, pneumonia, unspecified lung diseases, nasal congestion, nasal discomfort, pain in the throat, rhinitis, sneezing.
Gastrointestinal tract Very rare (<1/10,000)	Ascites, hemorrhagic enterocolitis, acute pancreatitis, mucosal ulcerations, stomatitis, diarrhea, vomiting, congestion, nausea.
Unknown (cannot be estimated based on available data)	Gastrointestinal bleeding, abdominal pain, abdominal discomfort, colitis, enteritis, cecitis, inflammation of the parotid gland.
Liver and biliary tract Rare (≥ 1/10,000 to <1/1,000)	Liver function disturbances, hepatitis.
Very rare (<1/10,000)	Hepatic veno-occlusive disease, enlarged liver, icterus, activation of viral hepatitis.

Unknown (cannot be estimated based on available data)	Cholestatic hepatitis, cytolytic hepatitis, cholestasis, liver toxicity with liver failure, increased bilirubin in the blood, abnormal liver function, increase in liver enzymes (ALAT, ASAT, alkaline phosphatase, and gamma-glutamyl transferase).
Skin and subcutaneous tissue Very common (≥ 1/10)	Alopecia.
Uncommon (≥ 1/1,000 to <1/100)	Total alopecia.
Rare (≥ 1/10,000 to <1/1,000)	Exanthem, dermatitis.
Very rare (<1/10,000)	Stevens-Johnson syndrome, toxic epidermal necrolysis, pruritus, erythema of the irradiated area, serious skin reactions, discoloration of palms, finger nails, and soles of the feet, toxic skin inflammation.
Unknown (cannot be estimated based on available data)	Palmar-plantar erythrodysesthesia (hand-foot syndrome), erythema multiforme, urticaria, blisters, erythema, swelling of the face, hyperhidrosis.
Bones, joints, muscles, and connective tissue Very rare (<1/10,000)	Rhabdomyolysis, cramps.
Unknown (cannot be estimated based on available data)	Scleroderma, muscle cramps, myalgia, arthralgia.
Kidneys and urinary tracts Very common (≥ 1/10)	Cystitis, microhematuria.
Common (≥ 1/100 til <1/10)	Hemorrhagic cystitis, macrohematuria.
Very rare (<1/10,000)	Suburethral bleeding, edema of the bladder wall, kidney failure, reduced kidney function. Interstitial inflammation, fibrosis, and sclerosis of the bladder. Increased creatinine in the blood.
Unknown (cannot be estimated based on available data)	Tubular necrosis, tubular disturbances, toxic nephropathy, hemorrhagic urethritis, ulcerative cystitis, bladder contractions, nephrogenic diabetes insipidus, atypical epithelial cells in the urinary bladder, increased urea in the blood.

Pregnancy, puerperium, and the perinatal period Unknown (cannot be estimated based on available data)	Premature birth
The reproductive system and breast Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100) Rare (≥ 1/10,000 to <1/1,000) Unknown (cannot be estimated based on available data)	Reduced spermatogenesis. Ovulation disturbances, reduced levels of female sex hormones. Oligospermia***, azoospermia***, irreversible ovulation disturbances, amenorrhea***. Infertility, ovarian failure, oligomenorrhea, testicular atrophy, reduced estrogen in the blood, increased gonadotropin in the blood.
Inborn, familial, and genetic diseases Unknown (cannot be estimated based on available data)	Fetal death, fetal malformation, delayed fetal development, fetal toxicity.
General symptoms and reactions at the administration site Very common (≥ 1/10) Common (≥ 1/100 to <1/10) Rare (≥ 1/10,000 to <1/1,000) Very rare (<1/10,000) Unknown (cannot be estimated based on available data)	Fever. Chills, asthenic conditions (e.g. fatigue, weakness, discomfort), mucositis. Chest pain. Multiple organ failure, headache, pain. Reactions at the administration site, e.g. phlebitis, thrombosis, necrosis, pain, swelling, erythema. Pyrexia, edema, influenza-like illness.
Examinations Uncommon (≥ 1/1,000 to <1/100)	ECG changes, reduced left ventricle ejection fraction (LVEF), increased LD, increased C-reactive protein.

Very rare (<1/10,000)	Weight increase

Source: The Danish Medicines Agency SPC, cyclophosphamide[58]
The text is abbreviated compared to the approved SPC. The full SPC is available at www.produktresume.dk.

Fludarabine phosphate

The most frequent side effects are myelosuppression (neutropenia, thrombocytopenia, and anemia), infections including pneumonia, cough, fever, fatigue, malaise, nausea, vomiting, and diarrhea. Other commonly presenting side effects are chills, edemas, discomfort, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis, and skin rash. Serious opportunistic infections have arisen among patients treated with fludarabine phosphate. Deaths caused by serious side effects have been reported.

Patients, who receive fludarabine phosphate, may experience the following side effects:

Infections and parasitic diseases Very common (≥ 1/10) Rare (≥ 1/10,000 to <1/1,000)	 Infections/opportunistic infections (as latent virus reactivation, such as e.g. progressive multifocal leukoencephalo-pathy, Herpes zoster virus, Epstein-Barr-virus) Pneumonia Lymphoproliferative diseases (EBV-associated)
Benign, malignant, and uspecified tumors (incl. cysts and polyps) Common (≥ 1/100 to <1/10)	 Myelodysplastic syndrome and acute myeloid leukemia (mainly associated with previous, concurrent, or later treatment with alkylating compounds, topoisomerase-inhibitors, or radiotherapy)
Blood and lymphatic system Very common (≥ 1/10) Common (≥ 1/100 to <1/10)	 Neutropenia, anemia, thrombocytopenia Myelosuppression
The immune system Uncommon (≥ 1/1,000 to <1/100)	 Autoimmune diseases (including autoimmune hemolytic anemia, Evans syndrome, thrombocytopenic purpura, acquired hemophilia, pemphigus)

Metabolism and nutrition Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100)	 Anorexia Tumor lysis syndrome (including kidney failure, metabolic acidosis, hyperkalemia, hypocalcemia, hyperuricemia, hematuria, urinary stones, hyperphosphatemia)
The nervous system Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100) Rare (≥ 1/10,000 to <1/1,000) Unknown	 Peripheral neuropathy Confusion Coma, seizures, agitation Cerebral bleeding
Eyes Common (≥ 1/100 to <1/10) Rare (≥ 1/10,000 to <1/1,000)	 Visual disturbances Blindness, optic neuritis, optic neuropathy
Heart Rare (≥ 1/10,000 to <1/1,000)	 Cardiac arrest, arrhythmia
Airways, thorax, and mediastinum Very common (≥ 1/10) Uncommon (≥ 1/1,000 to <1/100) Unknown	 Cough Pulmonary toxicity (including pulmonary fibrosis, pneumonitis, dyspnea) Pulmonary bleeding
Gastrointestinal tract Very common (≥ 1/10) Common (≥ 1/100 to <1/10) Uncommon (≥ 1/1,000 to <1/100)	 Vomiting, diarrhea, nausea Stomatitis Gastrointestinal bleeding, abnormal pancreatic enzymes
Liver and biliary tract Uncommon (≥ 1/1,000 to <1/100)	 Abnormal liver enzymes
Skin and subcutaneous tissues Common (≥ 1/100 to <1/10)	 Rash

Rare (≥ 1/10,000 to <1/1,000)	Skin cancer, toxic epidermal necrolysis, (Lyell-type), Stevens-Johnson-syndrome
Kidneys and urinary tract Unknown	Hemorrhagic cystitis
General symptoms and reactions at the administration site Very common (≥ 1/10) Common (≥ 1/100 to <1/10)	 Fever, fatigue, lethargy Edema, mucositis, chills, discomfort

Source: The Danish Medicines Agency SPC, fludarabine phosphate[59]
The text is abbreviated compared to the approved SPC. The full SPC is available at www.produktresume.dk.

TILs

No serious side effects of TIL infusion are expected. The patients can briefly experience fever, chills, and light dyspnea, and a small drop in saturation has been observed a few times. In addition, autoimmune reactions, including vitiligo and uveitis[60], can be seen.
Theoretically, there is a risk for the development of allergic reactions/anaphylactic shock. According to the literature this has not been seen yet.

Interleukin-2

Frequency and severity of side effects associated with interleukin-2 have in general proven to be dependent on route of administration, dose, and dosing interval. Most side effects are self-limiting and will go away within 1-2 days of treatment discontinuation.

See also the enclosed ”Vejledning til monitorering, dosismodifikationer og understøttende behandling under infusion af høj-dosis Interleukin-2” (”Guidelines for monitoring, dose modifications, and supportive treatment during infusion of high-dose interleukin-2”) (appendix 7)

The following side effects were reported from clinical studies and from post-marketing experience with interleukin-2:

Infections and parasitic diseases Common (≥1/100 to <1/10)	Respiratory infection.
Blood and lymphatic system (see further information below the table) Very common (≥1/10) Common (≥1/100 to <1/10) Uncommon (≥1/1,000 to <1/100) Rare (≥1/10,000 to <1/1,000)	Anemia, thrombocytopenia. Leukopenia, coagulopathy including disseminated intravascular coagulation, eosinophilia. Neutropenia Agranulocytosis, aplastic anemia, hemolytic anemia, neutropenic fever.
The immune system Uncommon (≥1/1,000 to <1/100) Rare (≥1/10,000 to <1/1,000)	Hypersensitivity reactions. Anaphylaxis.
The endocrine system Very common (≥1/10) Common (≥1/100 to <1/10)	Hypothyroidism Hyperthyroidism.
Metabolism and nutrition Very common (≥1/10) Common (≥1/100 to <1/10) Uncommon (≥1/1,000 to <1/100) Rare (≥1/10,000 to <1/1,000)	Anorexia. Acidosis, hyperglycemia, hypocalcemia, hypercalcemia, hyperkalemia, dehydration. Hypoglycemia. Diabetes mellitus
Mental disturbances Very common (≥1/10) Common (≥1/100 to <1/10):	Anxiety, confusion, depression, insomnia. Irritability, agitation, hallucinations.

The nervous system	
Very common (≥1/10)	Dizziness, headache, paresthesia, somnolence.
Common (≥1/100 to <1/10)	Neuropathy, syncope, speech disturbances, lost sense of taste, drowsiness.
Uncommon (≥1/1,000 to <1/100)	Coma, cramps, paralysis, muscle weakness
Unknown (cannot be estimated based on available data)	Intracranial/cerebral bleeding, cerebrovascular event, leukoencephalo-pathy (see further information below the table).
Eyes	
Common (≥1/100 to <1/10)	Conjunctivitis.
Rare (≥1/10,000 to <1/1,000)	Optic nerve disease including optic neuritis.
Heart	
Very common (≥1/10)	Tachycardia, arrhythmia, chest pain. Arrhythmia, cyanosis.
Common (≥1/100 to <1/10)	Cyanosis, transient ECG changes, myocardial ischemia, palpitations, cardiovascular disease including heart failure. Ventricular hypokinesis.
Uncommon (≥1/1,000 to <1/100)	Myocarditis, cardiomyopathy, cardiac arrest, pericardial exudate.
Rare (≥1/10,000 to <1/1,000)	Ventricular hypokinesis.
Unknown	Cardiac tamponade

Vascular diseases Very common (≥1/10)	Hypotension.
Common (≥1/100 to <1/10)	Phlebitis, hypertension.
Uncommon (≥1/1,000 to <1/100)	Thrombosis, thrombophlebitis, bleeding.
Airways, thorax, and mediastinum Very common (≥1/10)	Dyspnea, cough.
Common (≥1/100 to <1/10)	Pulmonary edema, pleural exudate, hypoxia, hemoptysis, epistaxis, nasal congestion. rhinitis.
Rare (≥1/10,000 to <1/1,000)	Pulmonary embolism, adult respiratory distress syndrome.
Gastrointestinal tract Very common (≥1/10)	Nausea with or without vomiting, diarrhea, stomatitis.
Common (≥1/100 to <1/10)	Dysphagia, dyspepsia, constipation, gastrointestinal bleeding including rectal bleeding, hematemesis, ascites, cheilitis, gastritis.
Uncommon (≥1/1,000 to <1/100)	Pancreatitis, intestinal obstruction, gastrointestinal perforation including necrosis/gangrene.
Rare (≥1/10,000 to <1/1,000)	Activation of latent Crohn's disease, pancreatitis, intestinal obstruction.
Liver and biliary tract Common (≥1/100 to <1/10)	Elevation of liver transaminases, increase in alkaline phosphatase, increase in lactate dehydrogenase, hyperbilirubinemia, hepatomegaly or hepatosplenomegaly.
Rare (≥1/10,000 to <1/1,000)	Cholecystitis, liver failure with lethal outcome.

Skin and subcutaneous tissues Very common (≥1/10)	Erythema/rash, skin exfoliation, pruritus, sweating.
Common (≥1/100 to <1/10)	Alopecia, urticaria.
Uncommon (≥1/1,000 to <1/100)	Vitiligo, Quincke's Edema.
Rare (≥1/10,000 to <1/1,000)	Vesiculobullous rash, Stevens-Johnson's syndrome.
Bones, joints, muscles, and connective tissue Common (≥1/100 to <1/10)	Myalgia, arthralgia.
Uncommon (≥1/1,000 to <1/100)	Myopathy, myositis.
Kidneys and urinary tract Very common (≥1/10)	Oliguria, increased serum urea, increased serum creatinine.
Common (≥1/100 to <1/10)	Hematuria, kidney failure, anuria.
General symptoms and reactions at the administration site Very common (≥1/10)	Reaction at the injection site*, pain at the injection site*, inflammation at the injection site*, fever with or without chills, discomfort and fatigue, pain, edema, weight increase, weight loss Necrosis at the injection site.
Common (≥1/100 to <1/10)	Mucositis, lump at the injection site, hypothermia.
Rare (≥1/10,000 to <1/1,000)	Necrosis at the injection site.

Source: The Danish Medicines Agency SPC, proleukin[61]
The text is abbreviated compared to the approved SPC. The complete SPC is available at www.produktresume.dk.

Comments:
* The frequency of reactions, pain, and inflammation at the injection site is lower than what is seen with continuous intravenous infusion.

Leukoencephalopathy

In the literature rare cases of leukoencephalopathy in connection with Proleukin have been reported, in particular among patients treated for HIV infection. In some cases, other risk factors such as opportunistic infections, co-administration of interferons, as well as several series of chemotherapy, which may predispose the patients to leukoencephalopathy, were present.

Capillary leak syndrome
Cardiac arrhythmia (supraventricular and ventricular), angina pectoris, myocardial infarction, respiratory insufficiency demanding intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental state changes can be linked with capillary "leak" syndrome. The frequency and severity of the capillary "leak" syndrome is lower after subcutaneous administration than for continuous intravenous infusion.

Serious manifestations of eosinophilia
During treatment most patients develop lymphocytopenia and eosinophilia with reactive lymphocytosis within 24-48 hours after cessation of treatment. These conditions are not considered to be side effects and may be attributed to the Proleukin mechanism of anti-tumor activity.

Cerebral vasculitis
Cerebral vasculitis, both isolated and in combination with other manifestations, has been reported. Cutaneous and leukocytoclastic hypersensitivity vasculitis have been reported. Some of these cases respond to treatment with corticosteroids.

Side effects associated with concurrent treatment with interferon alfa
The following side effects have been reported as rare with respect to parallel treatment with interferon alfa: Extracapillary IgA glomerulonephritis, oculo-bulbar myasthenia gravis, inflammatory arthritis, thyroiditis, bullous pemphigoid, rhabdomyolysis, as well as Stevens-Johnson's syndrome. Serious rhabdomyolysis and myocardial damage, including myocardial infarction, myocarditis, and ventricular hypokinesis, appear to be increased among patients treated with Proleukin (intravenously) and interferon alfa simultaneously.

Bacterial infection
Bacterial infection or worsening of bacterial infection, including septicemia, bacterial endocarditis, septic thrombophlebitis, peritonitis, pneumonia, and local infection surrounding the catheter site has been reported, mainly in association with intravenous administration.

Peginterferon alpha 2b

The following treatment-related side effects were reported among adults in clinical trials or through post-marketing surveillance of patients with chronic hepatitis C treated with peginterferon alfa-2b, including PegIntron monotherapy or PegIntron/ribavirin.

Infections and parasitic diseases Very common (≥ 1/10) Common (≥ 1/100 to < 1/10) Uncommon (≥ 1/1,000 to < 1/100)	 Viral infection*, pharyngitis * Bacterial infection (including sepsis), fungal infection, influenza, upper respiratory tract infection, bronchitis, herpes simplex, sinusitis, otitis media, rhinitis Infection at the injection site, lower respiratory tract infection
Blood and lymphatic system Very common (≥ 1/10) Common (≥ 1/100 to < 1/10) Very rare (< 1/10,000) Unknown (cannot be estimated based on available data)	 Anemia, neutropenia Hemolytic anemia, leukopenia, thrombocytopenia, lymphadenopathy Aplastic anemia Pure red cell aplasia
The immune system Uncommon (≥ 1/1,000 to < 1/100) Rare (≥ 1/10,000 to < 1/1,000) Unknown (cannot be estimated based on available data)	 Hypersensitivity towards the drug Sarcoidosis Acute allergic reactions including angioedema, anaphylaxis and anaphylactic reactions including anaphylactic shock, idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura, systemic lupus erythematosus
The endocrine system Common (≥ 1/100 to < 1/10)	 Hypothyroidism, hyperthyroidism

Metabolism and nutrition Very common (≥ 1/10)	Anorexia
Common (≥ 1/100 to < 1/10)	Hypocalcemia, hyperuricemia, dehydration, increased appetite
Uncommon (≥ 1/1,000 to < 1/100)	Diabetes mellitus, hypertriglyceridemia
Rare (≥ 1/10,000 to < 1/1,000)	Diabetic ketoacidosis
Mental disturbances Very common (≥ 1/10)	Depression, anxiety*****, emotional lability***,** decreased concentration, insomnia
Common (≥ 1/100 to < 1/10)	Aggression, agitation, anger, mood changes, abnormal behavior, nervosity, sleep disturbances, decreased libido, apathy, abnormal dreams, crying
Uncommon (≥ 1/1,000 to < 1/100)	Suicide, suicide attempt, suicidal ideation, psychosis, hallucinations, panic attacks
Rare (≥ 1/10,000 to < 1/1,000)	Bipolar conditions
Unknown (cannot be estimated based on available data)	Homicidal ideation, mania
The nervous system Very common (≥ 1/10)	Headache, dizziness
Common (≥ 1/100 to < 1/10)	Amnesia, failing memory, syncope, migraine, ataxia, confusion, neuralgia, paresthesia, hypoesthesia, hyperesthesia, hypertonia, drowsiness, attention disturbances, tremor, dysgeusia
Uncommon (≥ 1/1,000 to < 1/100)	Neuropathy, peripheral neuropathy
Rare (≥ 1/10,000 to < 1/1,000)	Seizures
Very rare (< 1/10,000)	Cerebrovascular bleeding, cerebrovascular ischemia, encephalopathy
Unknown (cannot be estimated based on available data)	Facial paralysis, mononeuropathies

Eyes Common (≥ 1/100 to < 1/10)	Visual disturbances, blurred vision, photophobia, conjunctivitis, eye irritation, lacrimation disorder, eye pain, dry eyes
Uncommon (≥ 1/1,000 to < 1/100)	Retinal exudates
Rare (≥ 1/10,000 to < 1/1,000)	Loss of visual acuity or field of vision, retinal hemorrhage, retinopathy, retinal artery occlusion, retinal vein occlusion, optic neuritis, papilledema, macular edema
Unknown (cannot be estimated based on available data)	Serous retinal detachment
Ear and labyrinth Common (≥ 1/100 to < 1/10)	Reduced hearing/hearing loss, tinnitus, dizziness
Uncommon (≥ 1/1,000 to < 1/100)	Ear pain
Heart Very common (≥ 1/10)	Palpitations, tachycardia
Uncommon (≥ 1/1,000 to < 1/100)	Myocardial infarction
Rare (≥ 1/10,000 to < 1/1,000)	Congestive heart failure, cardiomyopathy, arrhythmia, pericarditis
Very rare (< 1/10,000)	Cardiac ischemia
Unknown (cannot be estimated based on available data)	Pericardial effusion
Vascular diseases Common (≥ 1/100 to < 1/10)	Hypotension, hypertension, flushing
Rare (≥ 1/10,000 to < 1/1,000)	Vasculitis

Airways, thorax, and mediastinum Very common (≥ 1/10)	Dyspnea*, cough*
Common (≥ 1/100 to < 1/10)	Dysphonia, epistaxis, breathing problems, clogged airways, sinus congestion, nasal congestion, rhinorrhea, increased upper airway secretion, pharyngolaryngeal pain
Very rare (< 1/10,000)	Interstitial lung disease
Gastrointestinal tract Very common (≥ 1/10)	Vomiting*, nausea, abdominal pain, diarrhea, dry mouth*
Common (≥ 1/100 to < 1/10)	Dyspepsia, gastroesophageal reflux, stomatitis, mouth sores, glossalgia, gingival bleeding, constipation, flatulence, hemorrhoids, cheilitis, abdominal distension, gingivitis, glossitis, tooth disorder
Uncommon (≥ 1/1,000 to < 1/100)	Pancreatitis, mouth pain
Rare (≥ 1/10,000 to < 1/1,000)	Ischemic colitis
Very rare (< 1/10,000)	Colitis ulcerosa
Liver and biliary tract Common (≥ 1/100 to < 1/10)	Hyperbilirubinemia, hepatomegaly
Skin and subcutaneous tissues Very common (≥ 1/10)	Alopecia, pruritus*, dry skin*, rash*
Common (≥ 1/100 to < 1/10)	Psoriasis, photosensitivity reaction, maculopapular rash, dermatitis, erythematous rash, eczema, night sweats, hyperhidrosis, acne, furunculosis, erythema, urticaria, abnormal hair structure, nail disorder
Rare (≥ 1/10,000 to < 1/1,000)	Cutaneous sarcoidosis
Very rare (< 1/10,000)	Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema multiforme

Bones, joints, muscles, and connective tissue Very common (≥ 1/10)	Myalgia, arthralgia, musculoskeletal pain
Common (≥ 1/100 to < 1/10)	Arthritis, back pain, muscle spasms, pain in the extremities
Uncommon (≥ 1/1,000 to < 1/100)	Bone pain, muscle weakness
Rare (≥ 1/10,000 to < 1/1,000)	Rhabdomyolysis, myositis, rheumatoid arthritis
Kidneys and urinary tract Common (≥ 1/100 to < 1/10)	Changes in urinary frequency, polyuria, abnormal urine
Rare (≥ 1/10,000 to < 1/1,000)	Kidney failure, reduced kidney function
The reproductive system and breasts Common (≥ 1/100 to < 1/10)	Amenorrhea, chest pain, menorrhagia, menstruation irregularities, ovarian disorders, vaginal problems, sexual dysfunction, prostatitis, erectile dysfunction
General symptoms and reactions at the administration site Very common (≥ 1/10)	Reactions at the injection site*****, inflammation at the injection site, fatigue, asthenia, irritability, chills, fever, influenza-like symptoms, pain
Common (≥ 1/100 to < 1/10)	Chest pain, chest discomfort, pain at the injection site, discomfort, facial edema, peripheral edema, abnormal sensation, thirst
Rare (≥ 1/10,000 to < 1/1,000):	Necrosis at the injection site
Examination Very common (≥ 1/10)	Weight loss

Source: European Medicines Agency (EMA) SPC: PegIntron (EMA[7]).
The text is abbreviated compared to the approved SPC. The full SPC is available at the EMA home page: www.ema.europa.eu/ema/

Comments:
*****These side effects were common (≥ 1/100 to < 1/10) in clinical trials among patients treated with PegIntron monotherapy.

Risks and disadvantages associated with surgery and sample collection

Risks associated with the removal of tumor tissue

Prior to the inclusion it will be assessed, whether it is possible to remove part of the patient's own tumor tissue, either from the primary tumor or from a metastasis, through a smaller surgical procedure. The operation will be performed by physicians at the Department of Plastic Surgery at Herlev Hospital or by physicians from other surgical specialties, if necessary. Use of subcutaneous/cutaneous metastases or lymph nodes is prioritized. If readily accessible tumor tissue is not available, or if the removal is associated with serious risks for the patient, the patient cannot participate in the study.

Risks associated with biopsy collection

In association with biopsy collection there will be a small risk of infection and/or bleeding. Also, there may be discomfort such as pain or ecchymosis in the biopsy area.

Risks associated with blood sampling

In association with blood sampling the patient may experience discomfort such as pain or ecchymosis at the puncture site. The blood samples will also be associated with frequent visits at the hospital.

Monitoring and precautions

Hematological parameters

Close hematological surveillance of blood counts is indicated for all patients during the treatment. Leukocyte count, thrombocyte count, and hemoglobin values will be controlled at fixed intervals. Measurements will be made prior to commencing chemotherapy, Peg-Intron, and IL-2, and daily during treatment until the neutrophilocyte number is > 500/µl and the leukocyte number is > 1000/µl. Chemotherapy will not be administered to patients with leukocyte numbers below 500/µl and/or a thrombocyte number below 50,000/µl before commencing chemotherapy.

Kidney and urinary tract function

Before treatment start any obstruction of the efferent urinary tract, cystitis, or infection will be resolved. The patients will be treated with mesna and plenty of fluids to reduce the frequency and severity of bladder toxicity. If, during the treatment with cyclophosphamide, cystitis associated with micro- or macrohematuria is detected, the treatment will be stopped. The urine will be controlled for the presence of microscopic hematuria with a urine analysis test prior to commencing treatment with cyclophosphamide.

Cardiotoxicity

Cardiotoxicity is especially seen in association with administration of high doses of cyclophosphamide (120-240 mg/kg body weight). An electrocardiogram will be taken prior to treatment. Patients with known heart disease cannot be included in the study. If the patient experiences symptoms from the coronary circulation (e.g. chest pain, shortness of breath) the necessary examination-procedures will be performed.

Infertility

Treatment of men can increase the risk of irreversible infertility, and they will therefore be informed about the possibility of freezing down semen before treatment start. There is also a risk of fertility problems among women.

Live vaccines

Vaccination with live vaccines must be avoided during and immediately after treatment with chemotherapy due to the immunosuppressive effect.

Interactions:

Cyclophosphamide inhibits the cholinesterase activity and thereby reinforces the effect of depolarizing muscle relaxants, such as suxamethoniumchloride. This may result in longer-lasting apnea in association with anesthesia. The anesthesiologist must be informed, if a patient has received treatment with cyclophosphamide within 10 days prior to treatment with suxamethoniumchloride. The combination should be avoided.

Patients should not eat grapefruit or drink grapefruit juice, as grapefruit contains a substance, which may impair the activation of cyclophosphamide and thereby its effects.

Transfusion-related graft-versus-host reactions have been observed among patients treated with fludarabine phosphate after transfusion with non-irradiated/non-filtered blood. Therefore, patients who require blood transfusion and whom receive or have received treatment with fludarabine phosphate within ½ a year, may only receive irradiated or filtered blood. An agreement has been made with Blodbanken (the “blood bank”) at Herlev Hospital, that only irradiated blood will be ordered for these patients for half a year after treatment. All blood within the Capital Region of Denmark is filtered.

Results from a multi-dose study, which evaluated CYP substrates among chronic hepatitis C patients, who received pegylated interferon (PegIntron, 1.5 μg/kg) once weekly for 4 weeks, showed an increased activity of CYP2D6 and CYP2C8/9. Changes in the activity of CYP1A2, CYP3A4, or N-acetyltransferase were not observed.

Caution should be shown when co-administering peginterferon alfa-2b with drugs, which are metabolized by CYP2D6 and CYP2C8/9, especially drugs with a narrow therapeutic window, such as warfarin and phenytoin (CYP2C9) and flecainide (CYP2D6).

Effect evaluation, data analysis, and monitoring

Effect evaluation

Primary effect parameter

Paraclinical evaluation: to evaluate the immunological effects of the treatment, the patients will be followed by continuous *in vitro* analyses of the specific T-cell reactivity against tumor antigens. The immunological response towards tumor antigens before and after the treatment will be compared. These analyses will be performed on blood samples and tumor biopsies.

Secondary effect parameter

Clinical evaluation: The clinical effects of the treatment will be assessed using the objective response rate according to RECIST 1.1, survival, and progression-free survival.

Response criteria

RECIST

Clinical evaluation will be made according to the RECIST 1.1 Guidelines[62]:

Complete response (CR): All lesions disappear.
Partial response (PR): Defined as ≥ 30 % reduction of the sum of all measurable lesions' longest diameter.
Stable disease (SD): Defined as < 30 % reduction of the sum of all measurable lesions' longest diameter, or < 20 % increase in the sum of all measurable lesions' longest diameters.
Progressive Disease (PD): Defined as > 20 % increase in the sum of all measurable lesions' longest diameter *or* the presence of new lesions.

Complete and partial response must be verified by examination at the earliest 4 weeks after the response has been documented.

Immunological monitoring

Blood samples for immunological monitoring

Blood samples of 100 ml heparinized blood are taken for immunological monitoring, as well as 10 ml blood in dry glasses to freeze down serum; at surgery, before treatment start (at "baseline"), at discharge (approximately 1 week after T-cell infusion), and then 6 and 12 weeks after infusion of T cells (see schedule for examination plan and for follow-up). Thereafter, blood samples for immunological monitoring will be taken, when the patients show up for evaluation every third month, and until the patient leaves the study. Further, additional serum samples (10 ml blood

sample) are taken on day 0 before TIL-infusion, 2 hours after TIL-infusion, and then every second day until discharge.
This means, that a total of 500 ml blood will be taken for research in the period from the time of surgery until the patient reports to the outpatient clinic to receive the results of the first scan after the treatment (typically for a period of 3-4 months). These blood samples are taken in order to assess the effect of the treatment on the immune system for research purposes. The volume of blood, which will be taken for the study, does not surpass what the body itself can produce between each sampling. Research blood samples will not be taken, if the hemoglobin levels are not acceptable (> 6 mmol/l).

Mononuclear cells from peripheral blood (PBMCs) are isolated using Lymphoprep/Leucosep density gradient technique. The mononuclear cells are washed and resuspended in a freezing medium consisting of 90 % heat inactivated human AB serum and 10 % DMSO. The cells are frozen at -150 ^{o}C until analysis. A panel of relevant immunological assays for testing of antigen-specific immune reactivity will be applied, including measurement of cytokine production (multimer fluorescence staining, ELISPOT, ELISA), and proliferative and cytotoxic potential.

Tumor biopsies

Biopsy sampling from available tumor lesions or involved lymph nodes is planned. Depending on the localization and availability, excision-, punch-, crude needle-, or fine needle biopsies are taken. The biopsies are requested at the evaluation, 6 weeks after finished treatment, and in the case of progression, if possible (see the schedule for the examination plan and the schedule for follow-up). If the implicated areas are not readily accessible, the biopsy sampling will, if possible, take place under the guidance of ultrasound in sterile conditions at the ultrasound department at Herlev Hospital. The biopsy sampling will take place on an outpatient basis, and the biopsy size will here be approximately 5 mm^3.

Biopsies are examined for their content of immune cells. Further, TILs will be isolated from the lesions and analyzed for clonotype and specificity.

Statistics

This is a non-blinded, non-comparative study. Only descriptive statistics will be used, where the immunological and clinical response rate will be determined. Descriptive statistics will also be used to sum up response duration and patient characteristics. The study is designed as a pilot study and primarily aims at determining the safety and toxicity of the treatment. It is therefore not possible to formally determine a required sample size, which will allow determination of primary as well as secondary and tertiary end points. Based on experiences from previous clinical trials, inclusion of 20 patients[63] is deemed appropriate.

Data registration and analysis

The patient receives a patient number upon entry in the study in order to ensure patient anonymity. However, clinical personnel and selected individuals in the laboratory will be granted access to personal information, as this information is necessary to make sure the patient receives the correct treatment. The study responsible physician has access to the patient medical records to be able to request information about your illness, as this information shall be compared with the project specific analyses, which are made on cancerous tissue and blood samples.

All relevant data are registered in an eCRF (electronic Case Report Form), which is created together with the KFE. The principal investigator is responsible for eCRF creation and typing of data into the eCRF, when the study treatment has been completed. The eCRFs will be reported to the sponsor. Sponsor and principal investigator are responsible for data analysis for all included patients. Patient data and eCRF will be stored for 5 years according to existing guidelines for the storage of person sensitive information. A final report is compiled in collaboration between the members of the project group.

The analysis will include overviews of:

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

Upon termination of the study, person-referable data and any remaining samples will be coded. All patients, who receive T-cell infusion, will be included in the statistical analysis. Patients, who due to one of the following causes are excluded, will not be included in the statistical analysis:

- Does not have enough tissue to produce TILs
- Where TILs cannot be produced in the laboratory
- Where inclusion criteria at step 2 before starting chemotherapy are not fulfilled
- Withdraw their consent
- Has started another treatment

End of study report

Sponsor will inform the Danish Medicines Agency that the study has been concluded within 90 days. The study is deemed finalized at the last visit of the last patient. If the study is finished prematurely, the Danish Medicines Agency will also be informed with an explanation of the early ending.

Within one year of the conclusion of the study, sponsor will submit a final study report with the study results including publications based on the study to the Danish Medicines Agency and the National Committee on Health Research Ethics.

Amendments

Permission to make substantial changes to the protocol should be applied for from the Danish Medicines Agency and the National Committee on Health Research Ethics, and these changes cannot be implemented until approval has been received.
Changes in the protocol are considered substantial (substantial amendments), when they relate to

- The safety of the study participants
- The interpretation of the scientific documentation
- The completion or management of the study
- Quality and safety of the study drugs
- Other substantial changes

In the case of changes relating to

- extension relative to the date stated in the original approval
- new centers/changing of centers
- changing of principal investigator or sponsor
- when the study ends
- other small changes apart from typographical errors

the Danish Medicines Agency and the National Committee on Health Research Ethics will be informed, even though these authorities do not have to approve these changes.

Ethical considerations

Recruitment of study participants and informed consent

Patients with metastatic or locally advanced MM will be referred to the clinical trial from the oncology or plastic surgery department at Herlev Hospital or from one of the other oncological centers in Denmark, which treat patients with MM (Odense University Hospital, Aarhus University Hospital). Information about the study will be provided at scientific meetings for physicians at the involved departments. Referral of patients for the study is directed to the urogynecological (UG-team) visitation office, Department of Oncology at Herlev Hospital.

All patients will be informed about the study cf. appendix 1.

Insurance

The patients, who participate in the study, are covered by the hospital liability insurance.

Ethical aspects

Malignant melanoma has through several years shown increasing incidence and has a bad prognosis, when the illness is disseminated. Metastasizing MM can rarely be cured by either surgery, radiotherapy, chemotherapy, or immunotherapy, and the need for new treatment modalities is therefore considerable.
The purpose of this study is to improve the survival of patients with MM in the long term. Based on the current knowledge and the lack of treatment alternatives, there are no unacceptable risks or disadvantages associated with this study.

Participation is voluntary and preceded by both verbal as well as written information, and the treatment will be stopped in the event of unacceptable side effects, or at any time point if the patient so wishes. If the patient does not want treatment according to the protocol, the patient will receive treatment according to the usual guidelines of the department. The study is therefore deemed ethically sound.

The study follows the guidelines of the Declaration of Helsinki, and the study responsible physician will obtain permission from the National Committee on Health Research Ethics and the Danish Medicines Agency.

Research biobank

In relation to the present study, blood samples (110 ml/blood sample) and tumor biopsies will be taken and stored coded at -150 ºC in a research biobank at CCIT in room PA102, until all analyses linked to the study have been made or for a maximum of 15 years, after which any remaining material will be safely destroyed.
The samples may only be used for other research studies within other areas of research, if approved following a new application to the National Committee on Health Research Ethics. The National Committee on Health Research Ethics may according to the circumstances waive the requirement for consent in such future research projects.

The analyses will primarily be performed at CCIT, Department of Oncology, Herlev Hospital. Some special analyses of tumor tissue and blood samples may be performed at a foreign research institution, which we will make a specific collaboration with, and an independent Data Processor Agreement will be made. All patient information will in such cases be provided in coded form. If tissue and blood samples are sent abroad, they will be covered by the national legislation of the country, they are sent to. If the patient withdraws his or her informed consent, the biological material will be destroyed, if the patient does not want the material to be used.

Reporting to the Danish Data Protection Agency

The clinical trial is reported to the Danish Data Protection Agency. The law on treatment of personal data will be followed. Data pertaining to study subjects will be protected according to the law on treatment of personal data and the Health Act Section 3 regarding the legal position of patients.

Administrative aspects and publication

Patient identification

After entry into the study the patient will receive a number. This number will be used to identify the patient and will be used on electronic Case Report Forms (eCRFs). Data and patient material will be handled in a coded form and confidentially. The number will be awarded sequentially according to entry in the protocol and is not based on the patient's initials or birthdate.

Publications

The investigators are Inge Marie Svane, Rikke Andersen, Troels Holz Borch, and Marco Donia. Provided that the Vancouver rules, moreover, are fulfilled, the members of the project group will have shared copyright for the obtained results. Positive as well as negative results will be published in international scientific journals. Manuscripts will be prepared in collaboration between the investigators and the other members of the project group, with the investigators being primarily responsible for the preparation. The investigators will be co-authors on publications based on this study.
The order of authors will be determined according to the contribution of each author. The use of data from the study, both written and verbal, such as for e.g. conference participation, teaching, or the like can only take place after approval from the investigators. The investigators are obliged to publish results from the study and are naturally also interested in the results being disseminated and implemented in the everyday clinical practice. Publication is expected to be completed during 2017.

Economy

The study is initiated by the National Center for Cancer Immune Therapy (CCIT-DK) in collaboration with the Department of Oncology, Herlev Hospital, and is financed in part by these two departments, by the Capital Region of Denmark's research fund, and through operating and salary funds from private research foundations, which we continuously apply for.

The Committees on Health Research Ethics will be informed, if/when further funding is obtained. None of the physicians at the participating departments have any financial interest in the study, and there are no financial gains related to the study for the departments or their personnel. There are no financial connections between the funding bodies and the investigators.
The clinical trial is part of the study responsible physician Rikke Andersen's PhD project.

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Appendix 1

Guideline for providing verbal participant information

The verbal information regarding the project ”T cell therapy in combination with interferon-γ for patients with metastasized malignant melanoma” will be provided at the Department of Oncology, Herlev Hospital. The information session will be carried out by the study responsible physicians. In connection with the verbal information, written information material will be handed out. The session will take place in compliance with the guidelines from the Committee on Health Research Ethics.

Before the information session

- a time and a place for the session must be booked
- it should be stated that it constitutes an invitation to participate in a health science research project
- the right to take time to consider the information and the opportunity to bring an assessor to the session should be stated

The information session

- must be carefully planned
- must take place in an undisturbed setting and without interruptions
- the participant must be given sufficient time to read the written information, listen to the verbal information, and ask questions
- must include an understandable presentation of the research study without the use of technical or emotionally charged terms, and be provided in a considerate manner, adapted to the recipient’s individual preconditions regarding age, maturity, experience etc.
- must contain information about possible predictable risks, side effects, complications, and disadvantages, as well as the fact that there may be unpredictable risks and burdens associated with participation in a health science research project.
- must contain information about other treatment options
- must contain information about the fact that information about health conditions, strictly private circumstances, and other confidential information may be passed on to and be handled by individuals, who have to carry out a legally required quality control of the study.
- must contain information about the participants’ rights to renounce knowledge regarding their own health
- the information is provided by the study responsible physician or by the thereto authorized person associated with the study

Period of reflection and obtaining consent

- The period of reflection depends on the nature of the study. As a rule, the period of reflection should be at least 24 hours.

- There should be a clear relationship between information and consent. This entails that the consent to study participation as a rule is given soon after the information has been provided, yet still taking into consideration the necessary period of reflection.

After the information session, the study subject will be informed,

- If during the study new information about effect, risks, side effects, complications, or disadvantages should arise.
- If the research project's experimental design should change considerably with respect to the safety of the study subject (pertains to study subjects who actively participate in the study).
- If, during the completion of the research study, significant information about the health of the study subject should arise, unless the study subject unequivocally has expressed that he or she does not want this.
- About the achieved results, as well as about possible consequences for the individual participant. This requires, that it is practically possible, and that the study subject wants this.
- If the study is discontinued, the study subject should be informed about the cause of this.

Appendix 2

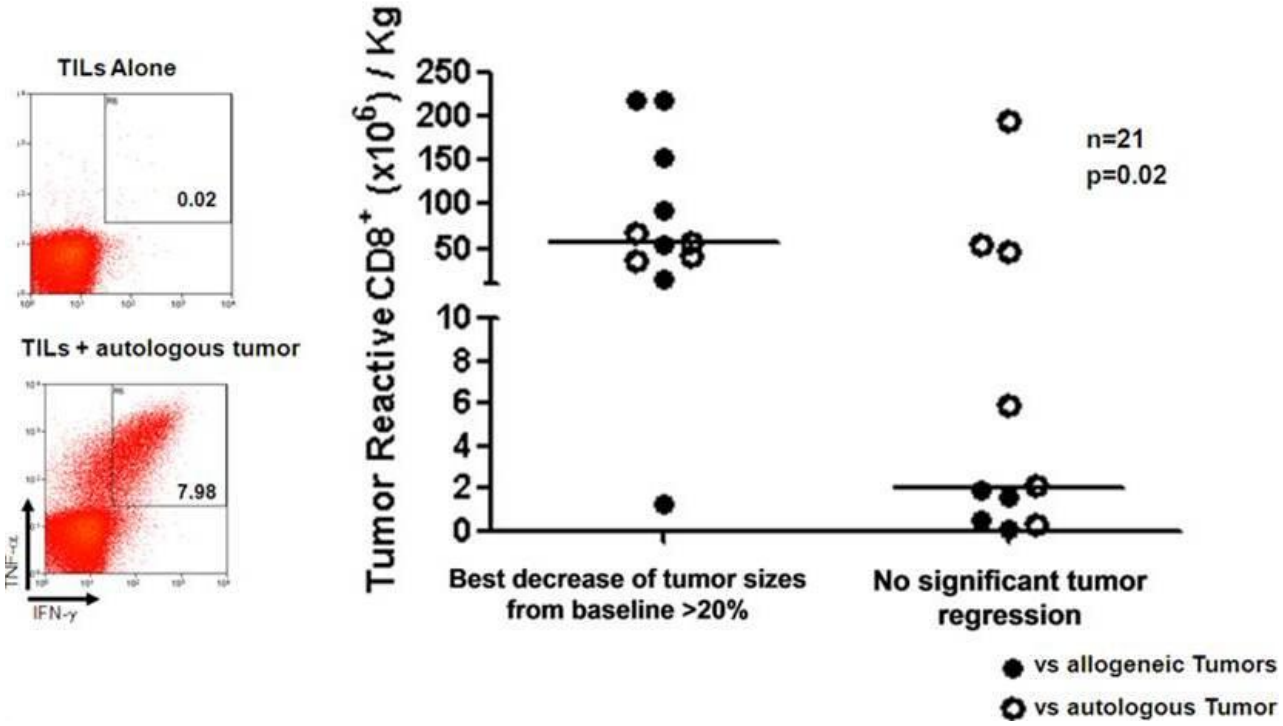
Patient characteristics and clinical results

	Patient Characteristics				Treatment Characteristics						Clinical Outcome	
Patient ID	Age	Sex	AJCC stage	Previous Treatments	Infused Cells ($x10^9$)	CD8%	CD4%	γδ%	Infused CD8 ($x10^9$)	IL-2	Response	Survival
Responders (n=11)												
MM909.01	60	F	M1c	1 (IL2)	28,7	78,1	19,5	0,03	22,4	Low	**CR (49 mo) NED**	**53 mo+**
MM909.11	41	M	M1a	3 (IL2, Ipi, DC)	74,7	97	2,4	0,01	72,5	Low	**CR (13 mo) NED**	**32 mo+**
MM909.15	48	F	M1b	1 (IL2)	85,7	31,3	54,9	10,7	26,8	Intermediate	**CR (27 mo+)**	**27 mo+**
MM909.17	49	M	M1c	2 (IL2, Ipi)	142,5	24,5	72	2,5	34,9	Intermediate	**PR (25 mo+) NED**	**25 mo+**
MM909.20	65	F	M1c	3 (IL2, Ipi, Tem)	82	88,5	10,5	0,04	92,7	Intermediate	**PR (12 mo)**	**19 mo+**
MM909.24	56	M	M1a	3 (Ipi, IL2, Vem)	110	67	20	0,05	73,7	Intermediate	**PR (15 mo+)**	**15 mo+**
MM909.26	46	M	M1c	1 (Ipi)	131,2	93	5	0,05	122,0	Intermediate	**PR (13 mo+)**	**13 mo+**
MM909.31	65	M	M1c	2 (IL2, Ipi)	117	53	47	0,22	62,0	Intermediate	**PR (11 mo)**	**12 mo+**
MM909.22	60	F	M1c	2 (IL2, Ipi)	123	21,6	78,1	0,02	26,6	Intermediate	**PR (8 mo+)**	**8 mo+**
MM909.36	40	F	M1c	3 (IL2, Ipi, Vem)	120	54,7	43,8	0,6	65,6	Intermediate	**PR (7 mo+)**	**7 mo+**
MM909.42	68	F	M1c	2 (IL2, Ipi)	83	8,2	91	0	6,8	Intermediate	**PR (2 mo+)**	**2 mo+**
	54 ± 4			**2,1 ± 0,8**	**100 ± 33**	**56 ± 31**	**40 ± 31**	**1,3 ± 3**	**55 ± 35**			
Non Responders (n=13)												
MM909.07	61	M	M1c	3 (IL2, CD137, DC)	12,7	89,2	9,8	0,17	11,3	Low	**SD (5mo)**	**11 mo**
MM909.03	62	M	M1c	2 (IL2, DC)	20	91,8	6,9	0,12	18,4	Low	**SD (4mo)**	**11.5 mo**
MM909.06	36	M	M1c	1 (IL2)	3,4	52,5	46,8	0,31	1,8	Low	**PD**	**4.6 mo**
MM909.02	47	M	M1b	2 (IL2, DC)	17,7	95,2	3,2	0,84	16,9	Low	**PD**	**7 mo**
MM909.16	60	M	M1c	2 (IL2, Ipi)	61,5	87,5	11,3	0,17	53,8	Intermediate	**SD (4 mo)**	**25 mo+**
MM909.18	51	M	M1c	2 (IL2, Ipi)	199,5	86,2	8,6	3,6	172,0	Intermediate	**SD (4 mo)**	**5.4 mo**
MM909.25	25	M	M1b	3 (Ipi, Il2, Ipi)	127	55	35	9,7	69,9	Intermediate	**SD (4 mo)**	**5.5 mo**
MM909.34	46	F	M1c	2 (IL2, Ipi)	110	92,2	4	0,93	101,4	Intermediate	**SD (4 mo)**	**5 mo**
MM909.37	36	F	M1c	2 (IL2, Ipi)	125	49,9	28,9	16,9	62,4	Intermediate	**SD (3 mo)**	**6 mo+**
MM909.40	53	M	M1c	3 (Ipi, IL2, Ipi)	78	27,7	57,8	9,53	21,6	Intermediate	**SD (2 mo+)**	**2 mo+**
MM909.14	43	F	M1c	4 (IL2, Ipi, Tem, DC)	85,7	17,3	82,1	0,25	14,8	Intermediate	**PD**	**3 mo**
MM909.29	52	F	M1c	4 (IL2, DC, Ipi, Vem)	81,6	37,3	10,1	51	30,4	Intermediate	**PD**	**6 mo**
MM909.27	62	F	M1b	3 (Ipi, IL2, Vem)	98	47,5	51,8	0,20	46,6	Intermediate	**PD**	**4 mo**
	48 ± 8			**2,5 ± 0,9**	**78 ± 56**	**64 ± 28**	**27 ± 25**	**7 ± 14**	**48 ± 47**			
Not Evaluated (n=2)												
MM909.43	48	F	M1c	2 (IL2, Tem)	99	31,9	67,1	0,17	31,6	Intermediate		
MM909.46	50	F	M1c	2 (IL2, Ipi)	75	31	54	13,5	23,3	Intermediate		

Patient characteristics and clinical results (unpublished) from studies of T cell therapy at CCIT/Department of Oncology, Herlev Hospital (clinicaltrials.gov identifier: NCT00937625). CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease

Appendix 3

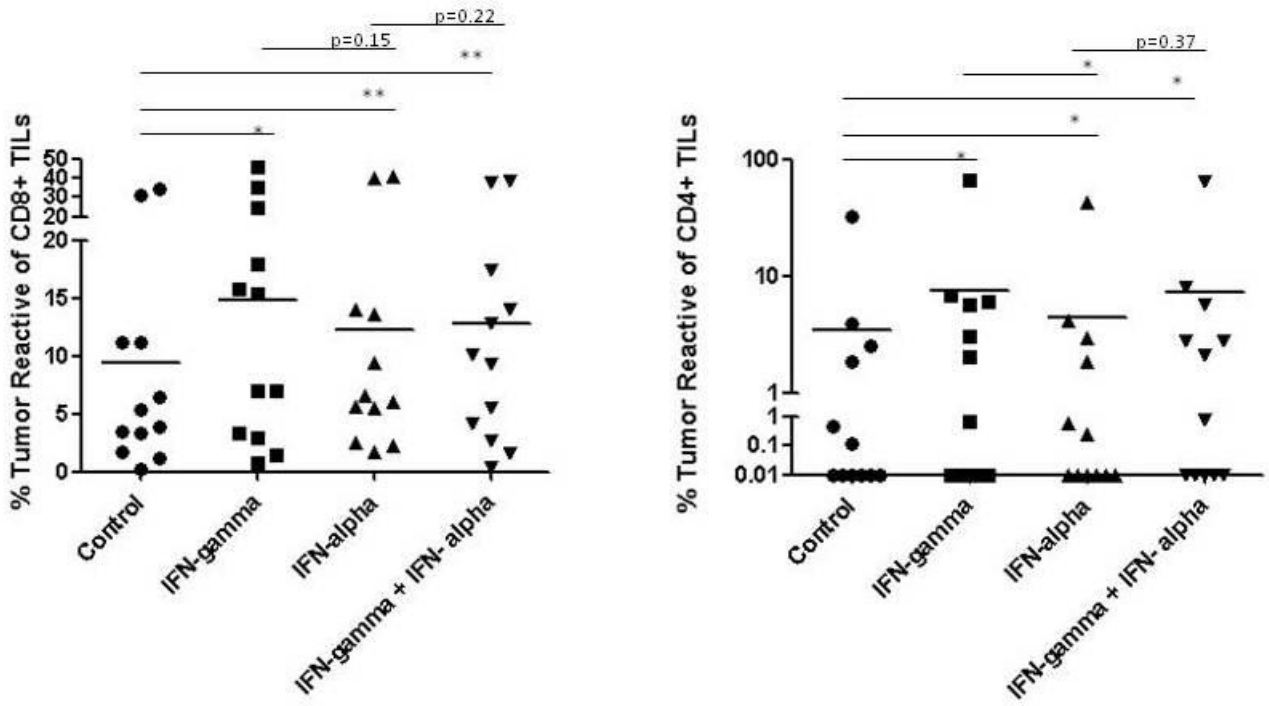
Anti-tumor response in the TIL infusion product



The TIL infusion product is incubated with autologous (arrow) or allogeneic tumor cell lines, after which the number of interferon (IFN)-γ and tumor necrosis factor (TNF)-α producing T cells are determined using intracellular cytokine staining. The number of reactive T cells, which are infused into the patient, is significantly correlated with clinical response. The reactivity of the infusion product has been analyzed for the first 21 patients. Donia M *et al.*, J Invest Dermatol 2013, and CCIT, unpublished.

Appendix 4

CD8+ and CD4+ TIL cytokine production is increased by stimulation with autologous tumor pretreated with interferon-alpha.



TILs were stimulated with autologous melanoma tumor cells *in vitro* for 4 hours (in the presence of brefeldin A). The figure shows the frequency of cytokine producing CD8+ (on the left) and CD4+ (on the right) TILs after stimulation with autologous tumor cells pretreated with nothing (control), Interferon-gamma (IFN-gamma), Interferon-alpha (IFN-alpha), and IFN-gamma + IFN-alpha, respectively.

Appendix 5

ECOG-function status/Performance status

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, no negative effects on activities of daily living (ADL).
1	Limited physically strenuous activity, but ambulatory and capable of performing light or sedentary work, e.g. light housework, office work.
2	Ambulatory and capable of taking care of him-/herself, but unable to do any kind of work. Ambulatory more than 50 % of waking hours.
3	Capable of taking care of him-/herself to a limited degree, bedridden or sitting more than 50 % of waking hours.
4	Completely disabled. Unable to take care of him-/herself. Always bedridden or sitting in a chair.
5	Dead

* Published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

Appendix 6

Common Terminology Criteria for Adverse Events (CTCAE) version 4

CTCAE[57] is a descriptive terminology, which can be used when reporting side effects. The adverse events (AEs) are graded according to the following:

- Grade 1: Mild AE
- Grade 2: Moderate AE
- Grade 3: Severe AE
- Grade 4: Life-threatening AE or AE leading to disability
- Grade 5: Death related to an AE

Appendix 7

Guidelines for monitoring, dose modifications, and supportive treatment during infusion of interleukin-2 (decrescendo-regime) associated with T cell therapy

Guidelines for protocol MM1413:
T cell therapy in combination with peginterferon for patients with metastatic malignant melanoma

Clinically responsible:
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Background and rationale

T-cell based immunotherapy is an experimental treatment regime with tumor-specific T cells, which *ex vivo* have been activated to show a specific reaction towards the cancer cells. This experimental type of treatment has been tested on patients with advanced malignant melanoma, and in this setting both complete and partial disease regression has been registered, where 50 % of the patients achieved measurable disease reduction.
Tumors are often infiltrated by large amounts of T cells (tumor-infiltrating lymphocytes), which specifically recognize tumor antigens but typically are inactive. It is possible in the laboratory to expand and reactivate such cytotoxic T cells for tumor cell killing. The patients' own tumor tissue is prepared in the laboratory, so that the cytotoxic T cells with the largest activity towards autologous tumor cells are activated and expanded *in vitro*. To maintain an immunological response towards the tumor, activating cytokines must be available to the specific T cells. Many "irrelevant" T cells will competitively reduce the availability of these cytokines to the T cells in question. To create an environment, which facilitates a T-cell mediated anti-tumor response, it is necessary, in addition to the presence of many tumor-specific T cells, to reduce irrelevant T cells and eliminate regulatory T cells.

Treatment

The treatment consists of one week of lymphocyte-depleting chemotherapy with two days of cyclophosphamide (60 mg/kg IV) and five days of fludarabine phosphate (25 mg/m^2 IV) followed by one infusion of tumor-infiltrating lymphocytes (TILs) (approximately 10^9-10^{10} cells).
After one week of chemotherapy and infusion of T cells on day 0, treatment with interleukin 2 is commenced, consisting of a continuous intravenous infusion on day 0-2: 18 MU/m^2 for 6 hours, 18 MU/m^2 for 12 hours, 18 MU/m^2 for 24 hours, followed by 4.5 MU/m^2 for 24 hours for 3 days.

Dose modification

The following treatment guideline only applies to IL-2.
The listed toxicity gradings are based on CTC criteria (version 4.0).

IL-2 is paused at the following toxicities:

- Hypotension according to the instructions of this guideline
- Newly arisen cardial arrhythmia, other than sinus tachycardia.
- Suspicion of or verified AMI.
- Agitation or persisting confusion.
- Bilirubin increase grade III/IV.
- Sepsis.
- Resting dyspnea.
- In the case of coagulation disturbances, defined as PP < 0.2.

In the case of pausing of IL-2, the patient should be reassessed every second hour, and the treatment cannot be resumed, until the toxicity is reduced to grade 0/1. When the IL-2 infusion is resumed, the planned dose is administered, so that the time of treatment is prolonged corresponding to the treatment pause. If the pause exceeds 24 hours, the treatment is shortened with this exceedance, so that the treatment is maximally prolonged with 24 hours.

Dose modification of IL-2 is primarily done with pausing. In the case of the following toxicities, IL-2 can be resumed at 50 % dose. IL-2 cannot be resumed until the toxicity is reduced to grade 0 or 1.

- In the case of an increase in creatinine grade III/IV
- In the case of grade II or III cerebral toxicity.

Contraindications for continued IL-2 treatment:

- Documented myocardial ischemia or infarction.
- Grade IV CNS toxicity.

Monitoring during IL-2 treatment

(Please refer to the relevant sections in the guidelines for supportive treatment)

- Vital functions:
- Pulse/BP every fourth hour.
- Temperature every eighth hour.
- Diuresis every 12 hours.
- Lung stethoscopy every 24 hours.
- Weight every 24 hours.

After treatment cessation, pulse/BP is measured for the last time after 4 hours, however BP is followed until normalization.

Monitoring of patients with systolic BP below 80 mm Hg, who are not responding to volume treatment are followed with:

- Pulse/BP every second hour.
- Diuresis every second hour.
- Lung stethoscopy every second hour.

Guidelines for supportive treatment

Influenza-like symptoms

During treatment with cytokines, the so called ”Flu-like Syndrome” usually arises, that is influenza-like symptoms such as fatigue, joint pain, fever, and chills. These constitutional symptoms are triggered by IL-2 and Interferon through a number of secondary cytokines, in particular TNFα and IL-1. The degree of these constitutional symptoms varies a lot from person to person. This may be due to genetic causes, as the TNFα production is determined by a genetic polymorphism in the HLA region.

The clinical picture

The degree of influenza-like symptoms varies a lot. Frequently, pronounced fatigue presents, as well as routinely fever, where the maximal increase in temperature varies but where the temperature may be between 38 and 41 ºC. In the case of a rapid increase in temperature, chills usually present. These side effects are usually not critical but can, however, be perceived by patients as burdensome.

Treatment principles

The fever is perceived as an effect and not a side effect of the treatment and therefore shall not be treated with antipyretic drugs such as paracetamol and NSAIDs. Only if the temperature surpasses 41 ºC, should treatment with paracetamol be initiated. It is of paramount importance, that the patients prior to the treatment are advised about these things, and that the fever is considered an important part of the treatment. It should be noted that opioids also have an antipyretic effect, and that their use therefore should be carefully considered.

References

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- Edwards, M.J *et al*: Passive immunization against tumor necrosis factor inhibits interleukin-2-induced microvaascular alterations and reduces toxicity. Surg. 1992.
- Hobohm U: Fever therapy revisited. British Journal of Cancer 2005.
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Nausea

The pathogenesis for IL-2 triggered nausea is unknown.

The clinical picture

Nausea and vomiting can arise during IL-2 treatment. It can vary from single bouts of vomiting without nausea to nearly permanent severe nausea with frequent vomiting. On the contrary, loss of appetite is seen in nearly all patients.

Treatment principles
General guidelines cannot be given, as there are large individual differences in the occurrence of nausea and vomiting. Both centrally as well as peripherally acting antiemetics can be used.

Treatment guideline
The following drugs can be used in the case of nausea, administered either perorally or IV. Prednisolone may not be used due to its immunosuppressive effects. Vogalene should be avoided due to the risk of orthostatic hypotension.

- Emend (Aprepitan) 125 mg on day 1, 80 mg on day 2 and 3
- Motilium (Domperidon) 20 mg up to 4 times pr. day
- Zofran (Ondansetron) 16 mg for 24 hours
- Kytril (Granisetron) 1-2 mg/24 hours
- Temesta (Lorazepam) 1-2 mg

References

- Siegel, J.P *et al*: Interleukin-2 toxicity. J. Clin. Oncol. 1991

Fluid balance/hypotension

Interleukin-2 usually leads to fluid retention. In the majority of the older studies with high-dose intravenous infusion, the most frequent dose-limiting side effect was a complex disturbance in the fluid balance. Interleukin-2 affects the regulation of the fluid balance through the so-called "capillary leak syndrome" and through a direct effect on the kidney function.

Reduced renal perfusion
Independently of the capillary-leak associated hypovolemia, oliguria is seen upon infusion of IL-2. It is evoked by an effect on the renal perfusion. IL-2 leads via leukotrienes to a disturbed renal vasoregulation. The large preglomerular vessels respond with pronounced vasoconstriction and subsequent reduction of the glomerular perfusion. This effect counteracts the physiological renovascular autoregulation, which in the case of a reduction in the glomerular flow actually was supposed to lead to a dilation of the preglomerular vessels. The pronounced reduction in the glomerular flow leads to a reduced function of the glomeruli and thereby oliguria, increasing to anuria.

Capillary leak syndrome

The underlying pathophysiology of the capillary leak syndrome is an increased permeability of the capillaries. There are several molecular mechanisms involved in this: in part an activation of endothelial cells via secondary cytokines, and in part a direct toxic effect of activated mononuclear cells, in particular on activated endothelial cells. The result is an increased permeability of the capillaries and an outflow of liquid and low molecular weight substances into the extravasal space. Hereby, interstitial edemas arise in nearly all organs, as well as an intravasal hypovolemia.

The clinical picture
The symptoms of the affected regulation of the fluid balance is complex. On one hand the capillary leak syndrome causes interstitial edemas and intravasal hypovolemia with subsequent arterial hypotonia. On the other hand, the rapidly developing renovascular autoregulatory disturbance leads to oliguria, fluid retention, and intravasal hypervolemia. Therefore, during infusion with IL-2 a reduced renal perfusion with oliguria and intravasal hypervolemia will occur. Later, as a consequence of the capillary leakage, an outflow of liquid to the extracellular space, edemas, and intravasal hypovolemia with consequent arterial hypotonia will occur (see below).

The following is a schematic attempt to describe interleukin-2's effect on the fluid balance:

<u>Reduced renal perfusion</u>
1) Reduced glomerular perfusion
2) Oliguria
3) Fluid retention
4) Intravasal hypervolemia
5) Edemas

<u>Capillary leak syndrome</u>
1) Increased capillary permeability
2) Outflow of water, electrolytes, and albumin
3) Interstitial edemas
4) Intravasal hypovolemia
5) Arterial hypotonia

Edemas can arise in all organs and are partly the cause of some of the organ side effects mentioned below. Interstitial pulmonary edema may be particularly problematic. This, however, should not arise during adequate treatment. The disturbances in the fluid balance frequently become more complicated due to fever, peripheral vasodilation, and diarrhea.

Treatment principles
To avoid intravasal hypovolemia, sufficient fluids should be administered. Large fluid infusions over a short time should, however, be avoided, as a pulmonary edema may arise. A fluid retention

of approximately 5-10 % of the body weight may be necessary during a five-day IL-2 infusion to prevent intravasal hypovolemia and thereby arterial hypotonia.
In the case of oliguria, dopamine or loop diuretics, which increase the glomerular perfusion, may be used. Both substances should be dosed correctly to maintain an adequate diuresis and may not be overdosed, as a too strong diuresis leads to volume depletion and thereby arterial hypotonia.
NSAID drugs cannot be used simultaneously with the IL-2 treatment due to a risk of aggravation of renal insufficiency.

Treatment guideline

- Concurrent with the IL-2 treatment, infusion of potassium-sodium-glucose infusion fluid at 100 ml/hour is administered for the entire IL-2 treatment period.
- In the case of a drop in systolic blood pressure of more than 30 mm Hg or to below 90 mm Hg, the infusion speed of potassium-sodium-glucose infusion fluid is increased to 125 ml/hour.
- At systolic blood pressure < 80 mm Hg or a clinically affected patient, 2 x 1000 ml NaCl infusion is given within 2 hours.
- If the blood pressure is not restored after the above, the IL-2 infusion is paused, and the patient is reassessed again after 2 hours with the aim to resume the IL-2 infusion.
- If the systolic blood pressure continues to drop, and the patient is clinically shocked, the patient should be transferred to the intensive care unit for treatment with pressors.

It is important, that the primary blood pressure, which is measured, and which forms the basis for assessment of blood pressure drop during treatment, is measured resting and without stress.

Voluven or HES infusion fluid is not used anymore, after two large studies have indicated a negative effect on renal function, and the Danish study showed an increased mortality in the HES treatment group.

In the case of hyponatremia below 130 mmol/L, NaCl is used instead of KNAG infusion in the same volumes. Hypokalemia can be compensated as usual according to the departmental instructions.

At diuresis below 360 ml/12 hours, furosemide is administered as a continuous infusion at 120 mg/24 hours. The infusion product is produced as two times furosemide 60 mg in 250 ml NaCl for infusion over 12 hours. The infusion is administered over a minimum of 24 hours and can be continued for as long as requested. Intravenous bolus infusion or tablet furosemide cannot be used.

References

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retention. Int.J. Immunophath. Pharmaol. 1993.
- Ognibene, F.P *et al*: Interleukin-2 administration causes reversible hemodynamic changes and left ventricular dysfunction similar to those seen in septic shock. Chest 1988.

Hematopoiesis

During treatment with IL-2, a drop in all hematopoietic cell lines (granulocytes, thrombocytes, and erythrocytes) in the peripheral blood can be seen. It is not caused by, as in chemotherapy, a toxic effect on the hematopoiesis. The bone marrow is not hypoplastic, and nadir is seen already at 1-2 days after cessation of the IL-2 infusion. The mechanism behind the hematopoietic toxicity is today not fully understood, probably it relates to an inhibition of the last steps of differentiation and an inhibited release of hematopoietic cells from the bone marrow through secondary cytokines. Unlike this, the lymphopenia, which usually presents after a few hours of IL-2 infusion, is a physiological reaction, as IL-2 activated lymphocytes in the peripheral blood express several adhesion antigens, which lead to adhesion to the capillary endothelium and subsequent extravasation. Upon cessation of IL-2 treatment the lymphopenia is reversible and is usually followed by lymphocytosis. These lymphocytes correspond to morphologically highly activated lymphatic cells, which can be confused with immature cells. After IL-2 infusion a pronounced eosinophilia is often seen, triggered by IL-5. The eosinophilia may in extreme cases constitute 50 % of the leukocytes. This phenomenon is not completely understood and has according to current knowledge no therapeutic or pathophysiological importance.

The clinical picture

As described above, a pronounced lymphopenia is seen during IL-2 infusion followed by lymphocytosis 1-2 days after cessation of treatment. Whether this has a clinical significance is not known. Anemia, thrombocytopenia, and granulocytopenia may occur, and among 10-20 % of patients, infusion of blood or thrombocytes is indicated. The hematological toxicity is reversible within a couple of days after cessation of IL-2 infusion. The granulocytopenia is very rarely serious but a functional disturbance of the granulocyte ability for chemotaxis may appear. This may lead to a reduced ability to fight bacterial infections, which predominantly is seen among patients with an intravenous catheter.

Treatment principles

Erythrocytes and thrombocytes may be substituted according to standard guidelines. Lympho- and granulocytopenia does not require treatment and does not warrant prophylactic antibiotics. All clinically uncharacteristic treatment courses should, however, lead to the consideration of bacterial infection.

Upon suspicion of bacterial infection (e.g. unusually high fever, unusually pronounced tachycardia, disturbances in coagulation, or affected general condition) the patient should be treated as for a bacteremia. This is important, as many of the side effects of IL-2 are similar to a bacteremia. If a patient is still having a high fever 3 hours after pausing of IL-2, the patient should be treated as

having a bacteremia. Nephrotoxic antibiotics should be avoided due to the simultaneous nephrotoxicity.

Treatment guideline
Upon suspicion of bacterial infection:

- Follow the usual procedures of the department
- Nephrotoxic antibiotics should be avoided

References

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- Klempner, M.S *et al*: An acquired chemotactic defect in neutrophils from patients receiving interleukin-2 immunotherapy. N. Engl. J. Med. 1990.
- Wanebeo, H.J *et al*: Production of and response to interleukin-2 in peripheral blood lymphocytes of cancer patients. Cancer. 1986.

Coagulation

Activated monocytes and endothelial cells can produce and release coagulation promoting substances. Of central pathogenetic importance is here the induction of Factor 3, which activates the exogenous coagulation cascade. The coagulation disturbance can be even more complex, as the malignant condition in itself can lead to hypercoagulopathy. Finally, the synthesis of coagulation factors in the liver may be reduced by IL-2.

The clinical picture
During IL-2 treatment a drop in coagulation factor 2,7,10 (PP) may be seen. Clinically relevant coagulation disturbances, such as disseminated intravascular coagulation (DIC) or thromboembolic complications, are, however, only seen rarely. In the case of severe coagulation disturbances, the possibility of sepsis should always be considered, and the treatment adapted accordingly.

Treatment principles
To avoid disruption of IL-2 infusion, K-vitamin should be given intravenously at the following PP values.

- At PP < 0.30 10 mg Konakion IV is given, this can be repeated until PP is above 0.30.
- At PP < 0.20 Interleukin-2 treatment in paused. Konakion 10 mg IV is given, whereafter a new blood sample is taken after 2 hours, the injection can be repeated. The Interleukin-2 treatment can be resumed at 100 % dose, when PP is above 0.30.
- In the case of abnormal bleeding tendency, which can be related to abnormal coagulation factors, fresh, frozen plasma (type-specific) can be given, contact e.g. the coagulation laboratory.

Patients treated with blood thinner will continue this treatment during the immunotherapy, and intervention is only needed, if PP falls below 0.20. In this case IL-2 is paused, and Konakion 1 mg IV may be used until PP is above 0.20, whereafter IL-2 can be resumed.

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- Fleischmann, J.D *et al*: Fibrinolysis, thrombocytopenia, and coagulation abnormalities complicating high-dose interleukin-2 immonotherapy. J. Lab. Clin. Med. 1991.

Skin

Several of the secondary cytokines, which are induced by IL-2, lead to a generalized dilatation of the peripheral vessels, in particular in the skin and mucosa. Histologically numerous activated T lymphocytes are found underneath the basement membrane. It is unclear, if these cells represent a proliferation of already present subcutaneous lymphocytes, or if they are extravasated from the dilated capillaries in the skin. In the skin these activated T cells release additional cytokines, which enhance the inflammatory reaction.

The clinical picture

The most distinctive clinical manifestation is a pronounced erythema, which is seen in nearly all patients, who receive high-dose IL-2. The erythema, however, has no clinical significance. Inflammatory skin conditions with generalized pruritus and hives can develop. Further, it is known that autoimmune skin conditions, in particular psoriasis, are worsened during IL-2 treatment.

Essential fatty acids to relieve skin problems

Good symptomatic effects on skin dryness and pruritus has been found with plant oil based dietary supplements with the essential fatty acids omega-3, omega-6, and omega-9. This should be recommended to the patient as basis treatment alongside of skin lotion. The dietary supplements described below can be purchased in health food stores and other products can probably be used; however, types which contain the three mentioned types of essential fatty acids are recommended. The following products have so far been used by patients: “Perfect balance” or “Livets olie” (“Life’s oil”). The recommended daily dose is listed on the product. The ingestion of the oil is easiest if used together with beverages e.g. juice. For skin areas with annoying itching, the oil can additionally be applied directly to the skin a couple of times per day.

Treatment principles

The generalized erythema does not require treatment. In the case of pruritus and hives, systemic antihistamine products may be effective. Drug-induced exanthems, which may go unnoticed during IL-2 treatment, pose a particular problem. The same may be the case for reactions to radiocontrast

agent. Drug-induced exanthems may necessitate cessation of further IL-2 treatment. A few cases of severe exfoliative reactions have been described. It should here be noted that glucocorticoids block both side effects, as well as the antitumoral effect. In severe allergic reactions local glucocorticoids can be used, while systemic treatment is only considered if vitally indicated, simultaneously with cessation of the IL-2 treatment.

Treatment guideline
Dietary supplements with essential fatty acids should be used as basis treatment along with skin lotion, following the above guidelines. In the absence of effect hereof, the following may be used:

- Itching: Tablet Atarax 25 mg as needed for maximally 5 days. In the absence of effect tablet Tavegyl or Zyrtec may be used.
- Severe itching: Local glucocorticoid creams.
- **Systemic glucocorticoids may only be used if vitally indicated!**

References

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- Gaspari, A.A *et al*: Dermatologic changes associated with interleukin-2 administration. JAMA 1987.
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Dermatological long-term side effects

Dermatological long-term side effects are common. Most frequently they consist of dryness and itching of the skin, which may persist for months after treatment. Drug-induced exanthems, caused by the supportive medication, may be particularly long-lasting and annoying. Pre-existing autoimmune skin disorders are usually worsened. This is seen in particular for psoriasis and can develop into a psoriatic arthritis. One death has been reported of a patient with pemphigus, who shortly after IL-2 treatment had a lethal worsening of his/her skin condition. Of particular interest is the manifestation of vitiligo among melanoma patients. Vitiligo is an immunological reaction directed towards normal melanocytes but also towards melanoma cells due to a large shared antigen pool. Vitiligo is not seen during IL-2 treatment of patients with other tumors and is in melanoma patients associated with the effects of the immune therapy. Serious skin conditions such as pemphigus, lupus, and dermatomyositis are contraindications for IL-2 treatment.
Good symptomatic effects on skin dryness and itching by plant oil-based dietary supplements with the essential fatty acids omega-3, omega-6, and omega-9 has been found. This should be recommended for patients as a basis treatment, alongside of moisturizing cream. The recommended daily dose is listed on the product. The ingestion of the oil is easiest if used together with beverages e.g. juice. For skin areas with annoying itching, the oil can additionally be applied directly to the skin a couple of times per day.

Treatment guideline

- Skin dryness, itching: Symptomatic treatment (oil baths, moisturizing cream, essential fatty acid dietary supplements).
- Psoriasis: confer with the department of dermatology, treatment with local glucocorticoids is permitted.

References

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- Staunton, M.R.*et al*: Life-threatening bullous skin eruptions during interleukin-2-therapy. J. Nat. Canc. Inst., 1990.

The central nervous system

Effects on the central nervous system during high-dose IL-2 treatment are frequent and caused by several mechanisms. An interstitial edema may arise as a consequence of the disturbances in the fluid balance. Secondary cytokines have a direct as well as an indirect toxic effect on the CNS. The mechanism behind this is only partially known. Probably these compounds exert their effects through the central neurotransmitter system. Brain metastases represent a special problem, as the IL-2 mediated inflammation of brain metastases can lead to a peritumoral edema. This reaction, which in peripheral metastases only rarely give rise to problems, can in the brain lead to generalized seizures and in rare cases incarceration.

The clinical picture

Studies have shown that up to 40 % of the patients in high-dose IL-2 treatment have mental side effects. Clinically significant side effects, however, are rare but include the full spectrum of psychiatric disorders such as depression, hallucinatory psychoses, schizoaffective psychoses, and comatose conditions. Most frequent are sleep disturbances.

Treatment principles

Mild mental changes should not be treated but observed. More severe psychiatric conditions should lead to pausing, treatment with systemic glucocorticoids, and usually discontinuation of the treatment. It can be difficult to discern an IL-2 triggered depression from the reactive depression, which may arise due to the processing of the disease. In case of doubt, a psychiatric consultation may be necessary. The patient should prior to the treatment be informed that the treatment may trigger a depression, and that a previous endogenous depression may predispose to this. Patients with a clinically significant psychiatric condition cannot be offered treatment with IL-2.

Treatment guideline

In the case of lasting confusion, coma/cramps, severe motor weakness, or focal neurological deficits, the treatment is discontinued. Glucocorticoid treatment with Solumedrol 80 mg IV daily is initiated immediately and until the symptoms have disappeared. Additional cerebral CT/MRI-imaging to check for cerebral metastases should be performed, if the symptoms do not decrease

within a couple of days. At degree II and III CNS toxicity treatment can after consultation with the treatment responsible and careful information of the patient be resumed at 50 % dose. At degree IV CNS toxicity, the treatment is discontinued.

References

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Lungs

An interstitial pulmonary edema may develop due to disturbances in the fluid balance. However, only rarely does it develop into a fulminant pulmonary edema. The cause is, as in a toxin provoked pulmonary edema, an increased capillary permeability. Therefore, no increase in pulmonary or central venous pressure is seen. The IL-2 provoked pulmonary edema can appear despite of hypovolemia. Unlike a cardiogenic pulmonary edema, the alveolar fluid is rich in albumin, and protein-rich pleural exudates may also appear.

The clinical picture

At high-dose IL-2 the clinical symptoms usually develop in the following order:

- Clear respiratory sound
- Light tachypnea increasing to hyperventilation (low pCO_2, normal pO_2)
- Moist respiratory sounds
- Respiratory insufficiency (drop in pO_2)

Existing lung conditions such as asthma bronchiale, chronic obstructive pulmonary disease, and restrictive lung disease can make the clinical picture very complex. Patients with these diseases should therefore not be treated with high-dose IL-2, and all patients with known lung disease or a smoking anamnesis should prior to treatment perform a pulmonary function test and have a forced expiratory volume for 1 second (FEV1) > 70 %.

Treatment principles

In the case of a mild pulmonary edema, the IL-2 infusion can be continued, if the diuresis can be increased, i.e. if there is no intravasal hypovolemia present. In this situation the mild pulmonary edema can be treated rapidly. A simultaneous intravasal hypovolemia is, however, an indicator for a considerable capillary leak syndrome and should lead to a pausing of the IL-2 infusion. In the case of resting dyspnea, the IL-2 infusion should be paused.

Treatment guideline

In the case of resting dyspnea, the following is done:

- IL-2 injection is paused, and nasal oxygen is administered.
- If the above is without effect, furosemide, 10-20 mg intravenously, is given. Coincidence of

dyspnea and hypotension should be expected, and the attention should therefore be directed towards simultaneous treatment thereof.
- Depending on arterial puncture and clinical condition, high-pressure ventilation may be considered. If high-pressure ventilation is indicated, injection of Solumedrol 80 mg IV should be tried first, and may be repeated.
- In the case of need for respiratory treatment/steroid the current treatment series is stopped, and the patient is reassessed after 2 weeks.

References

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Liver

Interstitial edema and secondary cytokines probably play a role here, as well as hepatitis-like changes due to activated inflammatory cells, endothelium, and Kupffer cells.

The clinical picture

Frequently, during IL-2 treatment an increase in transaminases without a concurrent drop in liver function is seen. Simultaneously, an increase in alkaline phosphatase is seen. A mild liver synthesis disturbance is seen among 5-30 % of the patients. In particular, the synthesis of coagulation factors and albumin is of clinical importance. In the case of severely reduced liver function, a bacterial infection should always be considered. Further, see the section on coagulation.

References

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Heart

Arrythmia and myocardial infarction is described but the exact mechanism is unclear. Secondary mediators, as well as the interstitial edema associated with fever, may cause arrythmia. The triggering factors for acute myocardial infarction are probably pre-existing arteriosclerosis in connection with fever, endothelial activation through secondary cytokines, and possibly activation of the coagulation system. In addition, an IL-2 triggered myocarditis probably plays a role for the development of arrythmia and myocardial infarction (AMI).

The clinical picture

All types of arrythmia may occur, including the febrile sinus tachycardia. In refractory sinus tachycardia and atrial flutter, hyperthyreosis should be considered in differential diagnostics. It may be triggered by an iodine-containing radiocontrast agent or be due to an IL-2 triggered thyroiditis.

Treatment principles
In clinically significant arrythmia or myocardial ischemia, the IL-2 infusion should be paused until restitution from the toxicity, and until the cardiac toxicity is clarified.

Treatment guideline
Upon suspicion of AMI, IL-2 treatment is stopped, and the treatment is paused, until AMI is confirmed or denied. Upon suspicion of AMI, ECG and cardiac enzymes are evaluated. The patient is moved to the coronary unit for continuous monitoring with scope. In the case of evidence of morbus ischaemicus cordis, the treatment is discontinued. Arrythmias are treated as usual.

References

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Gastrointestinal tract

Three factors play a role in the gastrointestinal toxicity: Mucosal edema, motility disturbances, and a general stress impact.

The clinical picture
The most frequently affected organs are the ventricle and the colon. Gastritis and ventricular ulcers, which may lead to strong bleeding, have been described. Mucosal edemas of the colon prevent reabsorption of fluids, and a characteristic diarrhea presents: watery diarrhea, one to five times a day. A few cases of ventricular and colon perforation during IL-2 treatment have been described. The pathophysiology behind these very rare side effects is unknown.

Treatment principles
The treatment is in all cases symptomatic. Prophylactic treatment with antacids and H2 blockers is not recommended but can be used in ulcer anamnesis or in treatment-induced dyspepsia. The diarrhea is difficult to treat. Loperamide may give slower peristalsis, and the fluid reabsorption in

the colon may be improved to some degree. If larger losses of fluid occur from the gastrointestinal tract, these must be equalized in order to avoid intravasal hypovolemia and arterial hypotonia.

Treatment guideline

In the case of clinical suspicion of fungus, treatment is initiated according to the usual guidelines of the department. Also, general good oral hygiene. In the case of diarrhea, more than 3-4 loose stools, tablet Imodium 4 mg is administered, followed by Imodium 2 mg at each loose stool. In the case of gastritis, treatment with proton-pump inhibitors is used.

References

- Rahman, R *et al*: Unusual gastrointestinal complications of interleukin-2 therapy. J. Immunother. , 1991.
- Schwartzentruber, D *et al*: Colonic perforation: An unusual complication of therapy with high-dose interleukin-2. Cancer, , 1988.
- Sparano, J.A *et al*: Symptomatic exacerbation of Crohn disease after treatment with high-dose interleukin-2. Ann. Med., 1993.

Chronic toxicity

Unlike the acute IL-2 toxicity, which disappears shortly after discontinuation of the infusion, a number of side effects, which may last from weeks to months, and which may be worsened by continued immunotherapy, appear. These side effects consist mainly of autoimmune reactions, which are more frequent among patients, who respond to the treatment. It is possible, that this association is seen, because the immune system of these patients is more easily "stimulated". More likely, however, it is that these autoimmune phenomena more frequently appear in responding patients, because these patients receive a longer-lasting treatment with IL-2. All chronic side effects usually disappear by themselves, and they respond well to glucocorticoid treatment. Treatment with glucocorticoids, however, should only be used on vital indication, as the effects on the tumor are also inhibited.

Thyroid gland

Autoimmune disorders in the thyroid gland are frequent, and subclinical conditions often become clinically manifest during IL-2 treatment. This is more common upon subcutaneous administration than by infusion. In a few studies of long-term treatment up to 50 % of participants show thyroid function disturbances, both hyper- and hypothyreosis. This, however, is significantly less frequent in high-dose IL-2 infusion and presents in between 1 and 20 %. Before, during, and after the treatment, thyroid parameters should be controlled (TSH, T3, and T4).

Treatment guideline

In the case of clinical disturbances in thyroid function, the endocrinology department should be consulted but the treatment may continue.

References

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- Wejl, N. I. *et al*: Hypothyroidism during immunotherapy with interleukin-2 is associated with antithyroid antibodies and response to treatment. J. Clin. Oncol. , 1993.

Lymphadenopathy

During IL-2 treatment a generalized lymphadenopathy with lymph nodes up to 4 cm in diameter is frequently seen. These reactive lymph node changes may be misinterpreted as tumor progression. Typically, the peripheral lymph nodes are not tender upon pressure. Lymphadenopathy may also be seen in a few locations and typically disappears after a couple of weeks.

Treatment suggestions

No treatment is necessary. The differential diagnosis between lymphadenopathy and progression may be difficult but by having a pause of at least 2 weeks between the last treatment and the evaluation, misinterpretation is avoided.

Joint pain

Transient joint pain is seen as a result of unspecific inflammatory reactions. Actual rheumatological joint disorders have so far only been observed among patients, who have been treated for a long time. Newly arised conditions, as well as exacerbations of existing conditions such as spondylosis, psoriatic arthropathy, and rheumatoid arthritis, have been observed. Usually the symptoms decrease for weeks to months after treatment discontinuation.

References

- Baron, N.W *et al*: Scintigraphic findings in patients with shoulder pain caused by interleukin-2. AJT, 1990.
- Scheibenbogen, C *et al*: Rheumatic disease following immunotherapy. Ann. Rheum. Dis,. 1993.

Other autoimmune disorders

In principle, all clinically relevant autoimmune disorders may be induced or worsened by IL-2 treatment. In animal experimental studies, severe conditions such as rheumatoid arthritis, lupus erythematosus, myositis, nephrotic syndrome, and many others can be seen during long-term IL-2 treatment. Autoantibodies are frequent, but do not all have clinical relevance. Further, potentially life-threatening autoimmune conditions represent a contraindication for IL-2 treatment.
All autoimmune phenomena during IL-2 treatment can be treated with high-dose glucocorticoids but at the same time as this the potential therapeutic effect is abolished.

Accompanying medication

Pain treatment
During the treatment with IL-2 and interferon, analgesics with antipyretic effect should as far as possible be avoided, cf. section on influenza-like symptoms. Opioids have, like paracetamol and NSAIDs, an antipyretic effect, and use during the immunotherapy should therefore be carefully considered.
Treatment with NSAID simultaneously with IL-2 is contraindicated due to an increased risk of renal insufficiency.

Tapering off of antihypertensive medication
As IL-2 may lead to a considerable drop in blood pressure, in particular in patients in anti-hypertensive therapy, a tapering off of all antihypertensive medication before treatment initiation is recommended. Antihypertensive medication is paused for as long as the treatment lasts. Most antihypertensive medications can be readily discontinued but one should pay attention to 2 groups of drugs:

- Beta blockers. Fast tapering may lead to tachycardia and angina pectoris, why tapering should be done over 14 days.
- ACE inhibitors/Angiotensin II-antagonists. May often be readily discontinued. If the indication is cardiac insufficiency, the patient should be referred to a cardiologist and the ejection fraction should be evaluated, as this should be normal.

One may prior to treatment ask one's own general practitioner to supervise the tapering and subsequent resumption of the treatment.

Appendix 8

Febrile neutropenia during T cell therapy

There is indication to initiate empirical antibiotic treatment in severely granulocytopenic (< 0.5 bn/l) patients with fever (≥ 38.5 °C or ≥ 38.0 °C for ≥ 1 hour).

Examinations at fever onset:

- Culture for bacteria and fungi from:
  - blood (sterile glass vials)
  - urine for D+R
  - throat
  - potential foci of infection (e.g. wounds)
- C-reactive protein, hematology, fluid numbers (in blood)
- X-ray of thorax

Treatment
Initially

- Piperacillin/Tazobactam (Tazocin) dose: 4 g/0.5 g every 8. hours IV.
- Penicillin allergy: Ceftazidim (Fortum) (dose: 2 g / 8. hours IV).

Initially, Gentamicin is not used in febrile neutropenia during T cell therapy, as the combination of gentamicin and IL-2 can exacerbate the nephrotoxicity. In the case of an aggravation of the clinical condition, supplemental Gentamicin can be used, however, at maximally 3 doses.

In the case of positive cultures, possibly adjustment of the initial treatment.

Evaluation on day 3

Temperature dropping, clinical response:

As a rule, the treatment is continued unchanged for at least another 4 days corresponding to a total of 7 days of treatment; however, so that the patient must have been afebrile for at least 2 days before discontinuation. In cases of continued signs of infection and in all cases of septicemia with *Pseudomonas aeruginosa* antibiotic treatment, however, is continued until the granulocyte number is > 0.5 bn/l.

Temperature unchanged/increasing, clinical condition unchanged/aggravated:
Repetition of initial examinations, supplemented with culturing for *Aspergillus spp.* from the

nasal vestibules.

Necessary considerations:

- Lung infiltrates: Bronchoalveolar lavage (BAL) with adjustment of treatment depending on findings. Possibly ordering of expectorate testing for *Legionella*-PCR, "atypical pneumonia titers" (LAT (*Legionella* antibody test), MPT (*Mycoplasma pneumoniae* test), and CKT (Chlamydia complement fixation test)), LUT (*Legionella*-antigen urine test), and CT scan of the thorax.
- Signs of IV catheter infection: Addition of Vancomycin dose: 1 g/twelfth hour IV (dose reduction in case of renal insufficiency)
- Signs of oral candidiasis: At this time decision should be made about indication to add empirical systemic antifungal therapy with Amphotericin B-dose or Caspofungin-dose: 70 mg IV day 1, thereafter 50 mg IV daily (dose reduction in liver insufficiency).

No objective findings: Change of initial treatment to Meropenem-dose: 1 g/eighth hour IV.

Evaluation on day 8

In the case of continued fever, the examinations from day 3 are repeated, supplemented with:

- *Aspergillus* galactomannan-antigen
- CMV-antigen in blood (PCR-method)
- CT scan of liver and spleen (chronic disseminated candidiasis):
- Consider systemic antifungal therapy with Amphotericin B as monotherapy or in case of simultaneous suspicion of infection with *Aspergillus* spp.: Caspofungin-dose: 70 mg IV day 1, hereafter 50 mg IV daily (dose reduction in liver insufficiency).

Switching from intravenous to peroral antibacterial therapy

Can be considered in cases, where the granulocyte number is increasing, and the patient has become afebrile.

The treatment is changed to: Penicillin 1 MIU x 3 perorally + Ciprofloxacin-dose: 500 mg x 2 perorally.

In the case of penicillin allergy, penicillin is replaced by Roxithromycin-dose: 150 mg x 2 perorally.