T Cell Therapy for Patients with Advanced Renal Cell Carcinoma.

A pilot Study

EudraCT nr.: 2016-001454-18

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

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List of Abbreviations

ACT = Adoptive Cell Therapy

AE = Adverse Event
ALAT = Alanine-Aminotransferase
ASAT = Aspartate-Aminotransferase
AR = Adverse Reaction
ASAT = Aspartaet-Aminotransferase
BMI = Body mass index
CCIT = Center for Cancer Immune Therapy
$CD4^+$ Cells = Helper T cells
$CD8^+$ Cells = Cytotoxic T cells
CLL = Chronic lymphatic leukemia
CR = Complete Response
CTC = Common Toxicity Criteria
CTCAE = Common Terminology Criteria for Adverse Events
CTL = Cytotoxic T lymphocyttes
Cy = Cyclophosphamide
DMSO = DiMethyl SulfOxide
ECG = Electrocardiogram
eCRF = Elektronic Case Report Form
EF = Ejection fraction
ELISA = Enzyme-Linked ImmunoSorbent Assay
ELISpot = Enzyme-Linked ImmunoSpot
Flu = Fludarabine phosphate
FTC = Freshly disaggregated tumor cells
GCP = Good Clinical Practice
G-CSF = Granulocyte colony stimulating factor
GFR = Glomerular filtration rate
HLA = Human Leukocyte Antigen
IFN = Interferon
IFN-γ = Interferon-gamma
IFN-α = Interferon alpha
IMPD = Investigational Medical Product Dossier
IL-2 = Interleukin-2
KFE = Klinisk forskningsenhed / Clinical Research Unit
LAK = Lymphokine-Activated Killer
LDH = Lactate dehydrogenase
MHC = Major Histocompatibility Complex
MM = Malignant melanoma = skin cancer
mRCC = Metastatic Renal Cell Carcinoma
MUGA = Multigated acquisition
NED = No evidence of disease
NGS = Next generation sequencing

ORR = Overall Response Rate
OS = Overall survival
PBMC = Peripheral mononuclear blood cells
PD = Progressive Disease
Peginterferon = Pegylated interferon alpha 2b
PET =Positron Emission Tomography
PFS = Progression free survival
PR = Partial Response
PS = Performance status, ECOG scale 0-4
RCC = Renal Cell Carcinoma
RECIST = Response Evaluation Criteria In Solid Tumours
REP = Rapid Expansion Protocol
RR= Response rate
SAE = Serious Adverse Event
SAR = Serious Adverse Reaction
SD = Stable Disease
SUSAR = Suspected Unexpected Serious Adverse Reaction
TAA = Tumor Associated Antigens
TCL = Tumor cell line
TIL = Tumor Infiltrating Lymphocyttes
TKI = Tyrosine Kinase Inhibotor
TNF = Tumor necrosis factor
Treg = Regulatory T cells
VEK = Videnskabsetisk Komité / Health Research Ethics Committee

Synopsis

Indication and treatment

Patients with metastatic renal cell carcinoma (mRCC) will be treated in this pilot study. The treatment involves infusion of tumor-infiltrating lymphocytes (TILs) isolated from the patient's own tumor tissue as well as administration of lymphodepleting chemotherapy and the immune-stimulating cytokine, interleukin-2 (IL-2).

Rationale

T-cell therapy is an experimental personalized immunotherapy where TILs are isolated from the patient's own tumor tissue, expanded *in vitro* to billions of cells and then administered to the individual patient with the purpose of eliminating the remaining cancer cells. Lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate is administered to the patient before TIL infusion to reduce the number of irrelevant immune cells- as well as regulatory T-cells known to inhibit T-cell mediated cancer cell killing. IL-2 is administered after TIL administration to activate and stimulate further proliferation of the infused TILs.

Purpose

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

Study design

The trial is a pilot study.
Patients will be included and treated at the Department of Oncology at Herlev Hospital. Patients can be referred from other oncology or urology centers.

All eligible patients will be treated during hospital admission and one series of treatment will span approximately 3 weeks. Treatment will be administered only once.
It will take approximately 4-6 weeks from the tumor tissue is removed for TIL manufacturing to the start of treatment. In some cases, TILs will be isolated from tumor tissue in advance and cryo-preserved for later treatment. After the end of treatment, the patients will be followed with clinical- and imaging controls for up to 5 years in a specialized immunotherapy unit at the Department of Oncology, Herlev Hospital. Patients will be excluded upon clinical or radiological progression.

The inclusion period is expected to be approximately five years, starting in august 2016.
The study will be monitored by the Good Clinical Practice (GCP)-unit, and reported to the Danish Health and Medicines Authority, the Research Ethics Committee and the Danish Data Protection Agency.

Population

Patients with histologically verified mRCC will be eligible for treatment if they meet the inclusion criteria, including an acceptable performance status, acceptable kidney- and liver function, and the absence of major co-morbidities. A total of 6 patients will be included and treated. The treatment will only be completed in patients with successful manufacturing of TILs.
Prior clinical trials have shown that the success rate of TIL manufacturing from metastatic melanoma exceeds 90% [1]. To date, we have successfully generated viable TIL cultures from 23 out of 25 tumor samples from patients with renal cell carcinoma who have undergone radical or partial nephrectomy (our internal observation).
The actual success rate of TIL manufacturing in this trial may depend on additional factors e.g. previous treatments, but major difficulties in manufacturing TILs from the majority of patients are not envisaged.

Toxicity

A phase II trial of T-cell based immunotherapy for patients with metastatic melanoma, administered in combination with the same lymphodepleting chemotherapy and IL-2 (intravenous decrescendo regimen) has shown acceptable safety and toxicity at the Department of Oncology, Herlev Hospital[1]. Several other studies in metastatic melanoma have used the same lymphodepleting chemotherapy regimen and high-dose bolus IL-2 administration,(e.g. Dudley et al.[2]), with an acceptable safety profile.
Recently, a Phase III study with an identical regimen was initiated at the Department of Oncology, Herlev Hospital. So far toxicity has been acceptable.

Evaluation of clinical response

The patients will be clinically evaluated 6 and 12 weeks after treatment with TILs and every 3 months for 2 years and semiannually hereafter. Evaluation by diagnostic imaging will take place before the treatment and in connection with the clinical evaluations starting from 6 weeks after TIL treatment.

Immunological response evaluation

Blood samples of 110 ml will be collected at the time of surgery, before TIL infusion, at discharge and in connection with the clinical evaluations. Serum samples (10 ml blood samples) will be collected during hospitalization at day 0 before TIL infusion, 2 hours after TIL infusion and every 2 days hereafter until discharge. Immune cells will be isolated from the collected blood samples by

standard gradient centrifugation and cryo-preserved for later analysis. Flow-cytometric analyses will be used to assess the quantity and function of different immune cell subsets (e.g. $CD4^+$, $CD8^+$) before and after treatment at several time points.

Introduction and rationale

Renal Cell Carcinoma

Renal Cell Carcinoma (RCC) constituted 2,1% of cancers worldwide in 2008 and estimated 1,5% of cancer related deaths[3]. In Denmark, 800-900 patients are diagnosed each year with RCC with an increasing incidence over the last 10 years (21,1 in 100.000 for men and 11,05 in 100.000 for women). Patients typically range from 60-70 years of age with <25% having metastatic disease at the time of debut. Approximately 30% of patients undergoing surgery with a curative intent will develop locally recurrent or metastatic disease[4].

Younger patients with metastatic disease, and a suitable general condition, can be treated with IL-2, Interferon (INF) and sometimes surgery with a curative intent. The remaining patients with metastatic disease will be treated with different biological targeted agents, mainly Tyrosine Kinase Inhibotors (TKIs), in a palliative setting. Metastatic RCC is resitant to chemotherapy[5]. Therefore, there is an unmedt need for new and improved treatments in mRCC.

Tumor immunology

Substantial progress has been made in the understanding of the reactions of the immune system against cancer in recent years. It has become clear that the immune system reacts against certain tumors *in vivo* and that immunological response against cancer cells are associated with a better prognosis[6,7]. In addition, it has been shown that a small percentage of patients with widely metastatic cancers can be cured with a variety of immune-activating approaches, including transfer of T cells[8].

In RCC, several clinical trials have investigated cell-based therapy. Infusion of IL-2 and lymphokine-activated killer (LAK) cells were administered in several clinical trials with more than 500 RCC patients, but did not find clinical response rate or overall survival significantly greater than treatment with IL-2 alone[9]. Treatment with TIL based adoptive cell therapy (ACT) has been tried in several other clinical trials, but only with modest success and highly varying response rates[9]. This is decribed in further details below (p. 14-15).

Tumor infiltrating lymphocytes (TILs)

Tumors are often infiltrated by large amounts of T-cells (TILs) that specifically recognize tumor antigens but typically are inactive, or not sufficiently active, in the tumor microenvironment. The inactive state of the T-cells in the tumor tissue is characterized by abnormal intracellular signaling, apoptosis and reduced proliferative capability, which are probably caused by various immune inhibiting factors in the tumor environment[10,11]. However, it is possible to amplify and reactivate such TILs for tumor cell killing *in vitro* by use of activating factors like IL-2[12,13].

T cell therapy

T cell therapy, which is also called "Adoptive T cell Therapy" (ACT), is an immunotherapeutic cancer treatment that has shown very promising results, especially in metastatic melanoma (MM). This treatment, which uses the patient's own T cells for tumor cell killing, was developed at the American National Institute of Health and in recent years several studies from research centers in other countries in both USA and Europe have been published with more than 500 patients having received the treatment in total[2,14–18].

The treatment is defined as the infusion of T cells isolated from the patient's own tumor tissue after *ex vivo* activation and several rounds of expansion, and takes advantage of the high number of tumor reactive T cells in tumor tissue compared to peripheral blood[19].

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

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

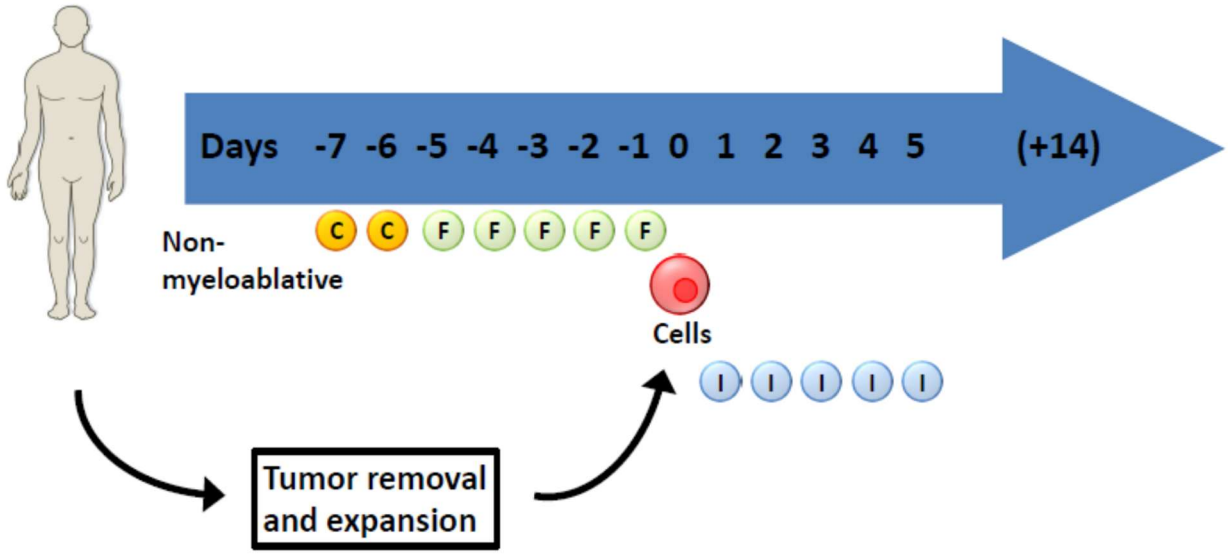

Figure 1: Course of treatment with T cell therapy
Tumor tissue for manufacturing of the T cell infusion product is removed immediately after inclusion. Treatment with Cyclophosphamide (C) and fludarabine phosphate (F) is commenced a week before the T cells are ready for infusion and are followed by infusion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

T cell therapy has shown very promising results in MM with overall response rates (ORR) around 50%, which has been confirmed in several phase I/II "single institution" studies[15–17,20,21]. Complete response (CR) has been observed in about 20% of the treated patients of which most CR are of long duration and with patients being free of disease more than 7 years after the treatment[14]. Thus, TIL based T cell therapy seems to have the potential of curing a significant number of the treated patients with MM.

Previous experiences with more than 500 patients with metastatic MM treated with T cell therapy has shown that the treatment is safe to administer to patients in a good performance status and adequate organ function, despite considerable - but temporary - toxicity. The observed acute side effects are reversible and consist of known and expected side effects to lymphodepleting chemotherapy and high dose IL-2.
So far, treatment with TIL based ACT in metastatic renal cell carcinoma (mRCC) has only been given with modest success and highly varying response rates. Furthermore, all the clinical trials are of an elder date. Importantly, none of the clinical trials investigating T cell therapy in mRCC used the same preparative chemotherapy regimen used today and the ex vivo methods for lymphocyte expansion have changes considerably since then[9].
The TIL/IL-2 conducted studies in mRCC patients are summarized in table 1 below:

Table 1: Clinical trials with TIL based ACT in mRCC

Author	Phase	Pre-conditioning chemotherapy	TIL	IL-2 dose and regimen	RCC Patients receiving TIL	ORR (%)	CR (%)	Comments
Topalian et al. (1988)	I	Cyclophosphamide	i.v.	High-dose bolus every 8h	4	25	0	Varying doses and combinations of TIL. Toxicities similar to IL-2 alone. No treatment-related mortalities.
Kradin et al. (1989)	I	None	i.v.	Continuous	7	29	0	TIL was infused every second day. Given in general medical wards. None required intensive-care monitoring.
Bukowski et al. (1991)	I	None	i.v.	Continuous	18	0	0	Escalating doses of IL-2. No unexpected toxicities.
Oldham et al. (1991)	I	Cyclophosphamide	i.v.	Continuous	9	0	0	Usual IL-2 toxicities were seen.
Thiounn et al. (1994)	III	None	i.v.	None	6	30	30	Patients were pretreated with IL-2.
Goedegebuure et al. (1995)	I	None	i.v.	Moderate bolus every 8h	8	0	0	
Figlin et al. (1997)	I/II	None	i.v.	Continuous + IFN-α-2a	55	34.6	9	CD8+ TIL or cytokine-primed TIL. No statistically significant difference between groups. No treatment-related deaths occured.
Figlin et al. (1999)	III	None	i.v.	Continuous	39	8	NR	Randomized study of IL-2 plus CD8+ TIL vs IL-2 alone. Serious adverse events comparable between groups. No side effects specifically associated with TIL therapy.
Dillman et al. (2004)	Retro-spective	None	i.v.	Hybrid bolus and continuous	9	11	0	No difference in survival by production method or amount of IL-2 given with TIL.

CCITs experience with T cell therapy

Clinical trial

The complicated methods of manufacturing TILs has been established at the Center for Cancer Immune Therapy (CCIT), Herlev Hospital, as one of the few places in the world[22], and a clinical trial has been conducted[1], with 31 patients with metastatic MM treated so far.
All patients were treated with classic lymphodepleting chemotherapy with cyclophosphamide and fludarabine phosphate followed by TIL infusion with approximately $100x10^9$ cells followed by the administration of IL-2.
In the original "T cell regimen" described by Dudley et al[15] very high dosages of IL-2 (720.000 IU/kg i.v.) where given as bolus injection every 8 hours until treatment limiting toxicity. It is unknown how high a dosage of IL-2 is necessary to maintain T cell expansion, and consequently CCIT has tested the treatment with low and intermediate doses of IL-2 to investigate whether clinical efficacy can be maintained while toxicity is decreased.
Low dosage subcutaneous IL-2 was given to 6 patients in a pilot study initiated in the summer of 2009 and the results are now published[15]. Two of the six treated patients achieved CR and are presently without evidence of disease (NED).
To achieve a higher response rate (RR), the dosage of IL-2 was then increased to an intermediary dosage and administered after the decrescendo-regimen equal to the decrescendo regimen used in Denmark as standard treatment of metastatic MM.
Additionally 25 patients have been treated after the increase in IL-2 dosage. Of the 31 treated patients, five achieved CR (48 (NED), 13 (NED), 47+, 32+ and 22+ months) and 7 patients achieved partial PR (45+ (NED), 12, 28+, 36+ (NED), 11, 11 and 8), of which 6 are having ongoing responses varying from 8-45 months. Thirteen patients had stable disease (SD) for 4-6 months and 5 patients progressed immediately after treatment. An ORR of 39% has been observed, which is comparable to other studies administering high dosage of IL-2. The lymphodepleting chemotherapy induced, as anticipated, myelosuppression with anemia, leucopenia and thrombocytopenia and all patients received prophylactic antibiotics and blood transfusions. All patients experienced transient grade III-IV toxicities during the 3 weeks of hospitalization but recovered quickly after the treatment. The above mentioned results have recently been published[23]. Recently, a Phase III trial comparing high-dose bolus IL-2 receiving T cell therapy and Ipilimumab in patients with MM has begun at CCIT with international collaborators. So far, 6 patients have been treated with TILs and high-dose bolus IL-2 and only expected and manageable toxicities have been observed (our internal observation).

In mRCC, the use of high-dose IL-2 is a well established first line treatment since the 90's[24]. Thus, in this trial we will combine infusion of TILs with high-dose IL-2.

Translational research

Further development of T cell therapy with optimizing and expansion to other cancer forms has a high priority at CCIT. Our already established platform for T cell therapy for MM gives us an

unique opportunity to study the interactions between tumor and immune system and thereby identify possible methods for optimization of T cell therapy, as well as extension to other tumor histologies.

Several studies has shown that the following T cell characteristics are important for achieving a clinical response after T cell therapy: long telomeres, short time spent in culture, a favorable T cell phenotype (CD27+, CD28+), a high absolute number of T cells and a high number of cytotoxic tumor-reactive T cells in the infusion product[25] as well as an increased persistence of T cell in the peripheral blood after infusion[14,22,26]. At CCIT, we have modified the original T cell expansion method from ”Standard TIL expansion” to ”Young TIL expansion” based on these characteristics and leading to a reduction of the length of cell manufacturing from 4-7 weeks to 2-4 weeks. A decreased amount of time in culture (Young TIL) provides the TILs with longer telomere sequences and more favorable phenotypes (CD27+, CD28+) with the ability of increased proliferation, an increased persistence in vivo and a higher antitumor activity, all of which are correlated to an increased clinical response[14,22]. This optimization of TIL production has made it possible to produce clinically usable TIL infusion products from more than 90% of the patients[27–30]. Furthermore, during the final expansion phase, the Rapid Expansion Protocol (REP), we have introduced the use of the Wave® bioreactor[31], which optimizes the conditions of proliferation of T cells and has made it possible to achieve a higher total number of cells as well as tumor reactive T cells in the TIL infusion product. We have standardized and harmonized TIL production methods between 3 European cancer research centers based on these TIL production protocols developed at CCIT and have initiated a randomized, multicenter TIL-based phase III trial with T cell therapy versus standard immunotherapy (clinicaltrials.gov identifier: NCT02278887) with the purpose of the approval of T cell therapy as standard treatment for patients with MM.

Recent studies suggest that TIL based ACT potentially can be used with success in other cancer forms including colon, breast, head and neck, kidney, ovary and sarcoma[32–37]. It has been shown that a high intratumoral presence of T cells is correlated with longer survival and functional analysis has shown that tumor infiltrating T cells show *in vitro* anti-tumor activity (Figure 2). We have successfully manufactured TILs from 23/25 samples obtained from RCC patients undergoing radical or partial nephrectomy, and in vitro expansion was similar characteristics as in MM (Figure 3.

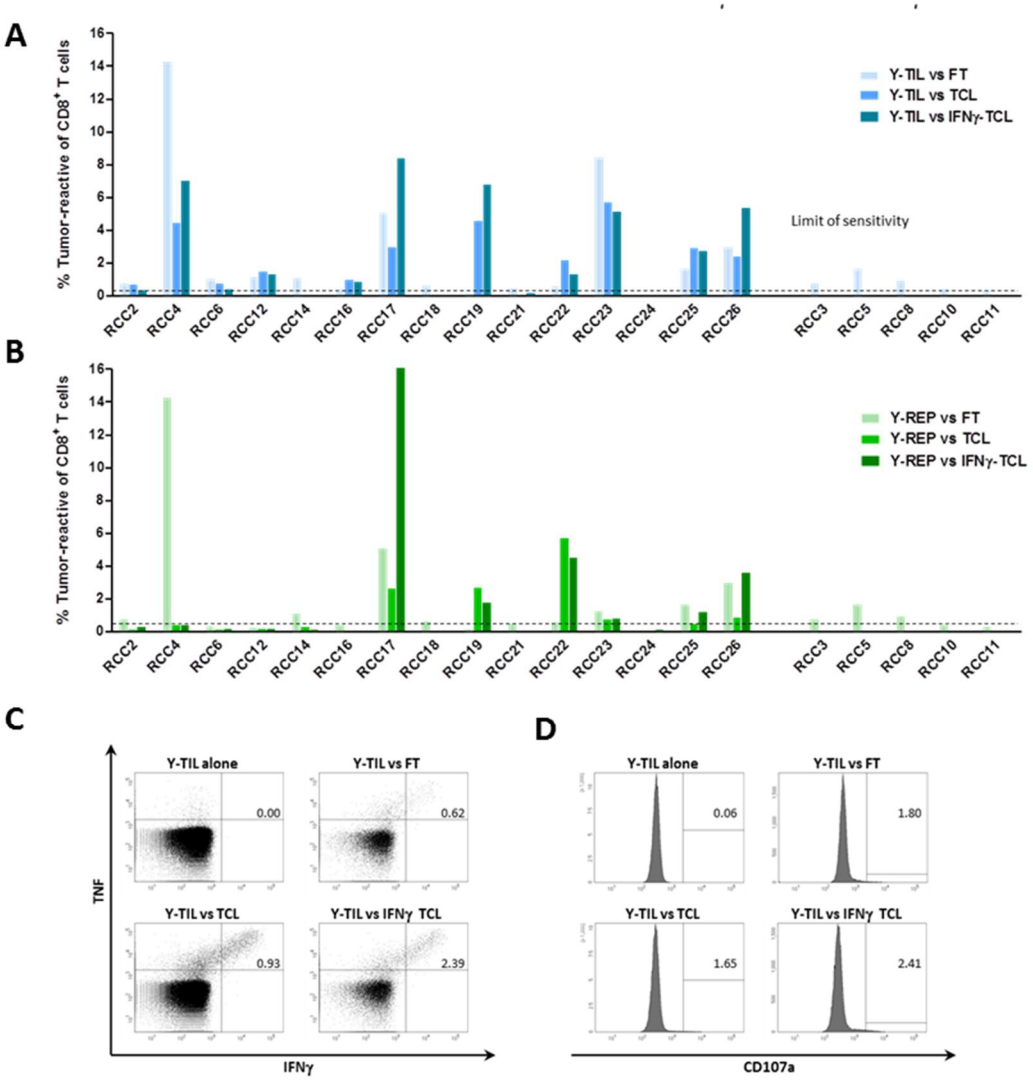

Figure 2: Anti-tumor reactivity of in vitro expanded CD8+ TILs in RCC

(A) *and* ***(B)*** *Bars show the proportion of tumor-reactive CD8+ T cells in* ***(A)*** *minimally expanded TIL cultures (Y-TIL) or* ***(B)*** *TIL cultures expanded to clinical numbers (Y-REP). Each bar shows one individual patient. The frequency of tumor reactive TILs was established by co-culture with autologous freshly disaggregated tumor cells (FTC) or short-term cultured tumor cell lines (TCL), with or without 72 hours of pre-treatment with IFNγ (IFNγ– TCL) which increases antigen presentation.. If autologous TCLs were not available TILs were only tested against FTC (RCC3, RCC5, RCC8, RCC10 and RCC11). Dotted line: limit of sensitivity.* ***(C)*** *and* ***(D)*** *Flow cytometry plots gated on CD8+ T cells showing cytokine expression (IFNγ and TNF) and CD107a up-regulation, which is a marker of T cell killing, from a representative patient (RCC26) after stimulation with autologous short-term cultured TCL and increased recognition after IFNγ pre-treatment of the TCL.*

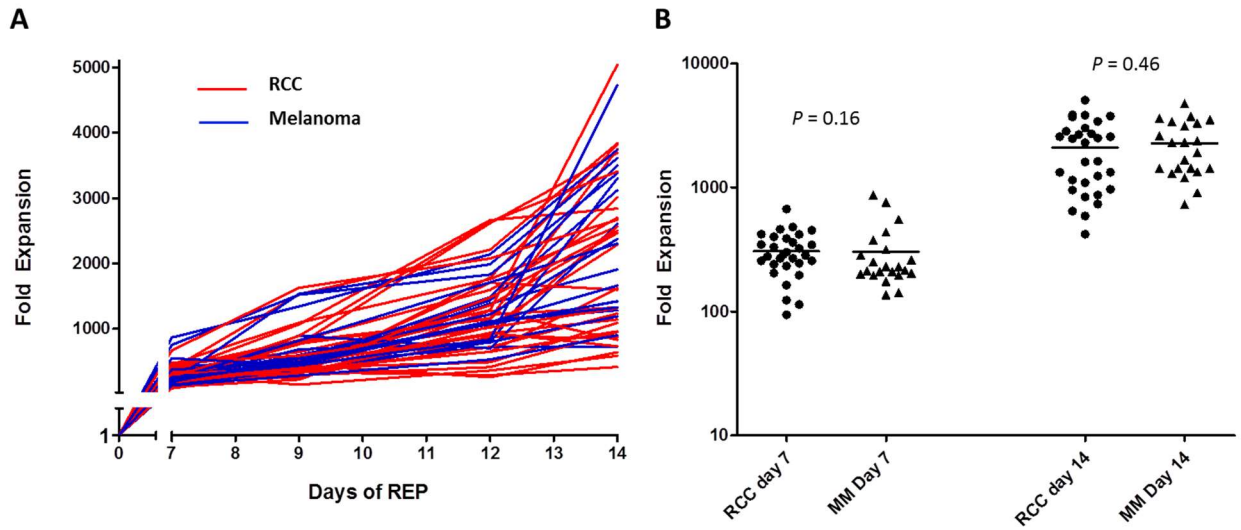

Figure 3. Clinical expansion in the REP with standard static conditions.
*Fold expansion in 14 days REP of minimally expanded TILs from RCC and MM was performed in parallel. (**A**) Fold expansion curves from 15 RCC patients in duplicates (n = 30) and 11 MM patients (n = 22). (**B**) No difference in median fold expansion on day 7 (P = 0.16) and day 14 (P = 0.46) for RCC (n = 30) and MM (n = 22).*

The rationale of the drugs used in the study

Lymphocyte depleting chemotherapy

Activating cytokines (the signal molecules IL-2, IL-7, IL-15, IL-21 etc.) need to be available for the tumor specific T cells to sustain an immunological response against tumor tissue. A large number of "irrelevant" T cells will decrease the availability of these cytokines for the relevant T cells through competition. Thus, a high number of tumor specific T cells with a high specificity as well as a reduction of irrelevant T cells and regulatory T cells (Tregs) are needed to create an environment that facilitates the T cell mediated antitumor response.
This study will use combination chemotherapy with two days treatment with cyclophosphamide and 5 days with fludarabine phosphate to create such an environment. This combination has been chosen based on earlier studies where it was shown safe and effective[38,39].

Cyclophosphamide

Cyclophosphamide is an alkylating drug that works by creating covalent bindings with biologically important macromolecules. Of special interest is the creation of a binding and linkage to DNA. Cell division can be prevented if the linkage is not canceled by the cells repair systems. The binding to

important proteins in the cell can damage important cellular functions and lead to cell death. Cyclophosphamide is among others used to treat breast cancer and in the treatment of hematological diseases as myelomatosis[40].

Fludarabine phosphate

Fludarabine phosphate is a pro-drug that is converted to the active triphosphate 2-fluoro-ara-ATP. It is an anti metabolite which inhibits DNA synthesis while simultaneously reducing RNA and protein synthesis. Fludarabine phosphate is used in the treatment hematological diseases as CLL among others[41].

Interleukin 2

IL-2 is physiologically produced by activated T-lymphocytes and stimulates the antigen specific and non-specific immune system through specific receptors[42].
Intermediate-dose interleukin-2 (IL-2) administered intravenously according to the decresendo-regimen[43] is used in Denmark as standard treatment for suitable patients with metastatic MM. Moreover, low-dose IL-2 is used for the treatment of metastatic RCC. As mentioned above, a Phase III trial comparing high-dose bolus IL-2 receiving T cell therapy and Ipilimumab in patients with MM has begun recently at CCIT with international collaborators.
In the present pilot study, IL-2 will be given as high-dose bolus infusions as mentioned above and which has been used in several other phase II studies[2,16,44].

The rationale of the treatment regimen used in the study

The ACT regimen has been determined according to our previous study carried out in MM[1].

Purpose and hypothesis

Primary

1) To assess tolerability and feasibility of the treatment.

Secondary

1) To clarify whether T cell therapy for patients with mRCC can induce a measurable immune response against tumor cells.

2) To describe objective responses using RECIST 1.1

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

Study design

The study is a pilot study for patients with advanced renal cell carcinoma. All patients will be included and treated at the Department of Oncology, Herlev Hospital. Patients can be referred to treatment from other centers in Denmark.
We expect to include and treat 6 patients within four years. Patients will only receive one course of treatment.
The patients will be evaluated for objective response after 6 and 12 weeks and approximately every 3 months for 2 years and the semiannually until disease progression.
The course of treatment can vary between patients depending on the time period from surgery to treatment. In most cases (e.g. TILs undergoing massive expansion and infusion without cryopreservation of intermediate products), the patients will receive treatment approximately 4-6 weeks after the operation and in these cases the course of treatment from surgery until the first evaluation with diagnostic imaging (6 weeks after treatment) will span across approximately 3 months.

Study population

Patients with histological verified mRCC will be candidates for this study. The patients need to have a good performance status, no major co-morbidities and acceptable organ functions.
Only patients in which it is possible to grow T cells from their tumor tissue will be offered treatment with T cells in the study and only patients receiving treatment will be included in the final study population.

Criteria of in- and exclusion

Criteria of inclusion

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

1. Histological proven mRCC with the possibility of surgical removal of tumor tissue of > 1 cm^3. Histology must include a clear cell component with or without a sarcomatoid dedifferentiation.
2. Metastatic disease irrespective of number of previous treatment lines. Treatment naïve pt's can be included.
3. Age: 18 – 70 years.
4. ECOG performance status of ≤1 (Appendix 2).
5. IMDC prognostic group 'Favorable' or 'Intermediary' (Appendix 3)
6. Life expectancy of > 6 months.
7. At least one measurable parameter after surgery in accordance with RECIST 1.1 –criteria's.
8. No significant toxicities or side effects (CTC ≤ 1) from previous treatments.
9. Normal ejection fraction (EF) measured by a multigated acquisition (MUGA) scan.
10. Crom EDTA clearance >40 ml/min

11. Sufficient organ function, including:

System	Laboratory value
Hematological	
ANC (Absolute Neutrophil Count)	≥ 1.500/µl
Leukocytes	≥ normal limit
Platelets	≥ 100.000/µl and < 700.000/µl
Hemoglobin	≥ 6,0 mmol/l (after blood transfusion if necessary)
Liver	
Total serum bilirubin	≤ 1,5 times upper normal limit
ASAT/ALAT	≤ 2,5 times upper normal limit
Alkaline phosphatase	≤ 5 times upper normal limit
Coagulation	
PP	> 40 unless the patient receives therapeutic anti-coagulation treatment.
INR	< 1,5 unless the patient receives therapeutic anti-coagulation treatment.

12. LDH ≤ 5 times upper normal limit as a measure of tumor burden
13. Women in the fertile age must use effective contraception. Likewise, men included in the study, as well as their partners, must use effective contraception. This applies from inclusion and until 6 months after treatment. Birth control pills, spiral, depot injection with gestagen, subdermal implantation, hormonal vaginal ring and transdermal depot patch are all considered safe contraceptives.
14. Signed statement of consent after receiving oral and written study information. The subject may also provide consent for Future Biomedical Research. However, the subject may participate in the main trial without participating in Future Biomedical Research
15. Willingness to participate in the planned controls.

Criteria of exclusion

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

1. A history of prior malignancies, exept curatively treated non-melanoma skin cancer and CIS of the cervix uteri. Patients treated for another malignancy can participate if they are without signs of disease for a minimum of 3 years after treatment.
2. Patients with cerebral metastases.
3. Patients with widespread bone or bone only metastases.
4. Known hypersensitivity to one of the active drugs or one or more of the excipients.
5. Severe medical conditions, such as severe asthma/COLD, ischemic hearth

disease/significant cardiac disease (e.g. NYHA class ≥2, also see appendix 4), poorly regulated insulin dependent diabetes mellitus among others, which can interfere with patient compliance and/or significantly increase the risk of side effects upon investigators clinical judgement.

6. Acutte/chronic infection with HIV, hepatitis, tuberculosis among others.
7. Severe allergies or previous anaphylactic reactions.
8. Active autoimmune disease, such as autoimmune neutropenia/thrombocytopenia or hemolytic anemia, systemic lupus erythematosis, Sjögren's syndrome, sclerodermia, myasthenia gravis, Goodpasteur's disease, Addison's disease, Hashimotos thyroiditis, active Graves disease.
9. Pregnant women and women breastfeeding.
10. Simultaneous treatment with systemic immunosuppressive drugs (including prednisolone, methotrexate among others).
11. Simultaneous treatment with other experimental drugs.
12. Simultaneous treatment with other systemic anti-cancer treatments.
13. Patients with active and uncontrollable hypercalcaemia.

Evaluation before entry in the study

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

- Medical history and clinical examination
- Performance status in accordance to the ECOG-scale
- Electrocardiogram
- Multigated acquisition (MUGA) scan. Can be supplemented with an exertion test if indicated.
- Cr-EDTA clearance*
- Pulmonary function tests
- Urine sample
- Laboratory analyses:
  A) Hematology: hemoglobin, leukocytes, granulocytes and platelets
  B) Blood chemistry: Sodium, potassium, magnesium, carbamide, creatinine, LDH, alkaline phosphatase, ASAT, ALAT, bilirubin, ionized calcium, CRP, TSH, Ca-ion, INR, PP
  C) Infections: Hepatitis B, Hepatitis C (IgG), HIV, HTLV-1(IgG), EBV, Syphilis and CMV
- Pregnancy test: Women in the fertile age must take a pregnancy test. This includes women who are not surgically sterilized, who are not postmenopausal and who have not used safe contraception's regularly within the last 6 months.
- Baseline tumor evaluation: A CT scan of the brain, chest, abdomen and pelvis is mandatory. Brain MRI scan is performed if brain metastases are suspected.
- Reviewing the checklist for inclusion/exclusion for treatment.

* If not performed before primary surgery/if potential entry in the study is due to progressive or recurrent disease. Is to be repeated within 2 weeks before hospitalization for T cell therapy if creatinine levels rises >20% after inclusion.

Examination plan during the course of treatment

	Inclusion	Operation	Befrore admission	Before T-cell infusion	Before discharge
Day			**-8**	**0**	**Approx. 10**
Performance Status	x		x	x	x
Clinical examination	x		x	x	x
Weight	x		x	x	x
Adverse events (CTC)			x	x	x
Screening blood tests[a]	x				
Immunological blood tests[b]		x	x		x
Random blood tests[c]			x	x	x
ECG	x		x		
MUGA	x				
Cr EDTA[d]	(x)		(x)		
Pulmonary function tests	x				
Urine sample			x		
Biopsy[e]		x			
Tumor Evaluation[f]	x		x		

Examination plan

a: *Screening blood samples: T-cell screening (blood sample-package): hemoglobin, platelets, leukocytes, differential-count, sodium, potassium, magnesium, carbamide, creatinine, ALAT, ASAT, alkaline phosphatase, bilirubin, LDH, INR, APTT, PP, ionized calcium, CRP, TSH, albumine, phosphate, T_3, T_4, Hepatitis B virus s-antigen (HBVSAG), Hepatitis B virus s-antibody (HBVSAB), Hepatitis B virus c-antibody (HBVCAB), Hepatitis C virus-antibody (HCVAB), Human immune defect virus type 1og 2 antibody and antigen (HIVCOMBO), HTLV type I-antibody + HTLV type II-antibody (IgG) (HTLVIGG), Ebstein-Barr virus-antibody (EBV), P-Treponema pallidum-antibody (TREPONE), Cytomegalovirus (CMV)*

b: *Immunological blood samples: HEREKS11. See the section about "Blood samples for immunological monitoring".*

c: *Random blood samples (are taken daily during hospital admission and until discharge): Hemoglobin, leukocyttes, diff.-count, platelets, creatinine, sodium, potassium, magnesium, carbamide, phosphate, LDH, ASAT, ALAT, bilirubin, alkaline phosphatase, albumin, ionized calcium, TSH, CRP, INR, PP, APTT.*

d: *Is to be performed before inclusion if not performed before primary surgery/if potential entry in the study is due to progressive or recurrent disease. Is to be repeated within 14 days prior to admission if a >20% rise in creatinine is observed.*

e: *Biopsy with core- or fine needle if possible and depending on location and accesibility*

f: *CT and MR scans can be used as mentioned above (page 23). The scan(s) before chemotherapy should preferably be performed within two week prior to admission.*

Treatment strategy

T cell therapy

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

Step 1: Screening, inclusion and surgical removal of tumor material followed by production and growth of TILs in the laboratory.

Step 2: Treatment during hospitalization with chemotherapy, TIL infusion and IL-2 administration.

Follow-up: Evaluation of the effect of treatment during follow-up.

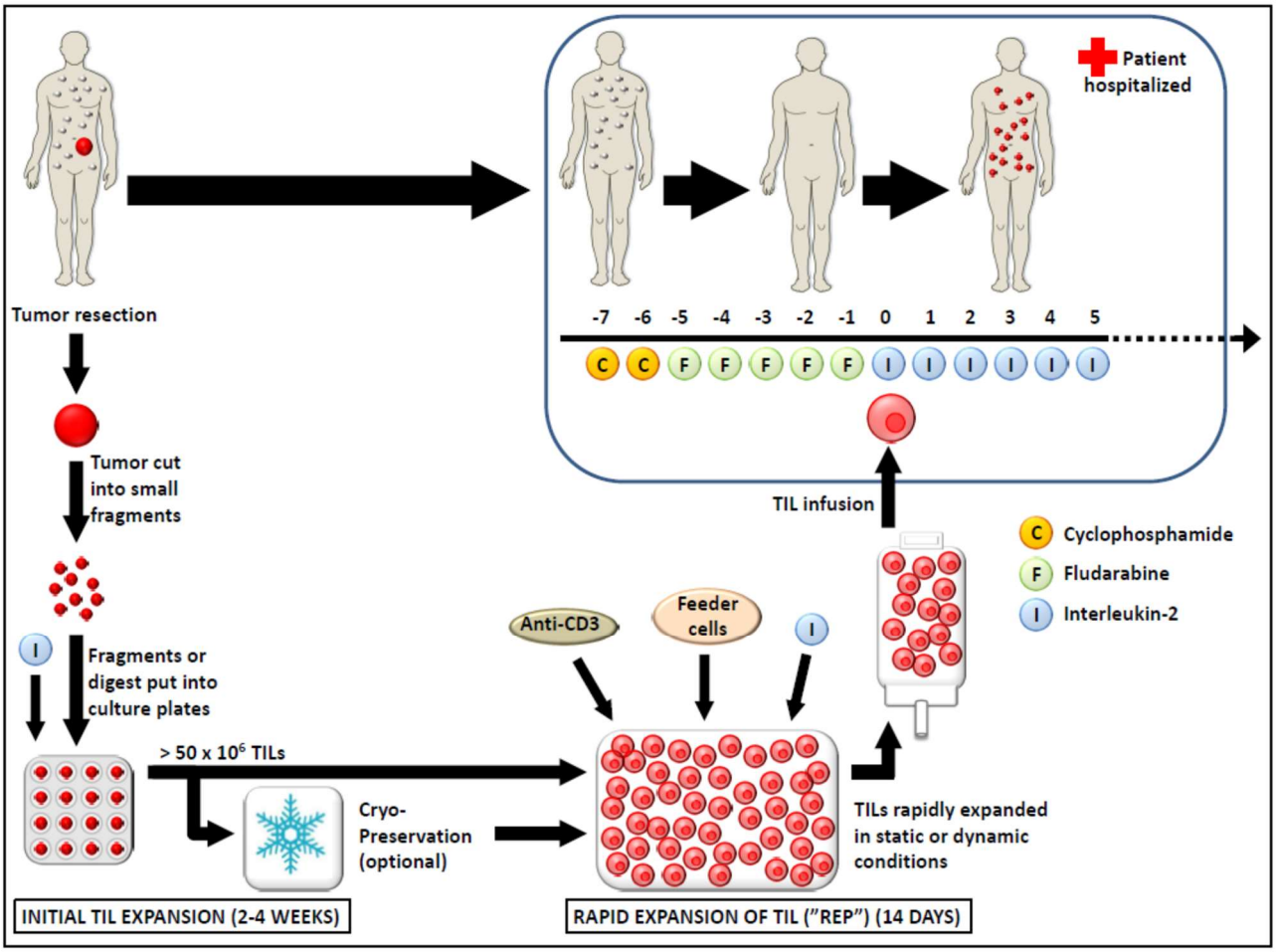

Figure 4: *Shows a schematic presentation of TIL isolation from tumor tissue followed by T cell expansion and treatment with T cell therapy.*
Tumor tissue (metastasis or primary tumor) of minimum 1 cm^3 is surgically removed from the patient and transported to the laboratory under sterile conditions where the tumor is separated into suitable fragments of 1-3 mm^3 and placed in growth wells with a growth media and IL-2. TILs are then initially grown for 2-4 weeks until a cell-number of minimum 50 x 10^6. At this point the cells can be cryo-preserved for later use or pass on to the Rapid Expansion Protocol (REP) in which the T cells for 2 weeks are stimulated with anti-CD 3 antibody, allogeneic, radiated PBMC (peripheral mononuclear blood cells), feeder cells and IL-2. After stimulation, the expanded TILs (now billions) are then washed, pooled and re-infused intravenously back in to the patient. Seven days of lymphodepleting chemotherapy is given to the patient before TIL infusion with cyclophosphamide (C) on day -7 to day -6 and Fludarabine (F) on day -5 to day -1 with the purpose of removing all existing lymphocytes in the patient to make room for the infused TILs and remove regulatory T cells (Tregs). High-dosage bolus IL-2 will be administered every eight hour from the day of T cell infusion (day 0) for up to 5 days (maximum of 15 doses) in order to active and stimulate the infused TILs for further expansion within the patient.

Medicinal products used in the study

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

Cyclophosphamide

Cyclophosphamide is given as an intravenous infusion for two consecutive days in a dosage of 60 mg per kg of body weight. The treatment takes place during hospital admission and with supplementary hydration and Mesna injections. Dosage is to be calculated based on the ideal body weight (male: 50 kg + 0,9 kg per cm above 150 cm, woman: 45 kg + 0,9 kg per cm above 150 cm) if Body Mass Index (BMI) is > 35. Dosage is to be reduced with up to 75% if renal function is reduced to GFR 30-70 ml/min and is contraindicated if GFR < 30 ml/min.

Fludarabine phosphate

Fludarabine phosphate is given as an intravenous infusion for 5 consecutive days in a dosage of 25 mg per m^2 body surface (starting the day after the last dosage of cyclophosphamide). The treatment takes place during hospital admission. Dosage is to be reduced with up to 80% if renal function is reduced to GFR 30-70 ml/min and is contraindicated if GFR < 30 ml/min.

TILs

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

Interleukin-2

Interleukin-2 will be administered at a dose of 600.000 IU/kg as an intravenous bolus over a 15-minute period every eight hours beginning 4-24 hours following the T cell infusion (day 0) and continuing for up to 5 days (maximum of 15 doses). Start of IL-2 administration depends on whether the patient has recovered from the TIL infusion.

Treatment plan

Year: Date:															
Week	-2	-1							0					1	
Treatment day Time (indic. time)	-8	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	4	7	14
Pt. is admitted	x														
Cyclophosphamide 60 mg/kg iv		x	x												
Fludarabinephos-phate 25 mg/m^2 iv				x	x	x	x	x							
TILs iv									x						
Pegfilgrastime 6 mg sc*									x						
Interleukin-2, 600.000 IU/kg/dose every eight hour bolus/i.v. (200 ml/hour)**									x	x	x	x	x		

Treatment plan
** See the section"Prophylactic treatment"*
*** Only one dose on day 0*

Concomitant treatment

Guidelines for supportive care and treatment

Supportive treatment is given on ordinary medical indications estimated by the physician responsible for the treatment. Any measures should be specified in the patient chart and flow sheet. The following is meant as guidance and other medications can be administered as it is seen fit. One exception is systemic corticosteroid which cannot be administered during treatment.

Prophylactic treatment

T cell therapy: Prophylactic treatment includes fluid therapy during cyclophosphamide treatment as well as supportive treatment.

In order to protect the mucosa of the bladder the following is administered:

- Tabl. Mesna (25% of the cyclophosphamide dosage i.v. x 4 daily on day -7 and -6).

In order to prevent deranging of electrolytes as well as low blood pressure during IL-2 treatment the following is administered:

- Fluid therapy in accordance with appendix 4.

In order to prevent opportunistic infections the following is administered:

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

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

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

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

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

In order to reduce the length of neutropenia, Pegfilgrastime, which is a human granulocyte stimulating factor (G-CSF), is administered in a dosage of 6 mg s.c. on day 0 approximately 2 hours after TIL infusion.

Supportive treatment

T cell therapy: During the infusion with T cells the patient can experience shivering which can be relieved with the administration of subcutaneous Pethidin in a dosage of 25 mg max x 4 if needed. Also, light breathing difficulties can be observed with a possible decrease in saturation which can be treated with oxygen by nasal catheter.

Neutropenia:
If simultaneous neutropenia and fever occurs it will be treated in accordance with the local instruction for “Febril neutropeni under T-celleterapi” (appendix 5).

Diarrhea:
Diarrhea will be treated immediately with the necessary supportive care and treatment, including Loperamide. Loperamide is to be withdrawn if blood or slime is observed in the feces during the diarrhea. If this occurs, suitable diagnostic microbiological samples should be collected to exclude an infectious cause. Patients should also be instructed in the intake of plentiful amounts of fluids to prevent dehydration following the diarrhea.

Anemia:
Transfusions with blood should be administered if hgb ≤ 6.0 mmol/L or if it is otherwise clinically indicated. Radiated and filtered blood will be given from day -7 and 6 months ahead.

Thrombocytopenia:
Transfusion with platelets is indicated if platelets <20/µl or if it is otherwise clinically indicated.

Interleukin-2: Doses may be skipped depending on patient tolerance. Toxicities will be managed, however the attending physician may elect to skip doses or discontinue treatment based on clinical judgement. If toxicities are easily reversed within 24 hours by supportive measurements then additional doses may be given. If more than 2 doses of interleukin-2 are skipped, interleukin-2 administration will be stopped. A detailed description of the IL-2 treatment and pausing/stopping rules can be found in appendix 5.

Local radiotherapy

Local radiotherapy can be prescribed for bone pains and soft tissue metastasesor if otherwise clinically indicated. Radiotherapy is preferably to be avoided within the 3 weeks period where the treatment takes place for the interest of the patient. Radiated areas cannot be used as parameters in the assessment of treatment response. If possible, not all evaluable areas should be included in the radiated area. If this is not possible it is no longer possible to evaluate a response to treatment and the patient is withdrawn from the study.

End of treatment

Normal end of treatment

Patients only receive one series of treatment consisting of a week of chemotherapy followed by one infusion of T cells (day 0) and up to 5 days of high-dose bolus IL-2. Patients will be followed at the department of oncology, Herlev Hospital, for up to 5 years or until progression.

Follow-up

Patients will be followed with clinical controls and diagnostic imaging at 6 and 12 weeks after treatment and every 3 months hereafter. After 2 years the controls will change to every 6 months for a total of up to 5 years or until progression. End of study is defined as 6 months after the last patients' treatment or after progression. OS and PFS will subsequently be followed for up to 5 years.
A clinical examination, toxicity assessment, blood samples including immunological blood samples and a PET/CT scan will be performed in connection with every control.
A tumor biopsy will be performed at the first control after treatment if the patient has remaining accessible tumor tissue and on progression.

Follow-up schedule

Year: Date:																												
Week nr.	6		12		24																							
Month nr.	1,5		3		6		9		12		15		18		21		24		30		36		42		48		54		60
Clinical ex. and subjective complaints	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0
Toxicity assessment	0		0		0																							
Weigth	0		0		0																							
PS	0		0		0		0		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		0		0		0		0
Biopsy [2)]	0																											
Tumor-evaluation [3)]	0		0		0		0		0		0		0		0		0		0		0		0		0		0		0

1) Blood samples (T-cell follow-up): hemoglobin, platelets, leukocytes, differential-count, sodium, potassium, creatinine, ALAT, ASAT, alkaline phosphatase, bilirubin, albumin, LDH, INR, APTT, PP, ionized calcium,phosphate, magnesium, carbamide, CRP, TSH, T_3, T_4, immunological blood samples – HEREKS11 (See the section about "Blood samples for immunological monitoring")
2) See the section about "Tumor biopsies".
3) CT and MR scans can be used as mentioned above (page 23).

Early termination of treatment

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

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

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

Other treatment: Patients will be excluded at any time a new treatment with an experimental drug or other systemic anticancer treatment is initiated after inclusion in this protocol. The patient will be

excluded if systemic treatment with corticosteroids is initiated unless it is on vital indication an in agreement with the physician responsible for the protocol.

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

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

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

Subsequent treatment

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

Production of TILs

Acquisition of tumor tissue

The patients will receive oral and written information as described elsewhere and a written consent will be obtained before surgery. A tumor biopsy will be performed after sufficient tissue material for pathological examination has been removed. The biopsy will be labeled with date and patient code, placed in a sterile container and transported to the GMP facilities 54J7 or 54I6 at Herlev Hospital for further processing. See the IMPD for more details

Establishment of "Young TIL" cultures

T cells are expanded using a recently established method for "Young TILs"[22]. The tumor mass will be isolated with a scalpel, and cut into small 1-3 mm^3 fragments. Fragments (typically from 24 to 72 fragments in total) will be placed separately in the wells of a 24 well/plate. A TIL culture is established from each fragment by passive migration of T cells from tumor tissue in the IL-2 based media. IL-2 belongs to the group of homeostatic cytokines which are characterized by having a positive effect on the activation of tumor specific T cell and thereby tumor cell killing. T cell density is kept at about $1x10^6$ cells/ml growth media containing the immune stimulating cytokine IL-2. Cell cultures from the different fragments are pooled to a single cell culture. T cell expansion is performed unselected to produce a polyclonal TIL repertoire targeted against multiple epitopes to potentially achieve more effective tumor cell destruction *in vivo*. The establishment of "Young TIL"

cultures usually takes 2-4 weeks with a rate of success more than 90%. See the IMPD for more details.

Rapid Expansion Protocol (REP)

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

Handling and transportation of the infusion product

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

Phenotype- and clonotype determination

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

Adverse events, potential risks and precautions

Adverse Events

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

The investigator must attempt to identify all clinical and objective events from patients receiving treatment and determine their relation to the study medication. The investigator determines the relationship between AEs and treatment using the following guidelines:

Grading of Adverse Events

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

Events are graded using CTCAE version 4.0 (appendix 3)[45]. The following scale can be used if this grading is not applicable:

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

Patients experiencing AEs will be monitored with the relevant clinical evaluations and laboratory investigations assessed by the attending physician. All AEs must be monitored until satisfactory restitution or stabilization. Results of the monitoring must be recorded in the patient chart and eCRF.
Abnormal laboratory tests are not to be recorded in the eCRF unless they have caused a clinical event, resulted in termination of the treatment or otherwise meet the criteria of a serious adverse event (see the following).

Serious Adverse Events

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

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

Guidelines for adverse events possible relation to the treatment

0 No relation– no temporal relation, other etiologies very likely the cause

1 Possible relation – less clear temporal relation, other etiologies likely the cause

2 Probably related – clear temporal relation with recovery at termination of treatment, and not reasonably explained by the patient's known clinical condition

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

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

Adverse Reactions

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

Reporting of Adverse Events and Adverse Reactions

Investigator reports SAEs, SARs and SUSARs to sponsor within 24 hours. Sponsor reports SUSARs to the Danish Health and Medicines Authority within 7 days if considered life threatening or fatal, and otherwise within 15 days. Consequences for the study must be reported. Sponsor submits a yearly list that summarizes any SAEs and SUSARs as well as a report regarding the study patients' safety to the Danish Health and Medicines Authority and the Research Ethics Committee (investigator can report to the Research Ethics Committee as well).
Sponsor submits a final report to the Danish Health and Medicines Authority at the end of the study, with a description of all SAEs, SARs and SUSARs.

The following is not to be reported:

- deaths caused by the malignant disease or progression
- hospitalizations or prolongation of current hospitalization caused by the malignant disease:
    - weight loss
    - fatigue

- electrolyte derangement
- pain management
- anxiety
- palliative hospitalization
- stay at hospice or terminal care
- progression of the underlying disease

- hospitalizations or prolongation of current hospitalization if the sole reason for hospitalization or prolongation is:
  - fluid treatment or treatment of nausea
  - blood transfusion
  - platelet transfusion
  - febrile leucopenia/neutropenia
  - administration of investigational procedures
  - placement of a permanent intravenous catheter

These events are to be registered in the eCRF.

Known Adverse Reactions

Chemotherapy

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

Cyclophosphamide

The dose limiting toxicities in patients' receiving treatment with cyclophosphamide are myelosuppression (neutropenia, thrombocytopenia and anemia) and urotoxicity (cystitis, haematuria and hemorrhagic cystitis). Sufficient treatment with Mesna alongside rehydration markedly reduces the frequency and severity of the urotoxicity.
Other common adverse reactions are alopecia, nausea and vomiting.

Patients receiving treatment with Cyclophosphamide can experience the following adverse reactions described in appendix 8.

Fludarabine phosphate

The adverse reactions are myelosuppression (neutropenia, thrombocytopenia and anemia), infection including pneumonia, coughing, fatigue, limpness, nausea, vomiting and diarrhea. Other common adverse reactions are shivering, edema, malaise, peripheral neuropathy, visual disturbances, anorexia, mucositis, stomatitis and rash. Severe opportunistic infections have occurred in patients receiving treatment with fludarabine phosphate. Deaths have been recorded as a cause of severe adverse reactions.

Patients receiving treatment with fludarabine phosphate can experience the following adverse reactions described in appendix 9.

TILs

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

Interleukin-2

Frequency and severity of adverse reactions to IL-2 has generally shown to be related to the route of administration, dose and frequency of treatment. Most adverse reactions are self-limiting and will disappear within 1-2 days after cessation of treatment.

For further information see appendix 5 ”Vejledning til behandling med høj-dosis bolus interleukin-2 i forbindelse med T-celle terapi”.

Patients receiving treatment with IL-2 can experience the following adverse reactions described in appendix 6. Also see appendix 10 for a summary of product characteristics.

Leukoencephalopathy
Rare cases of leukoencephalopathy related to administration of proleukine have been described in the literature, especially in patients treated for HIV. In some cases, other risk factors such as opportunistic infections, co administration of interferons and multiple series of chemotherapy had predisposed the patients.

Capillary leak-syndrome
Arrhythmia (supraventricular and ventricular), angina pectoris, myocardial infarction, respiratory insufficiency requiring intubation, organ dysfunction, gastrointestinal bleeding or kidney infarction, edema and change of mental state can be associated with capillary leak-syndrome.

Severe manifestations of eosinophilia
Most patients will experience lymphocytopenia and eosinophilia during treatment with reactive lymphocytosis within 24-48 hours after the treatment has ended. These manifestations are not considered as adverse reactions but can be explained by the anti-tumor activity mechanism of Proleukine.

Cerebellar vasculitis
Cerebellar vasculitis has been reported both isolated and in combination with other manifestations. Cutaneous and leukocytoplastic hypersensitivity vasculitis has also been reported. Some of these cases respond well to treatment with corticosteroids.

Bacterial infection
Bacterial infection or worsening of infection, including septicemia, bacterial endocarditis, septic thrombophlebitis, peritonitis, pneumonia and local infections surrounding catheters has been reported, mainly in relation to intravenous administration.

Risks and disadvantages regarding surgery and test sampling

Risks associated with removing tumor tissue

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

Risks associated with biopsies

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

Risks associated with blood tests

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

Monitoring and precautions

Hematological parameters

Careful hematological monitoring of blood counts is indicated for all patients during treatment. Leukocyte count, platelet count and hemoglobin values will be controlled at fixed intervals. Measurements will be made before start of chemotherapy, IL-2 and daily during treatment until neutrophile counts is > 500/µl and leukocyte count is > 1000/µl. Chemotherapy will not be given to patients with a leukocyte count < 500/µl and/or platelet number < 50.000/ µl before the start of chemotherapy.

Kidney- and urine infections

Any obstruction of the efferent urinary tracts, cystitis or infection will be resolved before start of treatment. Patients will be treated with Mesna and fluid therapy to decrease the frequency and severity of bladder toxicity. Treatment will be terminated if cystitis associated with micro- or macroscopic haematuria occurs during treatment with Cyclophosphamide. The patients' urine will be controlled for the presence of microscopic haematuria before start of treatment with Cyclophosphamide.

Cardiotoxicity

Cardiotoxicity is especially seen when administrating high doses of Cyclophosphamide (120-240 mg/kg body weight). An electrocardiogram will be performed before the start of treatment. Patients with known heart disease will not be included in the study. Necessary investigational procedures will be performed if the patients experiences symptoms from the cardiovascular system (e.g. chest pains, shortness of breath). Myocarditis has been described following high dose Il-2 treatment, if suspected CKMB, TPN, ECG and cardiology consult should be performed.

Infertility

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

Live vaccines

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

Interactions

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

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

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

Effect evaluation, data processing and monitoring

Effect evaluation

Primary effect parameters

Paraclinical evaluation: Patients will be followed continually with *in vitro* analysis of the specific T cell reactivity against tumor antigens to evaluate the immunological effect of treatment. The immunological response against tumor antigens before and after treatment will be compared. These analyses will be done on blood samples and tumor biopsies.

Secondary effect parameters

Clinical evaluation: The clinical effect of treatment will be rated using the objective response rate in accordance with RECIST 1.1, overall survival (OS) and progression-free survival (PFS).

Response Criteria

RECIST

Clinical evaluation will be done in accordance with RECIST 1.1 Guidelines[46]:

Complete response (CR): All lesions disappear.
Partial response (PR): Defined as a ≥ 30 % reduction in the sum of all measurable parameters longest diameter.

Stabile disease (SD): Defined as a < 30 % reduction in the sum of all measurable parameters longest diameter or a < 20 % increase in the sum of all measurable parameters longest diameter.
Progression (PD): Defined as a > 20 % increase in the sum of all measurable parameters longest diameter *or* the appearance of new lesions.

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

Immunological monitoring

Blood samples for immunological monitoring

Blood samples with 100 ml heparinized blood for immunological monitoring and 10 ml blood in a dry glass for freezing of serum are taken: at surgery, before start of treatment (at "baseline"), at hospital discharge (approximately 1 week after T cell infusion) and 6 and 12 weeks after T cell infusion (see the examination plan and follow-up schedule). Blood samples will be taken for immunological monitoring every 3rd month at evaluation hereafter until the patient is withdrawn from the study. In addition, serum samples (10 ml blood sample) will be taken at day 0 before TIL infusion, 2 hours after TIL infusion and every 2nd day until hospital discharge.
A total of 500 ml of blood will be collected for research purposes in the period from time of surgery until the patient meet for the first evaluation after treatment. These blood samples are taken to assess the effect of treatment on the immune system for research purposes. The amount of blood taken during the course of the study does not exceed what the body itself is capable of producing between each test. Blood samples for research purposes will not be taken if the blood count is not acceptable (> 6 mmol/l).

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

Tumor biopsies

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

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

Establishment of autologous short term in vitro cultured tumor cell lines (as in [49, 50], or equivalent methods) and lymphocyte cultures (as in [51] or equivalent methods) will be attempted using these samples. These cultures will be primarily used to perform correlates of tumor-recognition in vitro (as in [51]) with immune parameters and clinical outcomes following therapy on an exploratory basis. The informed consent sheet will contain information about the possibility to generate cell lines and cell cultures, that under pseudonymized or anonymized form can be used for health research purposes in multiple countries, according to the Danish National Ethics Committee guidelines.

Specimens for Future Biomedical Research

Only leftovers from analyses specifically described in the protocol will be transferred to the biobank for future biomedical research, as described on page 46 "Research biobank including Future Biomedical Research Biobank". There will be no extra samples to be obtained during this study. These specimens may be used to study various causes for how subjects may respond to the immunotherapy. These speciemens will be stored to provide a resource for future trials conducted by CCIT focused on the study of biomarkers responsible for how immunotherapy works, other pathways immunotherapy may interact with, or other aspects of disease.

Additional research analyses

Isolation of tumor cells

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

Cytokine Release Assay

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

Gene-based arrays

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

1. Differences among patients in expression level of a panel of relevant normal genes in the tumor microenvironment which could influence the chance of benefit from treatment
2. Expression of tumor/patient specific mutated genes which could influence the chance of benefit from treatment

Analysis for identifying specific tumor gene expression signatures[47] and mutations in the tumor cells, leading to patient-specific neo-antigens derived from these mutations[48], will be performed.

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

Methods

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

Next Generation Sequencing (NGS) of tumors and normal cells of the individual patient will be used to obtain information on tumor-specific mutations. Gene sequencing will be performed on tumor cells (tumor gene profile) and leukocytes (normal gene profile). Tumor specific gene expression (neo antigens) will be performed by subtracting the two gene profiles from each other. Data regarding potential disease causing genes will be generated as a byproduct of this analysis, but this data will not be used or explored further upon, since only data regarding tumor specific genes will be processed more closely. Therefore, we do not expect to obtain explicit knowledge regarding disease causing genes. Furthermore, 'Targeted sequencing' on a limitied number of defined genes will be performed on the tumor tissue to obtain a 'immune profile' to determine which genes- and consequently which proteins- are expressed in the tumor tissue. Data will be handled according to national laws.

If by chance these analyses will discover known mutations with potential significant impact on patient's health, the case will be discussed with the Clinical Genetics Department, Rigshospitalet, unles the patient has chosen not to be informed as stated in the patient information. The following criteria will determine if further actions are indicated.

- There is a reasonable degree of possibility that a genetic disposition is present,
- there is solid documentation of a link between the genetic disposition and the development of disease,
- the tests used to determine the genetic disposition are well established,
- the disease in question can be prevented or treated, and

- the link between the genetic disposition and the development of disease has considerable importance for the patient.

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

In case that a patient dies/is dead, or do not want information regarding significant health issues, a medical assessment, using the 5 above mentioned criteria (p. 43), will determine whether relatives to the patient is to be informed, in accordance with Danish law (sundhedslovens § 43, stk. 2, nr.2.).

Statistics

The study is non-blinded and non-comparative. Descriptive statistics will be used to estimate the immunological- and clinical response rate. Descriptive statistics will also be used to sum up the duration of response and patient characteristics. The study is designed as a pilot study and the primary aim is to determine safety and toxicity to the treatment. A required sample size that allows determination of primary- as well as secondary- and tertiary end points can therefore not formally be calculated. A phase II study is planned if the pilot study does not give rise to SUSARs.

Data registration and -analysis

The patients are given a patient number at inclusion in the study to secure patient anonymity. Clinical personnel and selected persons in the laboratory will have access to patient information to secure proper treatment.
The principal investigator has access to patient charts to obtain information regarding the cancer disease to be able to compare this information with the project specific analysis performed on cancer tissue and blood tests.

All relevant data is registered in the eCRF (electronic Case Report Form) developed in cooperation with the clinical research unit, KFE. The principal investigator has the responsibility of manufacturing the eCRF and subsequent recording of data when the investigational treatment is finished and eCRFs are reported to sponsor. Sponsor and principal investigator are responsible for data analysis on all included patients. Patient data and eCRF will be stored for 5 years in accordance with current guidelines for storage of personal information. Drafting of a final report will be conducted in collaboration between the members of the study group.

Analyses will include:

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

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

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

End of study report

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

Amendments

An application to the Danish Health and Medicines Authority and the Research Ethics Committee will be made if substantial changes to the protocol are to be made. These can be implemented when approved. Changes to the protocol are considered substantial in accordance with 'Vejledning om anmeldelse, indberetningspligt m.v. (sundhedsvidenskabelige forskningsprojekter)', paragraph 6.0. on www.dnvk.dk and the schedule 'Skema om ændringer (ammendmendts) til kliniske forsøg' on sundhedsstyrelsen.dk.

Ethical aspects

Recruitment of study patients and informed consent

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

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

Insurance

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

Ethical aspects

Despite advances, especially with biological targeted treatments, there is no recommended adjuvant treatment for RCC at the moment, and up to half of the operated patients will develop metastasic disease at some point. Only a few patients with metastatic disease can be treated with a curative intent. The remaining patients will be treated in a palliative setting. Furthermore, metastatic RCC is resistant to chemotherapy[5]..Based on the current knowledge and the lack of treatment options, the risks and downsides associated with this study are assessed to be acceptable.

Participation is voluntary and is preceded by oral and written information and the treatment will be stopped in case of unacceptable adverse reactions or if the patient wishes so at any time.
The patient will receive treatment after the current guidelines at the department if she does not wish treatment according to the protocol. The study is therefore assessed as ethical proper.

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

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

Research biobank, including Future Biomedical Research Biobank

In connection with the current study, blood samples (110 ml/blood sample) and tumor biopsies will be stored in coded form at -150 °C in a research biobank at the CCIT in room PA102 until all analysis concerning the study is performed or for a maximum of 15 years, after which the remaining material will be destroyed.

Analyses will be primarily performed at CCIT. However, some special analyses on tumor tissue or blood test samples may be performed at a partner institution after establishing a specific written agreement. All patient relevant information will be sent in an anonymous way. In case the patient's cells will be sent to partner institutions located abroad, these will be handled according to national laws and regulations of the specific nation where these have been sent. In such a case, all patient information will be communicated in coded form. A written data processing agreement will be signed between the data controllers and the data processors abroad. If any gene analysis are to be performed abroad, the data processing agreement will include the 5 criteria (p. 42-43) regarding the discovery of known mutations with potential significant impact on patient's health, as well as the requirement, that the partner abroad reports back to the primary project managers in Denmark so

that relevant actions can be taken as described on p. 43. If data processing is to be performed in a third-country, permission wil be applied for at the Danish Data Protection Agency, or one of the agencies standard contracts will be used.

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

Reporting to the Research Ethics Committee

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

Administrative aspects and publication

Patient identification

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

Publications

The primary project managers are Inge Marie Svane and Magnus Pedersen. The members of the project group have joint copyright of the obtained results, given that the Vancouver rules are met. Positive as well as negative results will be published in international journals. Manuscripts will be produced in cooperation with the project managers and other members of the study group, with the project managers as primarily responsible for the preparation. The project managers are co-authors on publications made on the basis of this study. Author succession will be determined based on the individual contributions. Use of study data, oral as well as written, at congresses, teaching or the likes, is only to take place if accepted by the project managers. The project managers are obliged to publish results from the study and are naturally interested in the propagation and implementation of the results in clinic. Publications are expected to be completed in 2018.

Economy

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

The Research Ethics Committees will be informed if/when new funds support is obtained. None of the physicians involved in the study have any economic interests in the study and there is no potential economic gain for the departments or of personnel in connection with the study. There are no economic attachments between the financial supporters and the project managers.
The study is part of the principal investigator Magnus Pedersen's PhD project.

Appendix 1

Guidelines for verbal information regarding participation in a clinical trial

The verbal information regarding the clinical trial "T-cell therapy for patients with advanced renal cell carcinoma" will be provided at Department of Oncology, Herlev Hospital. The information will be given by a medical doctor involved in the clinical trial. The verbal information will be accompanied by written participant information leaflet. The consultation will be conducted according to the guidelines from the ethical committee:

Before the consultation

- A time and place for the consultation are agreed upon.
- Beforehand, the participant is informed that the consultation will be about participation in a clinical trial.
- The participant is informed of his/her right to have time to consider the participation in the clinical trial and the option to bring an assessor to the consultation.

Consultation regarding participation in the clinical trial

- Must be scheduled.
- Must take place in a place with no interruptions.
- The participant must be given adequate time to read the written information, listen to the verbal information and ask questions.
- Must contain an understandable presentation of the clinical trial without use of technical or value-laden terms, and must be given in consideration of the participant's individual prerequisites in terms of age, maturity, experience etc.
- Must contain information about any foreseeable risks, side effects, complications and disadvantages, as well as that there may be unforeseeable risks and burdens linked to participation in a clinical trial.
- Must contain information on alternative treatment options.
- Must contain information that information about health conditions, purely private conditions and other confidential information can be passed on to and processed by persons who must carry out a statutory quality control of the trial.
- Must contain information that the participants have the right to renounce knowledge of their health conditions.
- The information is provided by the medical doctor responsible for the project or by an authorized person involved in the trial.

Consideration period and obtaining consent

- The consideration period depends on the nature of the clinical trial. As a rule of thumb, the consideration periode should be at least one day.

- There must be a clear connection between information and consent. This implies that consent to trial participation is generally given as soon as possible after providing information, however taking into account the necessary consideration period.

After the information interview, the subject will be informed,

- If, during the implementation of the trial, new information about effect, risks, side effects, complications or disadvantages emerges.
- If the experimental design of the research project is changed significantly in relation to the subject's safety (applies to subjects who actively participate in the experiment).
- If, during the implementation of the trial, significant information about the subject's state of health emerges, unless he subject has unequivocally expressed that the subject does not want this.
- About the results that have been achieved, as well as about any consequences for the individual. This assumes that it is practically possible and the subject wants this.
- If the trial is interrupted, the subject must be informed of the reason for this.

Appendix 2

ECOG-funktionsstatus/Performance status

ECOG-FUNKTIONSSTATUS*	
Niveau	**ECOG**
0	Fully active, able to carry on all predisease performance without restriction
1	Restricted in physical strenous activity, but ambulatory and able to carry out work of a light and sedentary nature (e.g. light house work, office work)
2	Ambulatory and capable of self-care but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead

* Udgivet af 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 3

International mRCC Database Consortium (IMDC) prognostic model

IMDC prognostic model*		
Prognostic factor	**Observation**	Score
Karnofsky performance score	80 to 100 % 70% or lower	0 1
Interval from diagnosis to treatment	≥ 1 year ≤ 1 year	0 1
Hemoglobin	≥ LLN < 1 LLN	0 1
Ionized calcium	≥ ULN < ULN	0 1
Neutrophil count	≥ ULN ≤ ULN	0 1
Platelet count	≥ ULN ≤ ULN	0 1
LLN: lower limit of normal, ULN: upper limit of normal.		
Prognostic group: - Favorable prognostic group: 0 risk factors - Intermediary prognostic group: 1-2 risk factors - Bad prognostic group: ≥ 3 risk factors		

***** *Heng D et al: Prognostic Factors for Overall Survival in Patients With Metastatic Renal Cell Carcinoma Treated With Vascular Endothelial Growth Factor–Targeted Agents: Results From a Large, Multicenter Study. J Clin Oncol 2009 Dec 1;27(34):5793-9.*

Appendix 4

New York Heart Association (NYHA) classification of heart failure

- Class I: No symptoms with normal physical activity. Normal functional status.
- Class II: Mild symptoms with normal physical activity. Comfortable at rest. Slight limitation of physical status.
- Class III: Moderate symptoms with less than normal physical activity. Comfortable only at rest. Marked limitation of physical status.
- Class IV: Severe symptoms with features of heart failure with minimal physical activity even at rest. Severe limitation of functional status.

* Symptoms – Fatique, palpitations, chest pain, dyspnea, syncope

(The Criteria Committee of the New York Heart Association: Diseases of the Heart and Blood Vessels; Nomenclature and Criteria for Diagnosis, 6th ed Boston, Little, Brown 1964)

Appendix 5

Common Terminology Criteria for Adverse Events (CTCAE) version 4

CTCAE[45] is a descriptive terminology used for reporting of adverse events. Adverse events (AE) are graded as follows:

- Grade 1: Mild AE
- Grade 2: Moderate AE
- Grade 3: Severe or medcially significant AE
- Grade 4: Lifethreatening AE
- Grade 5: Death

Appendix 6

Treatment guideline for high dose interleukin-2 during T-cell therapy

Introduction

High dose interleukin 2 (HD IL-2, 600,000 to 720,000 IU/kg)(HD IL-2) is registered in the USA as treatment for advanced melanoma and clear cell renal cell carcinoma patients, resulting in 15% objective response rate, of which about half the patients experience a durable complete remission. This treatment was however, never registered in Europe because of lack of randomized controlled studies (showing improvement in overall survival for HD IL-2) and the severity of adverse events accompanying this treatment.
Here, HD IL- 2 is used as a concomitant treatment with the TIL (tumor infiltrating lymphocytes) treatment.
IL-2 has been discovered as a T cell growth factor. In vivo IL-2 leads to lymphocytosis, and when given as high dose intravenously IL-2 causes severe capillary leak syndrome, high fever, rigors, nausea, diarrhea, hypotension, renal insufficiency, dyspnea, severe fluid retention and weight gain, erythrodermia and sometimes mental disturbances. In addition, IL-2 stimulates other blood cells of the native immune system, such as natural killer cells.

Treatment schedule

HD IL-2 is given as 15 min bolus infusions of 720,000 IU/kg in 50 mL saline, every 8 hours for a maximum of 15 doses. Basic intake should be minimal (i.v. 50 ml saline per hour plus oral intake). The first HD IL-2 dose is infused as early as 4 hours after the TIL infusion, depending on whether the patient has recovered from the TIL infusion. HD IL-2 should be started within 24 hours after TIL administration.
Before initiation of HD IL-2 patients anti-hypertensive drugs will be discontinued. Patients will start paracetamol 6 times daily, and ranitidine 150 mg twice daily (orally). One hour before infusion granisetron 1 mg is administered to prevent nausea. HD IL-2 is given via a CVC.
Prior to and following every infusion of HD IL-2, the blood pressure, pulse, temperature and O2-saturation needs to be measured (during the first 4 hours every 60 min, thereafter before administration of the following dose. , Urine production and total intake every 8 hours. The patients should be weighed every 8 hours. Every 8 hours a physician needs to check the patient's condition (vital signs, heart, lungs, stomach, skin and extremities) and mental condition. During hospitalization a dedicated nurse (practitioner) is the first person of contact. He or she will inform the treating physician every 8 hours or sooner in case of changes in the patient's condition.
Toxicity can rapidly start within hours after the infusion, especially rigors and fever. In order to prevent rigors, patients may require indomethacin and/or pethidin (25-50 mg) prior to every infusion. Capillary leak syndrome, resulting in edema, weight gain and progressive dyspnea will

increase gradually during treatment. Hypotension and oliguria may start about 24 hours after initiation of IL-2. Nausea, anorexia and vomiting may occur later during the treatment. As soon as patients clearly experience insufficiency in fluid intake, the basic [basis] infusion will be adjusted to maximal 100 mL/hr.
The most significant blood abnormalities are an increase in creatinin and thrombocytopenia. Most adverse events will disappear soon after the last dose of IL-2.
When within 8 hours after infusion the patient has not recovered from toxicity despite the measurements taken, the next dose IL-2 will be skipped. When the patient does not recover within 24 hours, IL-2 dosing will be discontinued. Importantly, there is no correlation between the number of IL-2 infusions and response to treatment!

Table 1 - Schedule for monitoring

	No vasopressors needed	**ICU admission**
Vital signs	Every hour during first 4 hours, then every 4 hours	Every hour
Intake and output	Every 8 hours	Every hour
Weight	Every 8 hours	Daily
Mental status	Every 8 hours	Every 8 hours
Infusion site	CVC: every 8 hours	Every 8 hours
Laboratory tests		
Hb, Ht, L, diff, Thr	Daily	Daily
Na, K, Creat, Ur, Glu	Daily	Daily
ASAT, ALAT, tot bili	Daily	Daily
Ca++ Mg++ fosfate	Daily	Daily
INR, APTT	Daily from day 2	Daily
CK, bicarbonate	Daily	Daily
TSH, FT4	Once during HD IL-2 period	
Urine analyses	Once during HD IL-2 period or if indicated	if indicated
ECG	Once duirng HD IL-2 period or if indicated	if indicated
X-thorax	Once during HD IL-2 period or if indicated	if indicated

Toxicity

Blood pressure
Systolic blood pressure of the patient prior to start of IL-2 is:

< 100 mmHg:	target BP during IL-2: > 80 mmHg systolic
100-120 mmHg:	target BP during IL-2: > 85 mmHg systolic
> 120 mmHg:	target BP during IL-2: > 90 mmHg systolic

If the blood pressure drops below these target values, the first action to take is fluid challenge. Hypotension should in this case (the HD IL-2 treatment) be treated with Ringer's lactate solution. (RCT have shown that colloidal fluid resuscitation is not superior over crystalline fluid resuscitation). Do not give more than 1-1,5 L per 24 hours, which means two to three 500 mL of Ringer's solution in 30 minutes on top of the basic intake (50 mL/hr + oral intake, or 100 mL/hr without oral intake), because of capillary leak syndrome. When the patient stays hypotensive in spite of the fluid challenges, vasopressors are the next treatment to use (in ICU setting). This should be prevented as much as possible.
NB. Hypotension after IL-2 administration is cyclic and surges 4-6 hours after IL-2 administration. This should be taken into consideration in the calculation of the vasopressors.

Urine production
Oliguria (urine production below 10-20 ml/hour) will occur due to decreased intravascular volume and hypotension. Renal insufficiency with IL-2 is prerenal, transient and reversible. Primarily oliguria is treated with fluid challenge. When 1-1,5 L Ringer's solution does not improve diuresis, the patient should get a urine catheter with urimeter to follow urine production closely and the patient should be transferred to ICU for vasopressors (preferably low dose dopamine). The urine production should be >10-20 mL/hr before the next bolus of IL-2 can be given. Do not treat with diuretics!
Diuretics (furosemide) can be used after treatment with IL-2 has been discontinued completely and blood pressure is back to normal, to remove the sometimes severe peripheral edema (at least 1x daily 40 mg furosemide).

Dyspnea
Pulmonary edema can occur due to capillary leak syndrome, and by too much fluid administration. Measuring transcutaneous O_2 saturation is useful during treatment with IL-2 (should be performed with determining other vital signs (blood pressure, pulse and temperature). O_2 saturation should be kept at >95% during treatment, primarily through a nasal oxygen tube with a maximum of 4 L/O_2/min or with a oxygen mask with 40% O_2. When O_2 saturation cannot be kept at 95% or higher, the next bolus IL-2 can not be administered.
Pulmonary edema usually leads to changes in pulmonary sounds, especially crackles. When crackles occurs over the upper lung areas in a patient with an O_2 saturation of <95% (despite treatment), IL-2 should be discontinued and, depending on the blood pressure, furosemide should be

administered for maximal fluid excretion. Artificial breathing is seldomly required, but should not be denied when needed.
See table 2 and 3 for other toxicities and the actions to be taken.

Infections
During treatment with HD IL-2 it can be difficult to recognize an infection. TIL patients are during HD IL-2 infusion still neutropenic, have a central venous catheter (CVC) and often also diarrhea, which results in a high risk of infection. When a patient gets a high fever despite of prophylaxis with paracetamol or indometacin, or when a patient becomes hypotensive and does not respond to fluid challenges, an infection should certainly be considered. Broad spectrum antibiotics (according local guidelines for instances vancomycin/ceftazidim) should be started immediately after blood (from both CVC lines and peripherally) and other cultures have been taken. Check the renal function before defining the dose.

After discontinuation of IL-2 treatment
The patient should be closely observed until all vital signs have been stabilized. Intake/output and weight should be measured until release from the hospital. Lab values should also stabilized or improve.
Halt the basic infusion within 8 hours after the last administration of IL-2, even when the patient still has an inadequate oral intake. Only in case of very severe gastro intestinal events (severe vomiting or diarrhea) or severe weight loss until below baseline weight it may be necessary to hydrate the patient i.v.
Proceed with paracetamol or indometacin and ranitidine until at least 16-24 hours after discontinuing treatment with IL-2, because the patient can still get feverish. Make sure diuresis is adequate (> 20 ml/hr) after the blood pressure has stabilized (without treatment). Monitor the reaction to furosemide and repeat dose until the diuresis is high (200 mL/hour).

Table 2 – expected toxicity

Expected toxicity	Grade	Supportive measures	Discontinue IL-2
Cold chills	3	Pethidin 25-50 mg IV, q 1 hr, as needed	no
Fever	3	Paracetamol 500 mg po, q 4 hr or indometacin 50-75 mg, po, q 8 hr	no
Pruritus	3	Hydroxyzine HCL (Atarax) 10-20 mg po, q 6 hr, as needed	no
Skin	3	Carbomer hydrogel with 1% menthol	no
Nausea/vomiting/ anorexia	3	Granisetron 1 mg IV/day, as needed or Ondansetron 8 mg IV, q 8 hr, as needed	no
Diarrhea	3	Loperamide 2 mg po, q 3 hr, as needed or codeine 20 mg po, q 6 hr, as needed	no
Malaise	3 or 4	Bed rest or minimal activity	no
Blood bilirubin increased	3 or 4	Observation	no
Anemia	3 or 4	Blood transfusion	no
Platelet count decreased	3 or 4	Thrombocyte transfusion	When grade 4 despite of transfusion

Table 3 - Criteria for delaying or stopping IL-2 dosage

For each relative criterium:	Take action and possibly delay dose (depending on recovery)
More than 3 relative criteria:	Take action and always delay dose. Stop treatment if patient recovers too slowly
Each absolute criterium:	Take action and always delay dose. Stop treatment if patient recovers too slowly

Organ	**Relative criteria**	**Absolute criteria**
Heart	Sinus tachycardia (120-130/min)	No recovery of sinus tachycardia (>130/min)
		Atrial fibrillation
		Supraventricular tachycardia
		Ventricular arrhythmia (frequent PVC)
		Elevated cardiac enzymes (troponin, CK)
		Ischemia on ECG
Skin		Moist desquamation
Gastro-intestinal	Diarrhea, 1 L/8hr	Diarrhea 2 L/8hr
	Ileus/increased abdominal girth	Vomiting not responding to anti-emetics
	Bilirubin > 125 mmol/L	Severe abdominal bloating
		Severe stomach ache
Coagulation	Slight hemoptysis, hematemesis, blood in stools	Severe hemoptysis, hematemesis, rectal loss of blood or melaena
	Thrombocytes: 30-50 x 10^9/L	Thrombocytes: <30 x 10^9/L
Infection		Strong clinical suspicion or proven
Musculoskeletal	Weight gain > 15%	
	Tightness extremities (edema)	Sensory disturbance in extremities
Neurological	Vivid dreams	Hallucinations
	Emotionally labile	Persistent crying
		Changes in mental status that do not recover within 2 hours
		Cannot apply 'serial sevens' (100-7 etc)
		Disoriented
Lungs	Dyspnea at rest	4 L O_2/min or O_2 40% cap to keep O_2 saturation >95%
	3-4 L O_2/min to keep O_2 saturation >95%	Need for intubation
	Crackles over 1/3 of the lung	Crackles over 1/2 of the lung
		Pleural fluid for which drainage is needed
Kidneys	Urine production 80-160 ml/8 hr	Urine production < 80 ml/8hr
	Urine production 10-20 ml/hr	Urine production < 10 ml/hr
	Creatinin 200-250 umol/L	Creatinin > 250 umol/L

Table 4 – Grading symptoms according to CTCAE v. 4.0

Symptom	Grade 1	2	3	4
Cold chills	Mild sensation of cold; shivering; chattering of teeth	Moderate tremor of the entire body; narcotics indicated	Severe or prolonged, not responsive to narcotics	-
Fever	38.0-39.0ºC	>39.0-40.0ºC	>40.0ºC for ≤24 hours	>40.0ºC for >24 hours
Pruritus	Mild or localized; topical intervention indicated	Intense or widespread; intermittent; skin changes from scratching (e.g. edema, papulation, excoriations, lichenification, oozing/crusts); oral intervention indicated; limiting instrumental ADL	Intense or widespread; constant; limiting self care ADL or sleep; oral corticosteroid or immunosuppressive therapy indicated	-
Nausea/ vomiting/ anorexia	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); tube feeding or TPN indicated	Life-threatening consequences; urgent intervention indicated
Diarrhea	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated
Malaise	Uneasiness or lack of well being	Uneasiness or lack of well being; limiting instrumental ADL		
Blood bilirubin increased	>ULN - 1.5 x ULN	>1.5 - 3.0 x ULN	>3.0 - 10.0 x ULN	>10.0 x ULN
Anemia	Hemoglobin (Hgb) <LLN - 10.0 g/dL; <LLN - 6.2 mmol/L; <LLN - 100 g/L	Hgb <10.0 - 8.0 g/dL; <6.2 - 4.9 mmol/L; <100 - 80g/L	Hgb <8.0 - 6.5 g/dL; <4.9 - 4.0 mmol/L; <80 - 65 g/L; transfusion indicated	Life-threatening consequences; urgent intervention indicated

Platelet count decreased	<LLN - 75,000/mm3; <LLN - 75.0 x 10e9 /L	<75,000 - 50,000/mm3; <75.0 - 50.0 x 10e9 /L	<50,000 - 25,000/mm3; <50.0 - 25.0 x 10e9 /L	<25,000/mm3; <25.0 x 10e9 /L

Appendix 7

Febrile neutropenia during T-cell therapy

Neutrophils < 0,5 mia./l

Fever = temperature > 38° C (rectal measurement > 38,5° C)

Note: Clinical suspicion of or confirmed infection in a neutropenic and febrile patient should be treated as sepsis.

Patients receiving T-cell therapy expectedly develops neutropenia from approximately day 0 lasting 7 days with considerable individual variation. Either from infusion of T-cells or interleukin-2, the patient develops fever lasting until about 24 hours after stopping IL-2.

Adverse events related to IL-2 are clinically hard to distinguish from sepsis, thus, patients should be treated as per local guidelines for febrile neutropenia with empiric antibiotics, thorough microbiological testing and radiological examinations as needed.

Appendix 8

Summary of product characteristics for cyclophosphamide

Cyclophosphamide 500 mg Powder for Solution for Injection or Infusion

Summary of Product Characteristics Updated 06-Apr-2021 | Sandoz Limited

1. Name of the medicinal product

Cyclophosphamide 500 mg Powder for Solution for Injection or Infusion

2. Qualitative and quantitative composition

Each vial of Cyclophosphamide 500 mg Powder for Solution for Injection or Infusion contains 534.5 mg cyclophosphamide monohydrate equivalent to 500 mg cyclophosphamide.

Strength after reconstitution: 20 mg cyclophosphamide (anhydrous)/ml solution (for reconstitution volumes, see 6.6.)

For the full list of excipients, see section 6.1.

3. Pharmaceutical form

Powder for solution for injection/infusion

White crystalline powder

4. Clinical particulars

4.1 Therapeutic indications

Cyclophosphamide may be used alone or in combination with other chemotherapeutic agents, depending on the indication. Cyclophosphamide is indicated in the treatment of:

• Chronic Lymphocytic Leukemia (CLL)

• Acute Lymphocytic Leukemia (ALL)

• As conditioning for a bone marrow transplantation, in the treatment of Acute Lymphoblastic Leukemia, Chronic Myelogenous Leukemia and Acute Myelogenous Leukemia, in combination with whole body irradiation or busulfan.

• Hodgkin's lymphoma, Non-Hodgkin's lymphoma and Multiple Myeloma

• Metastatic ovarian, and breast, carcinoma

• Adjuvant treatment of breast carcinoma

• Ewing's sarcoma

• Small cell lung cancer

• Advanced or metastatic neuroblastoma,

• Life-threatening autoimmune diseases: severe progressive forms of lupus nephritis and Wegener's granulomatosis.

4.2 Posology and method of administration

Cyclophosphamide should only be used by clinicians experienced in the use of cancer chemotherapy. Cyclophosphamide should only be administered where there are facilities for regular monitoring of clinical, biochemical and haematological parameters before, during, and after administration and under the direction of a specialist oncology service.

Posology

Dosage must be individualised. Doses and duration of treatment and/or treatment intervals depend on the therapeutic indication, the scheme of a combination therapy, the patient's general state of health and organ function, and the results of laboratory monitoring (in particular, blood cell monitoring).

In combination with other cytostatics of similar toxicity, a dose reduction or extension of the therapy-free intervals may be necessary.

Use of hematopoiesis stimulating agents (colony-stimulating factors and erythropoiesis stimulating agents) may be considered to reduce the risk of myelosuppressive complications and/or help facilitate the delivery of the intended dosing.

Hematologic and solid tumours

a. For daily treatment:

3 – 6 mg/kg body weight (= 120 – 240 mg/m2 body surface area), injected intravenously

b. For intermittent treatment:

10 – 15 mg/kg body weight (= 400 – 600 mg/m2 body surface area), injected intravenously, with therapy-free intervals of 2 to 5 days.

c. For high-dose- intermittent treatment:

20 – 40 mg/kg body weight (= 800 – 1600 mg/m2 body surface area), injected intravenously, with therapy-free intervals of 21 to 28 days.

As preparation for a bone marrow transplantation

2 days 60 mg/kg or 4 days 50 mg/kg body weight injected intravenously.

If a busulfan-cyclophosphamide (Bu/Cy) regimen is applied, the first dose of cyclophosphamide must be administered at least 24 hours after the last dose of busulfan (see section 4.4 and 4.5).

Autoimmune diseases

Per month 500 – 1000 mg/m2 body surface area.

Patients with Hepatic Impairment

Severe hepatic impairment may be associated with a decreased activation of cyclophosphamide. This may alter the effectiveness of the cyclophosphamide treatment and should be considered when selecting the dose and interpreting response to the dose selected. (See section 4.4).

The dose must be reduced in patients with severe hepatic impairment. A dose reduction of 25 % is recommended in patients with serum bilirubin concentrations of 3.1 – 5 mg/100 ml (= 0.053 – 0.086 mmol/l).

Patients with Renal Impairment

In patients with renal impairment, particularly in patients with severe renal impairment, decreased renal excretion may result in increased plasma levels of cyclophosphamide and its metabolites. This may result in increased toxicity and should be considered when determining the dosage in such patients. (See section 4.4). A dose reduction of 50% for a glomerular filtration rate below 10 mL/minute is recommended.

Cyclophosphamide and its metabolites are dialyzable, although there may be differences in clearance depending upon the dialysis system being used. In patients requiring dialysis, use of a consistent interval between cyclophosphamide administration and dialysis should be considered. See section 4.4.

Elderly

In elderly patients, monitoring for toxicities and the need for dose adjustment should reflect the higher frequency of decreased hepatic, renal, cardiac, or other organ function, and concomitant diseases or other drug therapy in this population.

Paediatric population

Cyclophosphamide has been administered to children. The safety profile of cyclophosphamide in paediatric patients is similar to that of the adult population.

Dose modification due to myelosuppression

A leukocyte and platelet count should be regularly performed during treatment with cyclophosphamide. It is recommended to adjust the dose, if required, if signs of myelosuppression become evident.

Please refer to the table below. Urinary sediment should also be checked regularly for the presence of erythrocytes.

Leukocyte count/µl	Platelet count /µl	Dosage

Method of administration

Cyclophosphamide is inert until activated by enzymes in the liver. However, as with all cytotoxic agents, it is recommended that reconstitution should be performed by trained personnel, in a designated area.

Precaution to be taken before manipulating or administering the product

Those handling the preparation should wear protective gloves. Care should be taken to avoid splashing material into the eyes. The material should not be handled by women who are pregnant or who are breast-feeding.

The choice of solvent for reconstituting Cyclophosphamide containing cyclophosphamide depends on the route of administration to be used.

Infusion:

If the solution is to be used for IV infusion, Cyclophosphamide (containing cyclophosphamide) is reconstituted by adding sterile water for injection or 0.9% sterile sodium chloride solution.

Reconstituted Cyclophosphamide should be further diluted in 5% dextrose or 0.9% sodium chloride solution prior to infusion.

Direct injection:

If the solution is to be used for direct injection, Cyclophosphamide (containing cyclophosphamide) is reconstituted by adding 0.9% sterile sodium chloride solution.

Please note that only Cyclophosphamide reconstituted in 0.9% sterile sodium chloride solution is suitable for bolus injection.

Cyclophosphamide (containing cyclophosphamide) reconstituted in water is hypotonic and should not be injected directly.

For detailed instruction on reconstitution please refer to section 6.6.

Intravenous use

Intravenous administration should preferably be conducted as an infusion.

To reduce the likelihood of adverse reactions that appear to be administration rate-dependent (e.g. facial swelling, headache, nasal congestion, scalp burning), cyclophosphamide should be injected or infused very slowly. Duration of the infusion (ranging from 30 minutes to 2 hours) should be appropriate for the volume and type of carrier fluid to be infused.

Before intravenous use, the substance must be completely dissolved.

Drug products for intravenous use must be inspected visually for particulate matter and discolouration prior to administration whenever solution and container permit.

4.3 Contraindications

Cyclophosphamide is contraindicated in patients with:

• hypersensitivity to cyclophosphamide, any of its metabolites

• acute infections

• bone marrow aplasia or bone marrow depression prior to treatment

• urinary tract infection

• acute urothelial toxicity from cytotoxic chemotherapy or radiation therapy

• urinary outflow

• obstruction

• breastfeeding (see section 4.6)

Cyclophosphamide should not be used in the management of non-malignant disease, except for immunosuppression in life-threatening situations

Myelosuppression, Immunosuppression, Infections

Treatment with cyclophosphamide may cause myelosuppression (anaemia, leukopenia, neutropenia and thrombocytopenia) and significant suppression of immune responses, which may result in severe, sometimes fatal, infections, sepsis and septic shock. Infections reported with cyclophosphamide include pneumonias, as well as other bacterial, fungal, viral, protozoal, and parasitic infections.

Latent infections can be reactivated. Reactivation has been reported for various bacterial, fungal, viral, protozoal, and parasitic infections.

Infections occurring during treatment with cyclophosphamide, including neutropenic fever, must be treated appropriately. Antimicrobial prophylaxis may be indicated in certain cases of neutropenia (at the discretion of the managing physician). In case of neutropenic fever, antibiotics and/or antimycotics must be given. Cyclophosphamide must be administered with the necessary caution (or not at all) in patients with severe *functional impairment* of *bone marrow* and patients with severe immunosuppression.

Close haematological monitoring is required for all patients during treatment. Haematological parameters must be checked prior to each administration and regularly during treatment. More frequent monitoring may be required if leukocyte counts drop below 3000 cells/microlitre (cells/mm³). Dose adjustment due to myelosuppression is recommended (see section 4.2).

Unless essential, cyclophosphamide should not be administered to patients with a leukocyte count below 2500 cells/microlitre (cells/ mm^3) and/or a platelet count below 50,000 cells/microlitre (cells/mm^3).

In principle, the fall in the peripheral blood cell and thrombocyte count and the time taken to recover may increase with increasing doses of cyclophosphamide.

The nadirs of the reduction in leukocyte count and thrombocyte count are usually reached in weeks 1 and 2 of treatment. The bone marrow recovers relatively quickly, and the levels of peripheral blood cell counts normalize, as a rule, after approximately 20 days.

Cyclophosphamide treatment may not be indicated, or should be interrupted, or the dose reduced, in patients who have or who develop a serious infection.

Severe myelosuppression must be expected particularly in patients pre-treated with and/or receiving concomitant chemotherapy and/or radiation therapy.

Urinary Tract and Renal Toxicity

Hemorrhagic cystitis, pyelitis, ureteritis, and haematuria have been reported with cyclophosphamide therapy. Bladder ulceration/necrosis, fibrosis/contracture and secondary cancer may develop. Urotoxicity may mandate interruption of treatment. Cases of urotoxicity with fatal outcomes have been reported.

Urotoxicity can occur with short-term and long-term use of cyclophosphamide. Hemorrhagic cystitis after single doses of cyclophosphamide has been reported. Cystectomy may become necessary due to fibrosis, bleeding, or secondary malignancy. Past or concomitant radiation or busulfan treatment may increase the risk for cyclophosphamide-induced hemorrhagic cystitis. Cystitis is, in general, initially abacterial. Secondary bacterial colonisation may follow.

Before starting treatment, it is necessary to exclude or correct any urinary tract obstructions. See section 4.3. Urinary sediment should be checked regularly for the presence of erythrocytes and other signs of uro/nephrotoxicity. Adequate treatment with mesna and/or strong hydration to force diuresis can markedly reduce the frequency and severity of bladder toxicity. It is important to ensure that patients empty the bladder at regular intervals. Haematuria usually resolves in a few days after cyclophosphamide treatment is stopped, but it may persist. Severe hemorrhagic cystitis usually requires a discontinuation of the treatment with cyclophosphamide.

Cyclophosphamide has also been associated with nephrotoxicity, including renal tubular necrosis.

Hyponatremia associated with increased total body water, acute water intoxication, and a syndrome resembling SIADH (syndrome of inappropriate secretion of antidiuretic hormone) have been reported in association with cyclophosphamide administration. Fatal outcomes have been reported.

Cardiotoxicity, Use in Patients with Cardiac Disease

Myocarditis and myopericarditis, which may be accompanied by significant pericardial effusion and cardiac tamponade, have been reported with cyclophosphamide therapy and have led to severe, sometimes fatal congestive heart failure. Histopathologic examination has primarily shown hemorrhagic myocarditis. Haemopericardium has been reported secondary to hemorrhagic myocarditis and myocardial necrosis. Acute cardiac toxicity has been reported with single doses as low as 20

Particular caution is required in patients with risk factors for cardiotoxicity and in patients with a pre-existing cardiac disease.

Pulmonary Toxicity

Pneumonitis and pulmonary fibrosis have been reported during and following treatment with cyclophosphamide. Pulmonary veno-occlusive disease and other forms of pulmonary toxicity have also been reported. Pulmonary toxicity leading to respiratory failure has been reported. While the incidence of cyclophosphamide-associated pulmonary toxicity is low, prognosis for affected patients is poor. Late onset of pneumonitis (greater than 6 months after start of cyclophosphamide) appears to be associated with a particularly high mortality. Pneumonitis may develop even years after treatment with cyclophosphamide. Acute pulmonary toxicity has been reported after a single cyclophosphamide dose.

Secondary Malignancies

As with all cytotoxic therapy, treatment with cyclophosphamide involves the risk of secondary tumours and their precursors as sequelae.

The risk of urinary tract cancer as well as the risk of myelodysplastic alterations, partly progressing to acute leukemias, is increased. Other malignancies reported after use of cyclophosphamide or regimens with cyclophosphamide include lymphomas, thyroid cancer, and sarcomas.

In some cases, the second malignancy developed several years after cyclophosphamide treatment had been discontinued. Malignancy has also been reported after in utero exposure.

The risk of bladder cancer can be markedly reduced by hemorrhagic cystitis prophylaxis.

Veno-occlusive Liver Disease

Veno-occlusive liver disease (VOLD) has been reported in patients receiving cyclophosphamide, mainly in patients receiving a cytoreductive regimen in preparation for bone marrow transplantation in combination with whole-body irradiation, busulfan, or other agents (see section 4.5). After cytoreductive therapy, the clinical syndrome typically develops 1 to 2 weeks after transplantation and is characterized by sudden weight gain, painful hepatomegaly, ascites, and hyperbilirubinemia/jaundice. However, VOLD has also been reported to develop gradually in patients receiving long-term low-dose immunosuppressive doses of cyclophosphamide.

As a complication of VOLD, hepatorenal syndrome and multiorgan failure may develop. Fatal outcome of cyclophosphamide-associated VOLD has been reported. Risk factors predisposing a patient to the development of VOLD include pre-existing disturbances of hepatic function, previous radiation therapy of the abdomen, and a low performance score.

VOLD incidence has been reported to reduce, if a time interval of at least 24 hours is observed between the last administration of busulfan and the first administration of cyclophosphamide (see section 4.2 and 4.5).

Genotoxicity

Cyclophosphamide is genotoxic and mutagenic, both in somatic and in male and female germ cells. Therefore, women should not become pregnant and men should not father a child during therapy with cyclophosphamide.

Women should not become pregnant during the treatment and for a period of 12 months following discontinuation of the therapy.

Men should not father a child during the treatment and for a period of 6 months following discontinuation of the therapy

Animal data indicate that exposure of oocytes during follicular development may result in a decreased rate of implantations and viable pregnancies, and in an increased risk of malformations. This effect should be considered in case of intended fertilisation or pregnancy after discontinuation of cyclophosphamide therapy. The exact duration of follicular development in humans is not known, but may be longer than 12 months. Sexually active women and men should use effective methods of contraception during these periods of time (see section 4.6.).

Fertility

Cyclophosphamide interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. Men treated with cyclophosphamide should be informed about sperm preservation prior to treatment (see section 4.6).

Impairment of Wound Healing

Cyclophosphamide may interfere with normal wound healing.

Nausea and Vomiting

Administration of cyclophosphamide may cause nausea and vomiting. Current guidelines on the use of antiemetics for prevention and amelioration of nausea and vomiting should be considered.

Alcohol consumption may increase cyclophosphamide-induced vomiting and nausea.

Stomatitis

Administration of cyclophosphamide may cause stomatitis (oral mucositis). Current guidelines on measures for prevention and amelioration of stomatitis should be considered.

Paravenous Administration

The cytostatic effect of cyclophosphamide occurs after its activation, which takes place mainly in the liver. Therefore, the risk of tissue injury from accidental paravenous administration is low.

In case of accidental paravenous administration of cyclophosphamide, the infusion should be stopped immediately, the extravascular cyclophosphamide solution should be aspirated with the cannula in place, and other measures should be instituted as appropriate. The area should subsequently be rinsed with physiological saline solution, and the arm or leg should rest.

Use in Patients with Renal Impairment

In patients with renal impairment, particularly in patients with severe renal impairment, decreased renal excretion may result in increased plasma levels of cyclophosphamide and its metabolites. This may result in increased toxicity and should be considered when determining the dosage in such patients. See section 4.2.

Use in Patients with Hepatic Impairment

Severe hepatic impairment may be associated with a decreased effect of cyclophosphamide. This may negatively alter the effectiveness of cyclophosphamide treatment and should be considered when selecting the dose and interpreting response to the dose selected. See section 4.2. Due to the porphyrogenic effect of Cycolphosphamide patients with acute porphyria should be treated with caution.

Use in Adrenalectomised Patients

Patients with adrenal insufficiency may require an increase in corticoid substitution dose when exposed to stress from toxicity due to cytostatics, including cyclophosphamide.

Use in Patients with Diabetes Mellitus

Caution is also advised in is patients with diabetes mellitus, since cyclophosphamide may interact with insulin and other hypoglycaemic agents (also see section 4.5).

Use in Patients who have recently undergone surgery

In general, cytostatics (among which agents cyclophosphamide) should not be administered to patients who had a surgery less than 10 days ago.

4.5 Interaction with other medicinal products and other forms of interaction

Cyclophosphamide is inactive, but is metabolised in the liver, mainly by CYP2A6, 2B6, 2C9, 2C19 and 3A4, into two active metabolites.

Planned co-administration or sequential administration of other substances or treatments with cyclophosphamide that could increase the likelihood or severity of toxic effects (by means of pharmacodynamic or pharmacokinetic interactions) requires careful individual assessment of the expected benefit and the risks.

Patients receiving such combinations must be monitored closely for signs of toxicity to permit timely intervention. Patients being treated with cyclophosphamide and agents that reduce its activation should be monitored for a potential reduction of therapeutic effectiveness and the need for dose adjustment.

Interactions negatively affecting the pharmacokinetics of cyclophosphamide and its metabolites

disease and mucositis has been reported with concomitant administration (see section 4.2 and 4.4).

- Ciprofloxacin: when administered prior to treatment with cyclophosphamide (used for conditioning prior to bone marrow transplant), ciprofloxacin may cause regression of the underlying disease.

- Chloramphenicol

- Azole-antimycotics (Fluconazole, Itraconazole): Azole-antimycotics are known to inhibit cytochrome P450 enzymes. Increased amounts of toxic degradation products of cyclophosphamide have been reported in combination with Itraconazole.

- CYP2B6 and CYP3A4 inhibitors (Nevirapin, Ritonavir): co-administration may reduce the efficacy of cyclophosphamide

- Prasugrel

- Sulfonamides, e.g. sulfadiazine, sulfamethoxazoel and sulfapyridine

- Thiotepa: a strong inhibition of cyclophosphamide bioactivation by thiotepa in high-dose chemotherapy regimens has been reported when thiotepa was administered 1 hour prior to cyclophosphamide.

- Ondansetron: There have been reports of a pharmacokinetic interaction between ondansetron and high-dose cyclophosphamide resulting in decreased cyclophosphamide AUC.

- Grapefruit (fruit or juice), Rifampicin, St. Johns worth: Co-administration with CYP3A4 Inhibitors or Inducers can reduce the efficacy or increase the toxicity of cyclophosphamide.

• An increase of the concentration of cytotoxic metabolites may occur with:

- Allopurinol: an increase of bone marrow suppression was reported.

- Azathioprine: increased risk of hepatotoxicity (liver necrosis)

- Chloral hydrate

- Cimetidine

- Disulfiram

- Glyceraldehyde

- Protease inhibitors: concomitant use of protease inhibitors may increase the concentration of cytotoxic metabolites. Use of protease inhibitor-based regimens was found to be associated with a higher incidence of infections and neutropenia in patients receiving cyclophosphamide, doxorubicin, and etoposide (CDE) than use of an NNRTI-based regimen. Increased incidence of mucositis is reported in combined therapy of cyclophosphamide (CDE) and saquinavir

- Inducers of human hepatic and extrahepatic microsomal enzymes (e.g., cytochrome P450 enzymes): The potential for hepatic and extrahepatic microsomal enzyme induction must be considered in case of prior or concomitant treatment with substances known to induce an increased activity of such enzymes such as rifampin, phenobarbital, carbamazepine, phenytoin, St. John's wort, benzodiazepines and corticosteroids.

- Dabrafenib

Pharmacodynamic Interactions and Interactions of Unknown Mechanism Affecting the Use of Cyclophosphamide

Combined or sequential use of cyclophosphamide and other agents with similar toxicities can cause combined (increased) toxic effects.

• Increased hematotoxicity and/or immunosuppression may result from a combined effect of cyclophosphamideand, for example

- ACE inhibitors: ACE inhibitors can cause leukopenia.

- Natalizumab

- Paclitaxel: Increased hematotoxicity has been reported when cyclophosphamide was administered after paclitaxel infusion.

- Thiazide diuretics (e.g. hydrochlorthiazide): An increase of bone marrow suppression was reported.

- Zidovudine

- Clozapine

• Increased cardiotoxicity may result from a combined effect of cyclophosphamide and, for example

- Trastuzumab

• Increased pulmonary toxicity may result from a combined effect of cyclophosphamide and, for example

- Amiodarone

- G-CSF, GM-CSF (granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor): reports suggest an increased risk of pulmonary toxicity in patients treated with cytotoxic chemotherapy that includes cyclophosphamide and G-CSF or GMCSF.

• Increased nephrotoxicity may result from a combined effect of cyclophosphamide and, for example

- Amphotericin B

- Indomethacin: acute water intoxication has been reported with concomitant use of indomethacin.

Other interactions

• Alcohol

A reduced antitumor activity was observed in tumour-bearing animals during ethanol (alcohol) consumption and concomitant oral low-dose cyclophosphamide medication. In some patients, alcohol may increase cyclophosphamide-induced vomiting and nausea.

• Etanercept

In patients with Wegener's granulomatosis, the addition of etanercept to standard treatment, including cyclophosphamide, was associated with a higher incidence of non-cutaneous solid malignancies.

• Metronidazole

Acute encephalopathy has been reported in a patient receiving cyclophosphamide and metronidazole. Causal association is unclear.

In an animal study, the combination of cyclophosphamide with metronidazole was associated with increased cyclophosphamide toxicity.

• Tamoxifen

Concomitant use of tamoxifen and chemotherapy may increase the risk of thromboembolic complications.

Interactions Affecting the Pharmacokinetics and/or Actions of Other Drugs

• Bupropion

Cyclophosphamide metabolism by CYP2B6 may inhibit bupropion metabolism.

• Coumarins

Both increased and decreased warfarin effects have been reported in patients receiving warfarin and cyclophosphamide.

• Cyclosporine

Lower serum concentrations of cyclosporine have been observed in patients receiving a combination of cyclophosphamide and cyclosporine than in patients receiving only cyclosporine. This interaction may result in an increased incidence of graft versus host disease (GVHD).

• Depolarising muscle relaxants

Cyclophosphamide treatment causes a marked and persistent inhibition of cholinesterase activity. Prolonged apnoea may occur with concurrent depolarizing muscle relaxants (e.g. succinylcholine, suxamethonium) as a result of a decreased pseudocholinesterase level. If a patient has been treated with cyclophosphamide within 10 days of general anaesthesia, the anaesthesiologist should be alerted.

• Digoxin, β- acetyldigoxin

Impaired absorption of digoxin and β-acetyldigoxin tablets have been reported during a concomitant cytotoxic treatment

• Vaccines

The immunosuppressive effects of cyclophosphamide can be expected to reduce the response to vaccination. Use of live

4.6 Fertility, pregnancy and lactation

Women of childbearing potential

Girls treated with cyclophosphamide during pre-pubescence generally develop secondary sexual characteristics normally and have regular menses.

Girls treated with cyclophosphamide during pre-pubescence subsequently have conceived.

Girls treated with cyclophosphamide who have retained ovarian function after completing treatment are at increased risk of developing premature menopause (cessation of menses before age of 40 years).

Contraception in males and females

Women should not become pregnant during the treatment and for a period of 12 months following discontinuation of the therapy.

Men should not father a child during the treatment and for a period of 6 months following discontinuation of the therapy

Sexually active women and men should use effective methods of contraception during these periods of time.

Pregnancy

There are very limited data from the use of cyclophosphamide in pregnant women. There are reports of serious multiple congenital aberrations after use during the first trimester.

Animal studies have shown teratogenicity and other reproduction toxicity (see section 5.3).

Considering the data from human case reports, animal studies and the mechanism of action of cyclophosphamide, its use during pregnancy, in particular during the first trimester, is not recommended.

In each individual case the potential benefit of the treatment should be weighed against the potential risk for the foetus.

Breastfeeding

Cyclophosphamide is excreted into the breast milk and can cause neutropenia, thrombocytopenia, low haemoglobin, and diarrhoea in children. Cyclophosphamide is contraindicated during breastfeeding (see section 4.3).

Fertility

Cyclophosphamide interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. In women cyclophosphamide may cause transient or permanent amenorrhea, and in boys treated with cyclophosphamide during pre-pubescence, oligospermia or azoospermia. Men treated with cyclophosphamide may develop oligospermia or azoospermia. Prior to treatment of men with cyclophosphamide, they should be informed of the possibility to store and keep viable sperm collected before treatment.

4.7 Effects on ability to drive and use machines

Patients undergoing treatment with cyclophosphamide may experience undesirable effects (including nausea, vomiting, dizziness, blurred vision, visual impairment) which could affect the ability to drive or use machines. The decision to drive or operate machinery should be made on an individual basis.

4.8 Undesirable effects

The frequency of adverse reactions reported in the table below are derived from clinical trials and from post marketing experience and are defined using the following convention: 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), very rare (< 1/10,000) not known.

Organ System Class (SOC)	Recommended MedDRA term	Frequency
Infections and infestations	Infections 1	Common
	Pneumonia2	Uncommon

	Ureteric cancer	Rare
	Tumour lysis syndrome	Very rare
	Non-Hodgkin's lymphoma	Not known
	Sarcoma	Not known
	Renal cell carcinoma	Not known
	Renal pelvis cancer	Not known
	Thyroid cancer	Not known
Blood and lymphatic system disorders	Myelosuppression4	Very common
	Leukopenia	Very common
	Neutropenia	Very common
	Febrile neutropenia	Common
	Thrombocytopenia	Uncommon
	Anaemia	Uncommon
	Disseminated intravascular coagulation	Very rare
	Haemolytic uremic syndrome	Very rare
	Agranulocytosis	Not known
	Lymphopenia	Not known
	Haemoglobin decreased	Not known
Immune system disorders	Immunosuppression	Very common
	Anaphylactic/Anaphylactoid reaction	Uncommon
	Hypersensitivity reaction	Uncommon
	Anaphylactic shock	Very rare
Endocrine disorders	SIADH (syndrome of inappropriate antidiuretic hormone secretion)	Rare
Metabolism and nutrition disorders	Anorexia	Uncommon
	Dehydration	Rare
	Hyponatremia	Very rare
	Blood glucose increased	Not known
	Blood glucose decreased	Not known
Psychiatric disorders	Confusional state	Very rare
Nervous system disorders	Peripheral neuropathy	Uncommon
	Polyneuropathy	Uncommon
	Neuralgia	Uncommon
	Convulsion	Rare
	Dizziness	Rare
	Dysgeusia	Very rare
	Hypogeusia	Very rare
	Paraesthesia	Very rare

Eye disorders	Blurred vision	Rare
	Visual impairment	Rare
	Conjunctivitis	Very rare
	Eye oedema 7	Very rare
	Lacrimation increased	Not known
Ear and labyrinth disorders	Deafness	Uncommon
	Tinnitus	Not known
Cardiac disorders	Cardiomyopathy	Uncommon
	Myocarditis	Uncommon
	Heart failure 8	Uncommon
	Tachycardia	Uncommon
	Ventricular arrhythmia	Rare
	Supraventricular arrhythmia	Rare
	Ventricular fibrillation	Very rare
	Angina	Very rare
	Myocardial infarction	Very rare
	Pericarditis	Very rare
	Atrial fibrillation	Very rare
	Ventricular tachycardia	Not known
	Cardiogenic shock	Not known
	Pericardial effusion	Not known
	Bradycardia	Not known
	Palpitations	Not known
	Electrocardiogram QT prolonged	Not known
Vascular disorders	Flushing	Uncommon
	Haemorrhage	Rare
	Thromboembolism	Very rare
	Hypertension	Very rare
	Hypotension	Very rare
	Pulmonary embolism	Not known
	Venous thrombosis	Not known
	Vasculitis	Not known
	Peripheral ischemia	Not known
Respiratory, thoracic and mediastinal disorders 89	Acute respiratory distress syndrome (ARDS)	Very rare
	Chronic pulmonary interstitial fibrosis,	Very rare
	Pulmonary oedema	Very rare
	Bronchospasm	Very rare
	Dyspnoea	Very rare

	Sneezing	Not known
	Pulmonary veno-occlusive disease	Not known
	Obliterative bronchiolitis	Not known
	Alveolitis allergic	Not known
	Pneumonitis	Not known
	Pleural effusion	Not known
Gastrointestinal disorders	Mucosal inflammation	Common
	Enterocolitis haemorrhagic	Very rare
	Acute pancreatitis	Very rare
	Ascites	Very rare
	Stomatitis	Very rare
	Diarrhoea	Very rare
	Vomiting	Very rare
	Constipation	Very rare
	Nausea	Very rare
	Abdominal pain	Not known
	Parotid gland inflammation	Not known
	Gastrointestinal haemorrhage	Not known
	Cecitis	Not known
	Colitis	Not known
	Enteritis	Not known
Hepatobiliary disorders	Hepatic function abnormal	Common
	Hepatitis	Rare
	Veno-occlusive liver disease	Very rare
	Hepatomegaly	Very rare
	Jaundice	Very rare
	Cholestatic hepatitis	Not known
	Hepatotoxicity 10	Not known
Skin and subcutaneous tissue disorders	Alopecia 11	Very common
	Rash	Rare
	Dermatitis	Rare
	Nail discolouration	Rare
	Skin discolouration 12	Rare
	Stevens-Johnson syndrome	Very rare
	Toxic epidermal necrolysis	Very rare
	Radiation erythaema	Very rare
	Pruritus (including itching due to inflammation)	Very rare
	Erythaema multiforme	Not known

	Hyperhidrosis	Not known
Musculoskeletal and connective tissue disorders	Rhabdomyolysis	Very rare
	Cramps	Very rare
	Scleroderma	Not known
	Muscle spasms	Not known
	Myalgia	Not known
	Arthralgia	Not known
Renal and urinary tract disorders	Cystitis	Very common
	Microhaematuria	Very common
	Haemorrhagic cystitis	Common
	Macrohematuria	Common
	Suburethral haemorrhage	Very rare
	Bladder wall oedema	Very rare
	Bladder fibrosis and sclerosis	Very rare
	Renal impairment	Very rare
	Blood creatinine increased	Very rare
	Renal tubular necrosis	Very rare
	Renal tubular disorder	Not known
	Nephropathy toxic	Not known
	Hemorrhagic ureteritis	Not known
	Bladder contracture	Not known
	Nephrogenic diabetes insipidus	Not known
	Atypical urinary bladder epithelial cells	Not known
	Blood urea nitrogen increased	Not known
Pregnancy, puerperium and perinatal conditions	Premature labour	Not known
Reproductive system and breast disorders	Impairment of spermatogenesis	Common
	Ovulation disorder (rarely irreversible)	Uncommon
	Amenorrhea 13	Rare
	Azoospermia/asperima 13	Rare
	Oligospermia 13	Rare
	Infertility	Not known
	Ovarian Failure	Not known
	Oligomenorrhoe	Not known
	Testicular atrophy	Not known
Congenital, familial and genetic disorders	Intra-uterine death	Not known
	Foetal malformation	Not known
	Foetal growth retardation	Not known

	Malaise	Common
	Chest pain	Rare
	Headache	Very rare
	Multiorgan failure	Very rare
	Injection/infusion site reactions	Very rare
	(thrombosis, necrosis, phlebitis, inflammation, pain, swelling, erythaema)	
Investigations	Blood lactate dehydrogenase increased	Uncommon
	C-reactive protein increased	Uncommon
	ECG changes	Uncommon
	Decreased LVEF	Uncommon
	Weight gain	Very rare
	Lower levels of female sex hormones	Uncommon
	Blood oestrogen level decreased	Not known
	Blood gonadotropin level increased	Not known

1 An increased risk for and severity of pneumonias (including fatal outcomes), other bacterial, fungal, viral, protozoal, and parasitic infections; reactivation of latent infections, including viral hepatitis, tuberculosis, JC virus with progressive multifocal leukoencephalopathy (including fatal outcomes), *pneumocystis jiroveci*, herpes zoster, *strongyloides*, sepsis and septic shock (including fatal outcomes).

2 including fatal outcomes

3 including acute myeloid leukemia, acute promyelocytic leukemia

4 manifested as Bone marrow failure, Pancytopenia, Neutropaenia, Agranulocytosis, Granulocytopenia,Thrombocytopaenia (complicated by bleeding), Leukopenia, Anaemia

5 manifested as myelopathy, peripheral neuropathy, polyneuropathy,neuralgia, dysesthesia, hypoesthesia, paresthesia, tremor, dysgeusia, hypogeusia,parosmia.

6 manifested as headache, altered mental functioning, seizures and abnormal vision from blurriness to vision loss

7 Observed in connection with an allergic reaction

8 Including fatal outcomes

9 While the incidence of cyclophosphamide-associated pulmonary toxicity is low, prognosis for affected patients is poor.

10 Hepatic failure, Hepatic encephalopathy, Ascites, Hepatomegaly, Jaundice, Blood bilirubin increased, Hepatic enzymes increased (ASAT, ALAT, ALP, gamma-GT)

11 May progress to baldness

12 Of the palms and heels

13 Persistent

Remark:

Certain complication such as thromboembolisms, disseminated intravascular coagulation, and haemolytic uremic syndrome may occur as a result of the underlying disorders, but the frequency of these complications may increase due to chemotherapy with Cyclophosphamide.

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected

There is no specific antidote for an overdosage of cyclophosphamide.

Cyclophosphamide and its metabolites are dialyzable. Therefore, rapid haemodialysis is indicated when treating any suicidal or accidental overdose or intoxication.

Overdosage should be managed with supportive measures, including appropriate, state-of-the-art treatment for any concurrent infection, myelosuppression, or other toxicity, should it occur.

Cystitis prophylaxis with mesna can help to prevent or reduce urotoxic effects in case of cyclophosphamide overdosage.

5. Pharmacological properties

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antineoplastic and Immunomodulating Agents; Antineoplastic agents. Alkylating agents. Nitrogen mustard analogues

ATC code: L01AA01.

Cyclophosphamide has been demonstrated to have a cytostatic effect in many tumour types.

Cyclophosphamide engages probably to the S-or G2-phase of the cell cycle.

It remains to be shown whether the cytostatic effect is entirely dependent on the alkylation of DNA or other mechanisms such as inhibition of chromatin transformation processes or inhibition of DNA polymerases play a role. The metabolite acrolein has no antineoplastic activity, but is responsible for the adverse urotoxic effect.

The immunosuppressive effect of cyclophosphamide is based on the fact that cyclophosphamide has an inhibitory effect on B-cells, CD4 + T-cells and to a lesser extent on CD8 +-T-cells. In addition, it is assumed that cyclophosphamide has an inhibitory effect on the suppressor that regulate the IgG2 class of antibodies.

Cross-resistance, especially with structurally related cytotoxic agents, e.g. ifosfamide, as well as other alkylating agents, cannot be excluded.

5.2 Pharmacokinetic properties

Cyclophosphamide is administered as an inactive prodrug that is activated in the liver.

Absorption

Cyclophosphamide is quickly and almost completely absorbed from parenteral sites.

Distribution

Less than 20% of cyclophosphamide is bound to plasma proteins. The protein binding of the metabolites of cyclophosphamide is higher but less than 70%. To what extent the active metabolites protein bound, is not known.

Cyclophosphamide is about in the cerebrospinal fluid and the mother's milk. Cyclophosphamide and metabolites can pass through the placenta.

Metabolism

Cyclophosphamide is activated in the liver to the active metabolites 4-hydroxy-cyclophosphamide and aldofosfamide (tautomeric form of 4-hydroxy-cyclophosphamide) through phase I metabolism by cytochrome P450 (CYP) enzymes. Different CYP isozymes contribute to the bioactivation of cyclophosphamide, including CYP2A6, 2B6, 2C9, 2C19 and 3A4, 2B6 in which the exhibits highest 4-hydroxylase activity. Detoxification is done mainly through glutathione-S-transferases (GSTA1, GSTP1) and alcohol dehydrogenase (ALDH1, ALDH3). Two to four hours after administration of cyclophosphamide, the plasma concentrations of the active metabolites are maximal, after which a rapid decrease of plasma concentrations takes place.

Elimination

The plasma half-life of cyclophosphamide is about 4 to 8 hours in adults and children. The plasma half-lives of the active metabolites are not known.

Following high-dose IV administration within the framework of allogeneic bone marrow transplantation, the plasma

5.3 Preclinical safety data

Acute toxicity

The acute toxicity of cyclophosphamide is relatively low. This was demonstrated in studies on mice, guinea pigs, rabbits and dogs.

Chronic toxicity

Chronic administration of toxic doses led to hepatic lesions manifested as fatty degeneration followed by necrosis. The intestinal mucosa was not affected. The threshold for hepatotoxic effects was 100 mg/kg in the rabbit and 10 mg/kg in the dog.

Mutagenicity and carcinogenicity

The mutagenic effects of cyclophosphamide have been demonstrated in various *in-vitro* and *in-vivo tests*. Chromosome aberrations following administration of cyclophosphamide have also been observed in humans. The carcinogenic effects of cyclophosphamide have been demonstrated in animal studies on rats and mice.

Teratogenicity

The *teratogenic effects* of cyclophosphamide have been demonstrated in various animals (mice, rats, rabbits, rhesus monkeys and dogs). Cyclophosphamide can cause skeletal, tissue as well as other malformations.

6. Pharmaceutical particulars

6.1 List of excipients

None

6.2 Incompatibilities

Not applicable

6.3 Shelf life

2 years

Chemical and physical in-use stability has been demonstrated for 24 hours at 2°C - 8°C for the reconstituted solution and for the diluted solution.

From a microbiological point of view, the reconstituted and diluted solution should be used immediately, unless reconstitution has taken place in controlled and validated aseptic conditions. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C- 8°C.

6.4 Special precautions for storage

Do not store above 25°C.

For storage conditions after reconstitution of the medicinal product, see section 6.3.

6.5 Nature and contents of container

Cyclophosphamide 500 mg Powder for Solution for Injection or Infusion is available in the following pack sizes:

1, 5 or 10 clear colourless 50 ml Type I-glass vials containing 500 mg cyclophosphamide sealed with uncoated bromobutyl stopper, and secured with a flip-off seal with a red PP button

Not all pack sizes may be marketed.

Vials are packed with or without a protective plastic overwrap (Onco-Safe). “Onco-Safe” does not come into contact with the medicinal product and provides additional transport protection, which increases the safety for the medical and pharmaceutical personnel.

Direct injection:

If the solution is to be used for direct injection, Cyclophosphamide (containing cyclophosphamide) is reconstituted by adding 0.9% sterile sodium chloride solution.

Infusion:

If the solution is to be used for IV infusion, Cyclophosphamide (containing cyclophosphamide) is reconstituted by adding sterile water for injection or 0.9% sterile sodium chloride solution.

The following quantities of water for injections or sodium chloride 0.9 % are added to the vials containing Cyclophosphamide, Powder for Solution for Injection or Infusion

Vial of 500 mg: 25 ml

Vial of 1000 mg: 50 ml

Vial of 2000 mg: 100 ml

Injecting the solvent into the vial for injection creates an abnormally high pressure, which disappears as soon as the second sterile needle has been inserted in the rubber stop of the vial for injection. The powder easily dissolves when the vial for injection is shaken vigorously to produce a clear solution. If the powder does not immediately dissolve, continue to shake the vial vigorously for up to several minutes until complete dissolution of the powder.The solution must be administered as soon as possible following its reconstitution.

After reconstitution the solution is clear and colourless to light yellow. Please check the vial before further use. Only clear solutions must be used.

Cyclophosphamide, Powder for Solution for Injection or Infusion reconstituted in water for injection has an osmolality of 92 mOsm/kg.

Cyclophosphamide, Powder for Solution for Injection or Infusion reconstituted in 0.9% sodium chloride has an osmolality of 353 mOsm/kg and a pH of 4.6

Intravenous use

Intravenous administration should preferably be conducted as an infusion.

Infusion:

Reconstituted Cyclophosphamide should be further diluted in 5% dextrose or 0.9% sodium chloride injection prior to infusion.

Direct injection:

Please note that only Cyclophosphamide reconstituted in 0.9% sterile sodium chloride solution is suitable for bolus injection.

Cyclophosphamide (containing cyclophosphamide) reconstituted in water is hypotonic and should not be injected directly.

The rules and regulations for handling cytostatics in general must be observed when reconstituting or handling Cyclophosphamide. Reconstitution must, to the extent possible, be performed in a *laminar air flow safety* cabinet. The person handling the product must wear a protective mask and protective *gloves*. In case of spills, the area must be thoroughly rinsed with water. If Cyclophosphamide, Powder for Solution for Injection or Infusion is stored (e.g. during transport) at the temperature exceeding the maximum temperature, cyclophosphamide may melt. Vials for injections containing melted cyclophosphamide can be visually recognised. Cyclophosphamide is a white powder. M*elted cyclophosphamide* is a *clear or* yellowish viscous liquid (usually found as droplets in the affected vials). Vials for injections containing melted cyclophosphamide may no longer be used.

7. Marketing authorisation holder

Sandoz Limited

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Watchmoor Park

Camberley, Surrey

Date of first renewal: 23/07/2019

10. Date of revision of the text

10/03/2021

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Appendix 9

Summary of product characteristics for fludarabinephosphat

Fludara 50mg powder for solution for injection or infusion

Summary of Product Characteristics Updated 18-Mar-2019 | SANOFI

1. Name of the medicinal product

Fludara 50 mg powder for solution for injection or infusion.

2. Qualitative and quantitative composition

Each vial contains 50 mg fludarabine phosphate.

One ml of reconstituted solution contains 25 mg fludarabine phosphate.

For the full list of excipients, see section 6.1.

3. Pharmaceutical form

Powder for solution for injection or infusion.

White lyophilisate for reconstitution.

4. Clinical particulars

4.1 Therapeutic indications

Treatment of B-cell chronic lymphocytic leukaemia (CLL) in adult patients with sufficient bone marrow reserves.

First line treatment with Fludara should only be initiated in adult patients with advanced disease, Rai stages III/IV (Binet stage C), or Rai stages I/II (Binet stage A/B) where the patient has disease related symptoms or evidence of progressive disease.

4.2 Posology and method of administration

Posology

The recommended dose is 25 mg fludarabine phosphate/m² body surface area given daily for 5 consecutive days every 28 days by intravenous route. Each vial is to be made up in 2 ml water for injection. Each ml of the resulting solution will contain 25 mg fludarabine phosphate (see section 6.6).

The required dose (calculated on the basis of the patient's body surface area) of the reconstituted solution is drawn up into a syringe. For intravenous bolus injection this dose is further diluted in 10 ml sodium chloride 9 mg/ml (0.9%). Alternatively, for infusion, the required dose drawn up in a syringe may be diluted in 100 ml sodium chloride 9 mg/ml (0.9%) and infused over approximately 30 minutes.

The duration of treatment depends on the treatment success and the tolerability of the drug.

In CLL patients, Fludara should be administered up to the achievement of best response (complete or partial remission, usually 6 cycles) and then the drug should be discontinued.

Patients with renal impairment

Doses should be adjusted for patients with reduced kidney function. If creatinine clearance is between 30 and 70 ml/min, the dose should be reduced by up to 50% and close haematological monitoring should be used to assess toxicity (see section 4.4).

Fludara treatment is contraindicated, if creatinine clearance is < 30 ml/min (see section 4.3).

Patients with hepatic impairment

No data are available concerning the use of Fludara in patients with hepatic impairment. In this group of patients, Fludara should be used with caution.

Paediatric population

The safety and efficacy of Fludara in children below the age of 18 years have not been established. Therefore, Fludara is not

Method of administration

Fludara should be administered under the supervision of a qualified physician experienced in the use of antineoplastic therapy.

It is strongly recommended that Fludara should be only administered intravenously. No cases have been reported in which paravenously administered Fludara led to severe local adverse reactions. However, unintentional paravenous administration must be avoided.

Precautions to be taken before handling the medicinal product

For instructions on handling and reconstitution of the medicinal product before administration, see section 6.6.

4.3 Contraindications

- Hypersensitivity to the active substance or to any of the excipients listed in section 6.1.
- Renal impairment with creatinine clearance < 30 ml/min.
- Decompensated haemolytic anaemia.
- Lactation.

4.4 Special warnings and precautions for use

Myelosuppression

Severe bone marrow suppression, notably anaemia, thrombocytopenia and neutropenia, has been reported in patients treated with Fludara. In a Phase I intravenous study in adult solid tumour patients, the median time to nadir counts was 13 days (range 3 – 25 days) for granulocytes and 16 days (range 2 - 32 days) for platelets. Most patients had haematologic impairment at baseline either as a result of disease or as a result of prior myelosuppressive therapy.

Cumulative myelosuppression may be seen. While chemotherapy-induced myelosuppression is often reversible, administration of fludarabine phosphate requires careful haematologic monitoring.

Fludarabine phosphate is a potent antineoplastic agent with potentially significant toxic side effects. Patients undergoing therapy should be closely observed for signs of haematologic and non-haematologic toxicity. Periodic assessment of peripheral blood counts is recommended to detect the development of anaemia, neutropenia and thrombocytopenia.

Several instances of trilineage bone marrow hypoplasia or aplasia resulting in pancytopenia, sometimes resulting in death, have been reported in adult patients. The duration of clinically significant cytopenia in the reported cases has ranged from approximately 2 months to approximately 1 year. These episodes have occurred both in previously treated or untreated patients.

As with other cytotoxics, caution should be exercised with fludarabine phosphate, when further haematopoietic stem cell sampling is considered.

Autoimmune disorders

Irrespective of any previous history of autoimmune processes or Coombs test status, life-threatening and sometimes fatal autoimmune phenomena (see section 4.8) have been reported to occur during or after treatment with Fludara. The majority of patients experiencing haemolytic anaemia developed a recurrence in the haemolytic process after rechallenge with Fludara. Patients treated with Fludara should be closely monitored for signs of haemolysis.

Discontinuation of therapy with Fludara is recommended in case of haemolysis. Blood transfusion (irradiated, see below) and adrenocorticoid preparations are the most common treatment measures for autoimmune haemolytic anaemia.

Neurotoxicity

The effect of chronic administration of Fludara on the central nervous system is unknown. However, patients tolerated the recommended dose, in some studies for relatively long treatment times (for up to 26 courses of therapy).

Patients should be closely observed for signs of neurologic effects.

When used at high doses in dose-ranging studies in patients with acute leukaemia, intravenous Fludara was associated with

Administration of Fludara can be associated with leukoencephalopathy (LE), acute toxic leukoencephalopathy (ATL) or reversible posterior leukoencephalopathy syndrome (RPLS).

These may occur:

• at the recommended dose

o when Fludara is given following, or in combination with, medications known to be associated with LE, ATL or RPLS,

o or when Fludara is given in patients with other risk factors such as cranial or total body irradiation, Hematopoietic Cell Transplantation, Graft versus Host Disease, renal impairment, or hepatic encephalopathy.

• at doses higher than the recommended dose

LE, ATL or RPLS symptoms may include headache, nausea and vomiting, seizures, visual disturbances such as vision loss, altered sensorium, and focal neurological deficits. Additional effects may include optic neuritis, and papillitis, confusion, somnolence, agitation, paraparesis/ quadriparesis, muscle spasticity and incontinence.

LE/ ATL/ RPLS may be irreversible, life-threatening, or fatal.

Whenever LE, ATL or RPLS is suspected, fludarabine treatment should be stopped. Patients should be monitored and should undergo brain imaging, preferably utilizing MRI. If the diagnosis is confirmed, fludarabine therapy should be permanently discontinued.

Tumour lysis syndrome

Tumour lysis syndrome has been reported in CLL patients with large tumour burdens. Since Fludara can induce a response as early as the first week of treatment, precautions should be taken in those patients at risk of developing this complication, and hospitalisation may be recommended for these patients during the first course of treatment.

Transfusion-associated graft-versus-host disease

Transfusion-associated graft-versus-host disease (reaction by the transfused immunocompetent lymphocytes to the host) has been observed after transfusion of non-irradiated blood in Fludara-treated patients. Fatal outcome as a consequence of this disease has been reported with a high frequency. Therefore, to minimise the risk of transfusion-associated graft-versus-host disease, patients who require blood transfusion and who are undergoing, or who have received treatment with Fludara should receive irradiated blood only.

Skin cancer

The worsening or flare up of pre-existing skin cancer lesions as well as new onset of skin cancer has been reported in some patients during or after Fludara therapy.

Impaired state of health

In patients with impaired state of health, Fludara should be given with caution and after careful risk/benefit consideration. This applies especially for patients with severe impairment of bone marrow function (thrombocytopenia, anaemia, and/or granulocytopenia), immunodeficiency or with a history of opportunistic infection.

Renal impairment

The total body clearance of the principle plasma metabolite 2-F-ara-A shows a correlation with creatinine clearance, indicating the importance of the renal excretion pathway for the elimination of the compound. Patients with reduced renal function demonstrated an increased total body exposure (AUC of 2F-ara-A). There are limited clinical data available in patients with impairment of renal function (creatinine clearance < 70 ml/min).

Fludara must be administered cautiously in patients with renal insufficiency. In patients with moderate impairment of renal function (creatinine clearance between 30 and 70 ml/min), the dose should be reduced by up to 50% and the patient should be monitored closely (see section 4.2). Fludara treatment is contraindicated if creatinine clearance is < 30ml/min (see section 4.3).

Older people

Since there are limited data for the use of Fludara in older people (> 75 years), caution should be exercised with the administration of Fludara in these patients (see also section 4.2).

foetal harm (see sections 4.6 and 5.3). Prescribers may only consider the use of Fludara, if the potential benefits justify the potential risks to the foetus.

Women should avoid becoming pregnant while on Fludara therapy.

Women of childbearing potential must be apprised of the potential hazard to the foetus.

Contraception

Women of child-bearing potential or fertile males must take effective contraceptive measures during and at least for 6 months after cessation of therapy (see section 4.6).

Vaccination

During and after treatment with Fludara vaccination with live vaccines should be avoided.

Retreatment options after initial Fludara treatment

A crossover from initial treatment with Fludara to chlorambucil for non responders to Fludara should be avoided because most patients who have been resistant to Fludara have shown resistance to chlorambucil.

Excipients

Each vial Fludara 50 mg powder for solution for injection/infusion contains less than 1 mmol sodium (23 mg), i.e. essentially 'sodium-free'.

4.5 Interaction with other medicinal products and other forms of interaction

In a clinical investigation using intravenous Fludara in combination with pentostatin (deoxycoformycin) for the treatment of refractory chronic lymphocytic leukaemia (CLL), there was an unacceptably high incidence of fatal pulmonary toxicity. Therefore, the use of Fludara in combination with pentostatin is not recommended.

Dipyridamole and other inhibitors of adenosine uptake may reduce the therapeutic efficacy of Fludara.

Clinical studies and in vitro experiments showed that during use of Fludara in combination with cytarabine the intracellular peak concentration and intracellular exposure of Ara-CTP (active metabolite of cytarabine) increased in leukaemic cells. Plasma concentrations of Ara-C and the elimination rate of Ara-CTP were not affected.

4.6 Fertility, pregnancy and lactation

Fertility

Women of childbearing potential must be apprised of the potential hazard to the foetus.

Both sexually active men and women of childbearing potential must take effective contraceptive measures during and at least for 6 months after cessation of therapy (see section 4.4).

Pregnancy

Pre-clinical data in rats demonstrated a transfer of Fludara and/or metabolites through the placenta. The results from intravenous embryotoxicity studies in rats and rabbits indicated an embryolethal and teratogenic potential at the therapeutic doses (see section 5.3).

There are very limited data of Fludara use in pregnant women in the first trimester.

Fludara should not be used during pregnancy unless clearly necessary (e.g. life-threatening situation, no alternative safer treatment available without compromising the therapeutic benefit, treatment cannot be avoided). Fludara has the potential to cause foetal harm. Prescribers may only consider the use of Fludara if the potential benefits justify the potential risks to the foetus.

Lactation

It is not known whether this drug or its metabolites are excreted in human milk.

However, there is evidence from preclinical data that fludarabine phosphate and/or metabolites transfer from maternal blood to milk.

4.8 Undesirable effects

Summary of safety profile

Based on the experience with the use of Fludara, the most common adverse events include myelosuppression (neutropenia, thrombocytopenia and anaemia), infection including pneumonia, cough, fever, fatigue, weakness, nausea, vomiting and diarrhoea. Other commonly reported events include chills, oedema, malaise, peripheral neuropathy, visual disturbance, anorexia, mucositis, stomatitis and skin rash. Serious opportunistic infections have occurred in patients treated with Fludara. Fatalities as a consequence of serious adverse events have been reported.

Tabulated list of adverse reactions

The table below reports adverse events by MedDRA system organ classes (MedDRA SOCs). The frequencies are based on clinical trial data regardless of the causal relationship with Fludara. The rare adverse reactions were mainly identified from the post-marketing experience.

System Organ Class	**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)
Infections and infestations	Infections / Opportunistic infections (like latent viral reactivation, e.g. Progressive multifocal leukoencephalopathy, Herpes zoster virus Esptein-Barr-virus), pneumonia			Lympho-proliferative disorder (EBV-associated)
Neoplasms benign, malignant and unspecified (incl cysts and polyps)		Myelodysplastic syndrome and Acute Myeloid Leukaemia (mainly associated with prior, concomitant or subsequent treatment with alkylating agents, topoisomerase inhibitors or irradiation)		
Blood and lymphatic system disorders	Neutropenia, anaemia, thrombocytopenia	Myelosuppression		
Immune system disorders			Autoimmune disorder (including Autoimmune haemolytic anaemia, Evan's syndrome, Thrombocytopenic purpura, acquired haemophilia, pemphigus)	
Metabolism and nutrition disorders		Anorexia	Tumour lysis syndrome (including renal failure, metabolic acidosis,	

Nervous system disorders		Peripheral neuropathy	Confusion	Coma, seizures, agitation
Eye disorders		Visual disturbances		Blindness, optic neuritis, optic neuropathy
Cardiac disorders				Heart failure, arryhthmia
Respiratory, thoracic and mediastinal disorders	Cough		Pulmonary toxicity (including pulmonary fibrosis, pneumonitis, dyspnoea)	
Gastro-intestinal disorders	Vomiting, diarrhoea, nausea	Stomatitis	Gastrointestinal haemorrhage, pancreatic enzymes abnormal	
Hepatobiliary disorders			Hepatic enzymes abnormal	
Skin and subcutaneous tissue disorders		Rash		Skin cancer, necrolysis epidermal toxic (Lyell type) Stevens-Johnson syndrome
General disorders and administration site conditions	Fever, fatigue, weakness	Oedema, mucositis, chills, malaise		

The most appropriate MedDRA term to describe a certain adverse event is listed. Synonyms or related conditions are not listed, but should be taken into account as well. Adverse event term representation is based on MedDRA version 12.0.

Within each frequency grouping, undesirable effects are presented in order of decreasing seriousness.

Postmarketing experience with frequency unknown

• Nervous system disorders

o Cerebral haemorrhage

o Leukoencephalopathy (see section 4.4)

o Acute toxic leukoencephalopathy (see section 4.4)

o Reversible posterior leukoencephalopathy syndrome (RPLS) (see section 4.4)

• Respiratory, thoracic and mediastinal disorders

o Pulmonary haemorrhage

• Renal and urinary disorder

o Haemorrhagic cystitis

Reporting of suspected adverse reactions

Reporting suspected adverse reactions after authorisation of the medicinal product is important. It allows continued monitoring of the benefit/risk balance of the medicinal product. Healthcare professionals are asked to report any suspected adverse reactions via Yellow Card Scheme at: www.mhra.gov.uk/yellowcard or search for MHRA Yellow Card in the Google Play or Apple App Store.

irreversible central nervous system toxicity characterised by delayed blindness, coma, and death. High doses are also associated with severe thrombocytopenia and neutropenia due to bone marrow suppression.

There is no known specific antidote for Fludara overdosage. Treatment consists of drug discontinuation and supportive therapy.

5. Pharmacological properties

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Antineoplastic agents, purine analogues

ATC-code L01B B05

Mechanism of action

Fludara contains fludarabine phosphate, a water-soluble fluorinated nucleotide analogue of the antiviral agent vidarabine, 9-ß-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase.

Fludarabine phosphate is rapidly dephosphorylated to 2F-ara-A which is taken up by cells and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2F-ara-ATP. This metabolite has been shown to inhibit ribonucleotide reductase, DNA polymerase α/δ and ε, DNA primase and DNA ligase thereby inhibiting DNA synthesis. Furthermore, partial inhibition of RNA polymerase II and consequent reduction in protein synthesis occur.

While some aspects of the mechanism of action of 2F-ara-ATP are as yet unclear, it is assumed that effects on DNA, RNA and protein synthesis all contribute to inhibition of cell growth with inhibition of DNA synthesis being the dominant factor. In addition, in vitro studies have shown that exposure of CLL lymphocytes to 2F-ara-A triggers extensive DNA fragmentation and cell death characteristic of apoptosis.

Clinical efficacy and safety

A phase III trial in patients with previously untreated B-chronic lymphocytic leukaemia comparing treatment with Fludara vs. chlorambucil (40mg / m² q4 weeks) in 195 and 199 patients respectively showed the following outcome: statistically significant higher overall response rates and complete response rates after 1st line treatment with Fludara compared to chlorambucil (61.1% vs. 37.6% and 14.9% vs. 3.4%, respectively); statistically significant longer duration of response (19 vs. 12.2 months) and time to progression (17 vs. 13.2 months) for the patients in the Fludara group. The median survival of the two patient groups was 56.1 months for Fludara and 55.1 months for chlorambucil, a non-significant difference was also shown with performance status. The proportion of patients reported to have toxicities were comparable between Fludara patients (89.7%) and chlorambucil patients (89.9%). While the difference in the overall incidence of haematological toxicities was not significant between the two treatment groups, significantly greater proportions of Fludara patients experienced white blood cell ($p=0.0054$) and lymphocyte ($p=0.0240$) toxicities than chlorambucil patients. The proportions of patients who experienced nausea, vomiting, and diarrhoea were significantly lower for Fludara patients ($p<0.0001$, $p<0.0001$, and $p=0.0489$, respectively) than chlorambucil patients. Toxicities of the liver were also reported for significantly ($p=0.0487$) less proportions of patients in the Fludara group than in the chlorambucil group.

Patients who initially respond to Fludara have a chance of responding again to Fludara monotherapy.

A randomised trial of Fludara vs. cyclophosphamide, adriamycin and prednisone (CAP) in 208 patients with CLL Binet stage B or C revealed the following results in the subgroup of 103 previously treated patients: the overall response rate and the complete response rate were higher with Fludara compared to CAP (45% vs. 26% and 13% vs. 6%, respectively); response duration and overall survival were similar with Fludara and CAP. Within the stipulated treatment period of 6 months the number of deaths was 9 (Fludara) vs. 4 (CAP).

Post-hoc analyses using only data of up to 6 months after start of treatment revealed a difference between survival curves of Fludara and CAP in favour of CAP in the subgroup of pretreated Binet stage C patients.

5.2 Pharmacokinetic properties

Plasma and urinary pharmacokinetics of fludarabine (2F-ara-A)

The pharmacokinetics of fludarabine (2F-ara-A) have been studied after intravenous administration by rapid bolus injection and short-term infusion as well as following continuous infusion and after peroral dosing of fludarabine phosphate (Fludara, 2F-ara-AMP).

Another metabolite, 2F-ara-hypoxanthine, which represents the major metabolite in the dog, was observed in humans only to a minor extent.

After single dose infusion of 25 mg 2F-ara-AMP per m² to CLL patients for 30 minutes 2F-ara-A reached mean maximum concentrations in the plasma of 3.5 - 3.7 µM at the end of the infusion. Corresponding 2F-ara-A levels after the fifth dose showed a moderate accumulation with mean maximum levels of 4.4 - 4.8 µM at the end of infusion. During a 5-day treatment schedule 2F-ara-A plasma trough levels increased by a factor of about 2. An accumulation of 2F-ara-A over several treatment cycles can be excluded. Postmaximum levels decayed in three disposition phases with an initial half-life of approximately 5 minutes, an intermediate half-life of 1 - 2 hours and a terminal half-life of approximately 20 hours.

An interstudy comparison of 2F-ara-A pharmacokinetics resulted in a mean total plasma clearance (CL) of 79 ± 40 ml/min/m² (2.2 ± 1.2 ml/min/kg) and a mean volume of distribution (Vss) of 83 ± 55 l/m² (2.4 ± 1.6 l/kg). Data showed a high interindividual variability. After intravenous and peroral administration of fludarabine phosphate plasma levels of 2F-ara-A and areas under the plasma level time curves increased linearly with the dose, whereas half-lives, plasma clearance and volumes of distribution remained constant independent of the dose indicating a dose linear behaviour.

Elimination

2F-ara-A elimination is largely by renal excretion. 40 to 60 % of the administered intravenous dose was excreted in the urine. Mass balance studies in laboratory animals with ^{3}H-2F-ara-AMP showed a complete recovery of radio-labelled substances in the urine.

Characteristics in patients

Individuals with impaired renal function exhibited a reduced total body clearance, indicating the need for a dose reduction. In vitro investigations with human plasma proteins revealed no pronounced tendency of 2F-ara-A protein binding.

Cellular pharmacokinetics of fludarabine triphosphate

2F-ara-A is actively transported into leukaemic cells, whereupon it is rephosphorylated to the monophosphate and subsequently to the di- and triphosphate. The triphosphate 2F-ara-ATP is the major intracellular metabolite and the only metabolite known to have cytotoxic activity. Maximum 2F-ara-ATP levels in leukaemic lymphocytes of CLL patients were observed at a median of 4 hours and exhibited a considerable variation with a median peak concentration of approximately 20 µM. 2F-ara-ATP levels in leukaemic cells were always considerably higher than maximum 2F-ara-A levels in the plasma indicating an accumulation at the target sites. In-vitro incubation of leukaemic lymphocytes showed a linear relationship between extracellular 2F-ara-A exposure (product of 2F-ara-A concentration and duration of incubation) and intracellular 2F-ara-ATP enrichment. 2F-ara-ATP elimination from target cells showed median half-life values of 15 and 23 hours.

5.3 Preclinical safety data

Systemic toxicity

In acute toxicity studies, single doses of fludarabine phosphate produced severe intoxication symptoms or death at dosages about two orders of magnitude above the therapeutic dose. As expected for a cytotoxic compound, the bone marrow, lymphoid organs, gastrointestinal mucosa, kidneys and male gonads were affected. In patients, severe side effects were observed closer to the recommended therapeutic dose (factor 3 to 4) and included severe neurotoxicity partly with lethal outcome (see section 4.9).

Systemic toxicity studies following repeated administration of fludarabine phosphate showed also the expected effects on rapidly proliferating tissues above a threshold dose. The severity of morphological manifestations increased with dose levels and duration of dosing and the observed changes were generally considered to be reversible. In principle, the available experience from the therapeutic use of Fludara points to a comparable toxicological profile in humans, although additional undesirable effects such as neurotoxicity were observed in patients (see section 4.8).

Embryotoxicity

The results from intravenous animal embryotoxicity studies in rats and rabbits indicated an embryolethal and teratogenic potential of fludarabine phosphate as manifested in skeletal malformations, foetal weight loss and post implantation loss. In view of the small safety margin between the teratogenic doses in animals and the human therapeutic dose as well as in analogy to other antimetabolites which are assumed to interfere with the process of differentiation, the therapeutic use of Fludara is associated with a relevant risk of teratogenic effects in humans (see section 4.6).

conducted, because the suspicion of an increased risk of second tumours due to Fludara therapy can exclusively be verified by epidemiological data.

Local tolerance

According to the results from animal experiments following intravenous administration of fludarabine phosphate, no remarkable local irritation has to be expected at the injection site. Even in case of misplaced injections, no relevant local irritation was observed after paravenous, intraarterial, and intramuscular administration of an aqueous solution containing 7.5 mg fludarabine phosphate/ml.

The similarity in nature of the observed lesions in the gastrointestinal tract after intravenous or intragastric dosing in animal experiments supports the assumption that the fludarabine phosphate induced enteritis is a systemic effect.

6. Pharmaceutical particulars

6.1 List of excipients

Mannitol

Sodium hydroxide (to adjust the pH to 7.7).

6.2 Incompatibilities

In the absence of compatibility studies, this medicinal product must not be mixed with other medicinal products.

6.3 Shelf life

Unopened vial

3 years.

Reconstituted and diluted solution

Chemical and physical in-use stability after reconstitution has been demonstrated for 7 days at 4 °C.

From a microbiological point of view, the product should be used immediately. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and should not be longer than 24 hours at 2 °C to 8 °C or 8 hours at room temperature

6.4 Special precautions for storage

This medicinal product does not require any special storage conditions.

For storage conditions of the reconstituted or diluted medicinal product, see section 6.3.

6.5 Nature and contents of container

10 ml colourless Type I glass vials containing 50 mg fludarabine phosphate.

Pack size: 5 vials per carton.

6.6 Special precautions for disposal and other handling

Reconstitution

Fludara should be prepared for parenteral use by aseptically adding sterile water for injection. When reconstituted with 2 ml of sterile water for injection, the powder should fully dissolve in 15 seconds or less. Each ml of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide (to adjust the pH to 7.7). The pH range for the final product is 7.2 - 8.2.

Dilution

The reconstituted solution is clear and colourless. It should be visually inspected before use.

Only clear and colourless solutions without particles should be used. Fludara should not be used in case of a defective container.

Handling and disposal

Fludara should not be handled by pregnant staff.

Procedures for proper handling should be followed according to local requirements for cytotoxic drugs.

Caution should be exercised in the handling and preparation of the Fludara solution. The use of latex gloves and safety glasses is recommended to avoid exposure in case of breakage of the vial or other accidental spillage. If the solution comes into contact with the skin or mucous membranes, the area should be washed thoroughly with soap and water. In the event of contact with the eyes, rinse them thoroughly with copious amounts of water. Exposure by inhalation should be avoided.

The medicinal product is for single use only. Any unused medicinal product, spillage or waste material should be disposed of in accordance with local requirements.

7. Marketing authorisation holder

Genzyme Europe B.V.

Paasheuvelweg 25

1105 BP Amsterdam

The Netherlands

8. Marketing authorisation number(s)

PL 12375/0039

9. Date of first authorisation/renewal of the authorisation

Date of first authorisation: 11 August 1994

Date of last renewal: 07 August 2009

10. Date of revision of the text

10 March 2019

LEGAL CLASSIFICATION

POM

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Appendix 10

Summary of product characteristics for proleukin

PROLEUKIN® (aldesleukin)

for injection, for intravenous infusion

Rx Only

WARNINGS

Therapy with Proleukin® (aldesleukin) should be restricted to patients with normal cardiac and pulmonary functions as defined by thallium stress testing and formal pulmonary function testing. Extreme caution should be used in patients with a normal thallium stress test and a normal pulmonary function test who have a history of cardiac or pulmonary disease.

Proleukin should be administered in a hospital setting under the supervision of a qualified physician experienced in the use of anticancer agents. An intensive care facility and specialists skilled in cardiopulmonary or intensive care medicine must be available.

Proleukin administration has been associated with capillary leak syndrome (CLS) which is characterized by a loss of vascular tone and extravasation of plasma proteins and fluid into the extravascular space. CLS results in hypotension and reduced organ perfusion which may be severe and can result in death. CLS may be associated with cardiac arrhythmias (supraventricular and ventricular), angina, myocardial infarction, respiratory insufficiency requiring intubation, gastrointestinal bleeding or infarction, renal insufficiency, edema, and mental status changes.

Proleukin treatment is associated with impaired neutrophil function (reduced chemotaxis) and with an increased risk of disseminated infection, including sepsis and bacterial endocarditis. Consequently, preexisting bacterial infections should be adequately treated prior to initiation of Proleukin therapy. Patients with indwelling central lines are particularly at risk for infection with gram positive microorganisms. Antibiotic prophylaxis with oxacillin, nafcillin, ciprofloxacin, or vancomycin has been associated with a reduced incidence of staphylococcal infections.

Proleukin administration should be withheld in patients developing moderate to severe lethargy or somnolence; continued administration may result in coma.

DESCRIPTION

Proleukin® (aldesleukin), a human recombinant interleukin-2 product, is a highly purified protein with a molecular weight of approximately 15,300 daltons. The chemical name is des-alanyl-1, serine-125 human interleukin-2. Proleukin, a lymphokine, is produced by recombinant DNA technology using a genetically engineered *E. coli* strain containing an analog of the human interleukin-2 gene. Genetic engineering techniques were used to modify the human IL-2 gene, and the resulting expression clone encodes a modified human interleukin-2. This recombinant form differs from native interleukin-2 in the following ways: a) Proleukin is not glycosylated because it is derived from *E. coli* ; b) the molecule has no N-terminal alanine; the codon for this amino acid was deleted during the genetic engineering procedure; c) the molecule has serine substituted for cysteine at amino acid position 125; this was accomplished by site specific manipulation during the genetic engineering procedure; and d) the aggregation state of Proleukin is likely to be different from that of native interleukin-2.

The *in vitro* biological activities of the native nonrecombinant molecule have been reproduced with Proleukin.[1,2]

Proleukin is supplied as a sterile, white to off-white, lyophilized cake in single-use vials intended for intravenous administration. When reconstituted with 1.2 mL Sterile Water for Injection, USP, each mL contains 18 million International Units (1.1 mg) Proleukin, 50 mg mannitol, and 0.18 mg sodium dodecyl sulfate, buffered with approximately 0.17 mg monobasic and 0.89 mg dibasic sodium phosphate to a pH of 7.5 (range 7.2 to 7.8). The manufacturing process for Proleukin involves fermentation in a defined medium containing tetracycline hydrochloride. The presence of the antibiotic is not detectable in the final product. Proleukin contains no preservatives in the final product.

Proleukin biological potency is determined by a lymphocyte proliferation bioassay and is expressed in International Units as established by the World Health Organization 1st International Standard for Interleukin-2 (human). The relationship between potency and protein mass is as follows:

18 million International Units Proleukin = 1.1 mg protein

CLINICAL PHARMACOLOGY

Proleukin® (aldesleukin) has been shown to possess the biological activities of human native interleukin-2.[1,2] *In vitro* studies performed on human cell lines demonstrate the immunoregulatory properties of Proleukin, including: a) enhancement of lymphocyte mitogenesis and stimulation of long-term growth of human interleukin-2 dependent cell lines; b) enhancement of lymphocyte cytotoxicity; c) induction of killer cell (lymphokine-activated (LAK) and natural (NK)) activity; and d) induction of interferon-gamma production.

The *in vivo* administration of Proleukin in animals and humans produces multiple immunological effects in a dose dependent manner. These effects include activation of cellular immunity with profound lymphocytosis, eosinophilia, and thrombocytopenia, and the production of cytokines including tumor necrosis factor, IL-1 and gamma interferon.[3] *In vivo* experiments in murine tumor models have shown inhibition of tumor growth.[4] The exact mechanism by which Proleukin mediates its antitumor activity in animals and humans is unknown.

Pharmacokinetics

Proleukin exists as biologically active, non-covalently bound microaggregates with an average size of 27 recombinant interleukin-2 molecules. The solubilizing agent, sodium dodecyl sulfate, may have an effect on the kinetic properties of this product.

The pharmacokinetic profile of Proleukin is characterized by high plasma concentrations following a short intravenous infusion, rapid distribution into the extravascular space and elimination from the body by metabolism in the kidneys with little or no bioactive protein excreted in the urine. Studies of intravenous Proleukin in sheep and humans indicate that upon completion of infusion, approximately 30% of the administered dose is detectable in plasma. This finding is consistent with studies in rats using radiolabeled Proleukin, which demonstrate a rapid (<1 min) uptake of the majority of the label into the lungs, liver, kidney, and spleen.

The serum half-life (T 1/2) curves of Proleukin remaining in the plasma are derived from studies done in 52 cancer patients following a 5-minute intravenous infusion. These patients were shown to have a distribution and elimination T 1/2 of 13 and 85 minutes, respectively.

Following the initial rapid organ distribution, the primary route of clearance of circulating Proleukin is the kidney. In humans and animals, Proleukin is cleared from the circulation by both glomerular filtration and peritubular extraction in the kidney.[5-8] This dual mechanism for delivery of Proleukin to the proximal tubule may account for the preservation of clearance in patients with rising serum creatinine values. Greater than 80% of the amount of Proleukin distributed to plasma, cleared from the circulation and presented to the kidney is metabolized to amino acids in the cells lining the proximal convoluted tubules. In humans, the mean clearance rate in cancer patients is 268 mL/min.

The relatively rapid clearance of Proleukin has led to dosage schedules characterized by frequent, short infusions. Observed serum levels are proportional to the dose of Proleukin.

CLINICAL STUDIES

Safety and efficacy were studied in a series of single and multicenter, historically controlled studies enrolling a total of 525 patients with metastatic renal cell carcinoma or melanoma. Eligible patients had an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) of 0 or 1 and normal organ function as determined by cardiac stress test, pulmonary function tests, and creatinine ≤1.5 mg/dL. Studies excluded patients with brain metastases, active infections, organ allografts and diseases requiring steroid treatment.

The same treatment dose and schedule was employed in all studies demonstrating efficacy. Proleukin was given by 15 min intravenous infusion every 8 hours for up to 5 days (maximum of 14 doses). No treatment was given on days 6 to 14 and then dosing was repeated for up to 5 days on days 15 to 19 (maximum of 14 doses). These 2 cycles constituted 1 course of therapy. Patients could receive a maximum of 28 doses during a course of therapy. In practice >90% of patients had doses withheld. Doses were withheld for specific toxicities (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection and "**ADVERSE REACTIONS**" section).

Metastatic Renal Cell Cancer

Two hundred fifty-five patients with metastatic renal cell cancer (metastatic RCC) were treated with single agent Proleukin in 7 clinical studies conducted at 21 institutions. Metastatic RCC patients received a median of 20 of 28 scheduled doses of Proleukin.

In the renal cell cancer studies (n=255), objective response was seen in 37 (15%) patients, with 17 (7%) complete and 20 (8%) partial responders (See Table I). The 95% confidence interval for objective response was 11% to 20%. Onset of tumor regression was observed as early as 4 weeks after completion of the first course of treatment, and in some cases, tumor regression continued for up to 12 months after the start of treatment. Responses were observed in both lung and non-lung sites (e.g., liver, lymph node, renal bed occurrences, soft tissue). Responses were also observed in patients with individual bulky lesions and high tumor burden.

TABLE 1: Proleukin Clinical Response Data

	Number of Responding Patients (response rate)	**Median Response Duration in Months (range)**
Metastatic RCC		
CR's	17 (7%)	80+* (7 to 131+)
PR's	20 (8%)	20 (3 to 126+)
PR's + CR's	37 (15%)	54 (3 to 131+)

(+) sign means ongoing

* Median duration not yet observed; a conservative value is presented which represents the minimum median duration of response.

Lack of efficacy with low dose Proleukin regimens

Sixty-five patients with metastatic renal cell cancer were enrolled in a single center, open label, non-randomized trial that sequentially evaluated the safety and anti-tumor activity of two low dose Proleukin regimens. The regimens administered 18 million International Units Proleukin as a single subcutaneous injection, daily for 5 days during week 1; Proleukin was then administered at 9 $x10^6$ International Units days 1-2 and 18 $x10^6$ International Units days 3-5, weekly for an additional 3 weeks (n=40) followed by a 2 week rest or 5 weeks (n=25) followed by a 3 week rest, for a maximum of 3 or 2 treatment cycles, respectively.

These low dose regimens yielded substantially lower and less durable responses than those observed with the approved regimen. Based on the level of activity, these low dose regimens are not effective.

Metastatic Melanoma

Two hundred seventy patients with metastatic melanoma were treated with single agent Proleukin in 8 clinical studies conducted at 22 institutions. Metastatic melanoma patients received a median of 18 of 28 scheduled doses of Proleukin during the first course of therapy. In the metastatic melanoma studies (n=270), objective response was seen in 43 (16%) patients, with 17 (6%) complete and 26 (10%) partial responders (See Table II). The 95% confidence interval for objective response was 12% to 21%. Responses in metastatic melanoma patients were observed in both visceral and non-visceral sites (e.g., lung, liver, lymph node, soft tissue, adrenal, subcutaneous). Responses were also observed in patients with individual bulky lesions and large cumulative tumor burden.

TABLE 2: Proleukin CLINICAL RESPONSE DATA

	Number of Responding Patients (response rate)	Median Response Duration in Months (range)
Metastatic Melanoma		
CR's	17 (6%)	59+* (3 to 122+)
PR's	26 (10%)	6 (1 to 111+)
PR's + CR's	43 (16%)	9 (1 to 122+)

(+) sign means ongoing

* Median duration not yet observed; a conservative value is presented which represents the minimum median duration of response.

INDICATIONS AND USAGE

Proleukin® (aldesleukin) is indicated for the treatment of adults with metastatic renal cell carcinoma (metastatic RCC).

Proleukin is indicated for the treatment of adults with metastatic melanoma.

Careful patient selection is mandatory prior to the administration of Proleukin. See "**CONTRAINDICATIONS**", "**WARNINGS**" and "**PRECAUTIONS**" sections regarding patient screening, including recommended cardiac and pulmonary function tests and laboratory tests.

Evaluation of clinical studies to date reveals that patients with more favorable ECOG performance status (ECOG PS 0) at treatment initiation respond better to Proleukin, with a higher response rate and lower toxicity (See "**CLINICAL PHARMACOLOGY**" section, "**CLINICAL STUDIES**" section and "**ADVERSE REACTIONS**" section). Therefore, selection of patients for treatment should include assessment of performance status.

Experience in patients with ECOG PS >1 is extremely limited.

CONTRAINDICATIONS

Proleukin® (aldesleukin) is contraindicated in patients with a known history of hypersensitivity to interleukin-2 or any component of the Proleukin formulation.

Proleukin is contraindicated in patients with an abnormal thallium stress test or abnormal pulmonary function tests and those with organ allografts. Retreatment with Proleukin is contraindicated in patients who have experienced the following drug-related toxicities while receiving an earlier course of therapy:

- Sustained ventricular tachycardia (≥5 beats)
- Cardiac arrhythmias not controlled or unresponsive to management
- Chest pain with ECG changes, consistent with angina or myocardial infarction
- Cardiac tamponade
- Intubation for >72 hours

- Renal failure requiring dialysis >72 hours
- Coma or toxic psychosis lasting >48 hours
- Repetitive or difficult to control seizures
- Bowel ischemia/perforation
- GI bleeding requiring surgery

WARNINGS

See boxed "**WARNINGS**"

Because of the severe adverse events which generally accompany Proleukin® (aldesleukin) therapy at the recommended dosages, thorough clinical evaluation should be performed to identify patients with significant cardiac, pulmonary, renal, hepatic, or CNS impairment in whom Proleukin is contraindicated. Patients with normal cardiovascular, pulmonary, hepatic, and CNS function may experience serious, life threatening or fatal adverse events. Adverse events are frequent, often serious, and sometimes fatal.

Should adverse events, which require dose modification occur, dosage should be withheld rather than reduced (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection).

Proleukin has been associated with exacerbation of pre-existing or initial presentation of autoimmune disease and inflammatory disorders. Exacerbation of Crohn's disease, scleroderma, thyroiditis, inflammatory arthritis, diabetes mellitus, oculo-bulbar myasthenia gravis, crescentic IgA glomerulonephritis, cholecystitis, cerebral vasculitis, Stevens-Johnson syndrome and bullous pemphigoid, has been reported following treatment with IL-2.

All patients should have thorough evaluation and treatment of CNS metastases and have a negative scan prior to receiving Proleukin therapy. New neurologic signs, symptoms, and anatomic lesions following Proleukin therapy have been reported in patients without evidence of CNS metastases. Clinical manifestations included changes in mental status, speech difficulties, cortical blindness, limb or gait ataxia, hallucinations, agitation, obtundation, and coma. Radiological findings included multiple and, less commonly, single cortical lesions on MRI and evidence of demyelination. Neurologic signs and symptoms associated with Proleukin therapy usually improve after discontinuation of Proleukin therapy; however, there are reports of permanent neurologic defects. One case of possible cerebral vasculitis, responsive to dexamethasone, has been reported. In patients with known seizure disorders, extreme caution should be exercised as Proleukin may cause seizures.

PRECAUTIONS

General

Patients should have normal cardiac, pulmonary, hepatic, and CNS function at the start of therapy. (See "**PRECAUTIONS**" section, "**Laboratory Tests**" subsection). Capillary leak syndrome (CLS) begins immediately after Proleukin® (aldesleukin) treatment starts and is marked by increased capillary permeability to protein and fluids and reduced vascular tone. In most patients, this results in a concomitant drop in mean arterial blood pressure within 2 to 12 hours after the start of treatment. With continued therapy, clinically significant hypotension (defined as systolic blood pressure below 90 mm Hg or a 20 mm Hg drop from baseline systolic pressure) and hypoperfusion will occur. In addition, extravasation of protein and fluids

into the extravascular space will lead to the formation of edema and creation of new effusions.

Medical management of CLS begins with careful monitoring of the patient's fluid and organ perfusion status. This is achieved by frequent determination of blood pressure and pulse, and by monitoring organ function, which includes assessment of mental status and urine output. Hypovolemia is assessed by catheterization and central pressure monitoring.

Flexibility in fluid and pressor management is essential for maintaining organ perfusion and blood pressure. Consequently, extreme caution should be used in treating patients with fixed requirements for large volumes of fluid (e.g., patients with hypercalcemia). Administration of IV fluids, either colloids or crystalloids is recommended for treatment of hypovolemia. Correction of hypovolemia may require large volumes of IV fluids but caution is required because unrestrained fluid administration may exacerbate problems associated with edema formation or effusions. With extravascular fluid accumulation, edema is common and ascites, pleural or pericardial effusions may develop. Management of these events depends on a careful balancing of the effects of fluid shifts so that neither the consequences of hypovolemia (e.g., impaired organ perfusion) nor the consequences of fluid accumulations (e.g., pulmonary edema) exceed the patient's tolerance.

Clinical experience has shown that early administration of dopamine (1 to 5 mcg/kg/min) to patients manifesting capillary leak syndrome, before the onset of hypotension, can help to maintain organ perfusion particularly to the kidney and thus preserve urine output. Weight and urine output should be carefully monitored. If organ perfusion and blood pressure are not sustained by dopamine therapy, clinical investigators have increased the dose of dopamine to 6 to 10 mcg/kg/min or have added phenylephrine hydrochloride (1 to 5 mcg/kg/min) to low dose dopamine (See "**ADVERSE REACTIONS**" section). Prolonged use of pressors, either in combination or as individual agents, at relatively high doses, may be associated with cardiac rhythm disturbances. If there has been excessive weight gain or edema formation, particularly if associated with shortness of breath from pulmonary congestion, use of diuretics, once blood pressure has normalized, has been shown to hasten recovery. **NOTE: Prior to the use of any product mentioned, the physician should refer to the package insert for the respective product.**

Proleukin® (aldesleukin) treatment should be withheld for failure to maintain organ perfusion as demonstrated by altered mental status, reduced urine output, a fall in the systolic blood pressure below 90 mm Hg or onset of cardiac arrhythmias (See "**DOSAGE AND ADMINISTRATION**" section, "**Dose Modifications**" subsection). Recovery from CLS begins soon after cessation of Proleukin therapy. Usually, within a few hours, the blood pressure rises, organ perfusion is restored and reabsorption of extravasated fluid and protein begins.

Kidney and liver function are impaired during Proleukin treatment. Use of concomitant nephrotoxic or hepatotoxic medications may further increase toxicity to the kidney or liver.

Mental status changes including irritability, confusion, or depression which occur while receiving Proleukin may be indicators of bacteremia or early bacterial sepsis, hypoperfusion, occult CNS malignancy, or direct Proleukin-induced CNS toxicity. Alterations in mental status due solely to Proleukin therapy may progress for several days before recovery begins. Rarely, patients have sustained permanent neurologic deficits (See "**PRECAUTIONS**" section "**Drug Interactions**" subsection).

Exacerbation of pre-existing autoimmune disease or initial presentation of autoimmune and inflammatory disorders has been reported following Proleukin alone or in combination with

interferon (See “**PRECAUTIONS**” section “**Drug Interactions**” subsection and “**ADVERSE REACTIONS**” section). Hypothyroidism, sometimes preceded by hyperthyroidism, has been reported following Proleukin treatment. Some of these patients required thyroid replacement therapy. Changes in thyroid function may be a manifestation of autoimmunity. Onset of symptomatic hyperglycemia and/or diabetes mellitus has been reported during Proleukin therapy.

Proleukin enhancement of cellular immune function may increase the risk of allograft rejection in transplant patients.

Serious Manifestations of Eosinophilia

Serious manifestations of eosinophilia involving eosinophilic infiltration of cardiac and pulmonary tissues can occur following Proleukin.

Laboratory Tests

The following clinical evaluations are recommended for all patients, prior to beginning treatment and then daily during drug administration.

- Standard hematologic tests-including CBC, differential and platelet counts
- Blood chemistries-including electrolytes, renal and hepatic function tests
- Chest x-rays

Serum creatinine should be ≤1.5 mg/dL prior to initiation of Proleukin treatment.

All patients should have baseline pulmonary function tests with arterial blood gases. Adequate pulmonary function should be documented (FEV_1 >2 liters or ≥75% of predicted for height and age) prior to initiating therapy.

All patients should be screened with a stress thallium study. Normal ejection fraction and unimpaired wall motion should be documented. If a thallium stress test suggests minor wall motion abnormalities further testing is suggested to exclude significant coronary artery disease.

Daily monitoring during therapy with Proleukin should include vital signs (temperature, pulse, blood pressure, and respiration rate), weight, and fluid intake and output. In a patient with a decreased systolic blood pressure, especially less than 90 mm Hg, constant cardiac rhythm monitoring should be conducted. If an abnormal complex or rhythm is seen, an ECG should be performed. Vital signs in these hypotensive patients should be taken hourly.

During treatment, pulmonary function should be monitored on a regular basis by clinical examination, assessment of vital signs and pulse oximetry. Patients with dyspnea or clinical signs of respiratory impairment (tachypnea or rales) should be further assessed with arterial blood gas determination. These tests are to be repeated as often as clinically indicated.

Cardiac function should be assessed daily by clinical examination and assessment of vital signs. Patients with signs or symptoms of chest pain, murmurs, gallops, irregular rhythm or palpitations should be further assessed with an ECG examination and cardiac enzyme evaluation. Evidence of myocardial injury, including findings compatible with myocardial infarction or myocarditis, has been reported. Ventricular hypokinesia due to myocarditis may be persistent for several months. If there is evidence of cardiac ischemia or congestive heart failure, Proleukin therapy should be held, and a repeat thallium study should be done.

Drug Interactions

Proleukin may affect central nervous function. Therefore, interactions could occur following concomitant administration of psychotropic drugs (e.g., narcotics, analgesics, antiemetics, sedatives, tranquilizers).

Concurrent administration of drugs possessing nephrotoxic (e.g., aminoglycosides, indomethacin), myelotoxic (e.g., cytotoxic chemotherapy), cardiotoxic (e.g., doxorubicin) or hepatotoxic (e.g., methotrexate, asparaginase) effects with Proleukin may increase toxicity in these organ systems. The safety and efficacy of Proleukin in combination with any antineoplastic agents have not been established.

In addition, reduced kidney and liver function secondary to Proleukin treatment may delay elimination of concomitant medications and increase the risk of adverse events from those drugs.

Hypersensitivity reactions have been reported in patients receiving combination regimens containing sequential high dose Proleukin and antineoplastic agents, specifically, dacarbazine, cis-platinum, tamoxifen and interferon-alfa. These reactions consisted of erythema, pruritus, and hypotension and occurred within hours of administration of chemotherapy. These events required medical intervention in some patients.

Myocardial injury, including myocardial infarction, myocarditis, ventricular hypokinesia, and severe rhabdomyolysis appear to be increased in patients receiving Proleukin and interferon-alfa concurrently.

Exacerbation or the initial presentation of a number of autoimmune and inflammatory disorders has been observed following concurrent use of interferon-alfa and Proleukin, including crescentic IgA glomerulonephritis, oculo-bulbar myasthenia gravis, inflammatory arthritis, thyroiditis, bullous pemphigoid, and Stevens-Johnson syndrome.

Although glucocorticoids have been shown to reduce Proleukin-induced side effects including fever, renal insufficiency, hyperbilirubinemia, confusion, and dyspnea, concomitant administration of these agents with Proleukin may reduce the antitumor effectiveness of Proleukin and thus should be avoided.[12]

Beta-blockers and other antihypertensives may potentiate the hypotension seen with Proleukin.

Delayed Adverse Reactions to Iodinated Contrast Media

A review of the literature revealed that 12.6% (range 11-28%) of 501 patients treated with various interleukin-2 containing regimens who were subsequently administered radiographic iodinated contrast media experienced acute, atypical adverse reactions. The onset of symptoms usually occurred within hours (most commonly 1 to 4 hours) following the administration of contrast media. These reactions include fever, chills, nausea, vomiting, pruritus, rash, diarrhea, hypotension, edema, and oliguria. Some clinicians have noted that these reactions resemble the immediate side effects caused by interleukin-2 administration, however the cause of contrast reactions after interleukin-2 therapy is unknown. Most events were reported to occur when contrast media was given within 4 weeks after the last dose of interleukin-2. These events were also reported to occur when contrast media was given several months after interleukin-2 treatment.[13]

Carcinogenesis, Mutagenesis, Impairment of Fertility

There have been no studies conducted assessing the carcinogenic or mutagenic potential of Proleukin.

There have been no studies conducted assessing the effect of Proleukin on fertility. It is recommended that this drug not be administered to fertile persons of either gender not practicing effective contraception.

Pregnancy

Pregnancy Category C.

Proleukin has been shown to have embryolethal effects in rats when given in doses at 27 to 36 times the human dose (scaled by body weight). Significant maternal toxicities were observed in pregnant rats administered Proleukin by IV injection at doses 2.1 to 36 times higher than the human dose during critical period of organogenesis. No evidence of teratogenicity was observed other than that attributed to maternal toxicity. There are no adequate well-controlled studies of Proleukin in pregnant women. Proleukin should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from Proleukin, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

Pediatric Use

Safety and effectiveness in children under 18 years of age have not been established.

Geriatric Use

There were a small number of patients aged 65 and over in clinical trials of Proleukin; experience is limited to 27 patients, eight with metastatic melanoma and nineteen with metastatic renal cell carcinoma. The response rates were similar in patients 65 years and over as compared to those less than 65 years of age. The median number of courses and the median number of doses per course were similar between older and younger patients.

Proleukin is known to be substantially excreted by the kidney, and the risk of toxic reactions to this drug may be greater in patients with impaired renal function. The pattern of organ system toxicity and the proportion of patients with severe toxicities by organ system were generally similar in patients 65 and older and younger patients. There was a trend, however, towards an increased incidence of severe urogenital toxicities and dyspnea in the older patients.

ADVERSE REACTIONS

The rate of drug-related deaths in the 255 metastatic RCC patients who received single-agent Proleukin® (aldesleukin) was 4% (11/255); the rate of drug-related deaths in the 270 metastatic melanoma patients who received single-agent Proleukin was 2% (6/270).

The following data on common adverse events (reported in greater than 10% of patients, any grade), presented by body system, decreasing frequency and by preferred term (COSTART)

are based on 525 patients (255 with renal cell cancer and 270 with metastatic melanoma) treated with the recommended infusion dosing regimen.

TABLE 3: ADVERSE EVENTS OCCURRING IN ≥10% OF PATIENTS (n=525)

Body System	% Patients	Body System	% Patients
Body as a Whole		**Metabolic and Nutritional Disorders**	
Chills	52	Bilirubinemia	40
Fever	29	Creatinine increase	33
Malaise	27	Peripheral edema	28
Asthenia	23	SGOT increase	23
Infection	13	Weight gain	16
Pain	12	Edema	15
Abdominal pain	11	Acidosis	12
Abdomen enlarged	10	Hypomagnesemia	12
Cardiovascular		Hypocalcemia	11
Hypotension	71	Alkaline phosphatase increase	10
Tachycardia	23	**Nervous**	
Vasodilation	13	Confusion	34
Supraventricular tachycardia	12	Somnolence	22
Cardiovascular disorder[a]	11	Anxiety	12
Arrhythmia	10	Dizziness	11
Digestive		**Respiratory**	
Diarrhea	67	Dyspnea	43
Vomiting	50	Lung disorder[b]	24
Nausea	35	Respiratory disorder[c]	11
Stomatitis	22	Cough increase	11
Anorexia	20	Rhinitis	10
Nausea and vomiting	19	**Skin and Appendages**	
Hemic and Lymphatic		Rash	42
Thrombocytopenia	37	Pruritus	24
Anemia	29	Exfoliative dermatitis	18
Leukopenia	16	**Urogenital**	
		Oliguria	63

[a] Cardiovascular disorder: fluctuations in blood pressure, asymptomatic ECG changes, CHF.

[b] Lung disorder: physical findings associated with pulmonary congestion, rales, rhonchi.

[c] Respiratory disorder: ARDS, CXR infiltrates, unspecified pulmonary changes.

The following data on life-threatening adverse events (reported in greater than 1% of patients, grade 4), presented by body system, and by preferred term (COSTART) are based on 525 patients (255 with renal cell cancer and 270 with metastatic melanoma) treated with the recommended infusion dosing regimen.

TABLE 4: LIFE-THREATENING (GRADE 4) ADVERSE EVENTS (n= 525)

Body System	# (%) Patients	Body System	# (%) Patients
Body as a Whole		**Metabolic and Nutritional Disorders**	
Fever	5 (1%)	Bilirubinemia	13 (2%)
Infection	7 (1%)	Creatinine increase	5 (1%)
Sepsis	6 (1%)	SGOT increase	3 (1%)
Cardiovascular		Acidosis	4 (1%)
Hypotension	15 (3%)	**Nervous**	
Supraventricular tachycardia	3 (1%)	Confusion	5 (1%)
Cardiovascular disorder[a]	7 (1%)	Stupor	3 (1%)
Myocardial infarct	7 (1%)	Coma	8 (2%)
Ventricular tachycardia	5 (1%)	Psychosis	7 (1%)
Cardiac arrest	4 (1%)	**Respiratory**	
Digestive		Dyspnea	5 (1%)
Diarrhea	10 (2%)	Respiratory disorder[c]	14 (3%)
Vomiting	7 (1%)	Apnea	5 (1%)
Hemic and Lymphatic		**Urogenital**	
Thrombocytopenia	5 (1%)	Oliguria	33 (6%)
Coagulation disorder[b]	4 (1%)	Anuria	25 (5%)
		Acute kidney failure	3 (1%)

[a] Cardiovascular disorder: fluctuations in blood pressure.

[b] Coagulation disorder: intravascular coagulopathy.

[c] Respiratory disorder: ARDS, respiratory failure, intubation.

The following life-threatening (grade 4) events were reported by <1% of the 525 patients: hypothermia; shock; bradycardia; ventricular extrasystoles; myocardial ischemia; syncope; hemorrhage; atrial arrhythmia; phlebitis; AV block second degree; endocarditis; pericardial

effusion; peripheral gangrene; thrombosis; coronary artery disorder; stomatitis; nausea and vomiting; liver function tests abnormal; gastrointestinal hemorrhage; hematemesis; bloody diarrhea; gastrointestinal disorder; intestinal perforation; pancreatitis; anemia; leukopenia; leukocytosis; hypocalcemia; alkaline phosphatase increase; BUN increase; hyperuricemia; NPN increase; respiratory acidosis; somnolence; agitation; neuropathy; paranoid reaction; convulsion; grand mal convulsion; delirium; asthma, lung edema; hyperventilation; hypoxia; hemoptysis; hypoventilation; pneumothorax; mydriasis; pupillary disorder; kidney function abnormal; kidney failure; acute tubular necrosis.

In an additional population of greater than 1,800 patients treated with Proleukin-based regimens using a variety of doses and schedules (e.g., subcutaneous, continuous infusion, administration with LAK cells) the following serious adverse events were reported: duodenal ulceration; bowel necrosis; myocarditis; supraventricular tachycardia; permanent or transient blindness secondary to optic neuritis; transient ischemic attacks; meningitis; cerebral edema; pericarditis; allergic interstitial nephritis; tracheo-esophageal fistula.

In the same clinical population, the following fatal events each occurred with a frequency of <1%: malignant hyperthermia; cardiac arrest; myocardial infarction; pulmonary emboli; stroke; intestinal perforation; liver or renal failure; severe depression leading to suicide; pulmonary edema; respiratory arrest; respiratory failure. In patients with both metastatic RCC and metastatic melanoma, those with ECOG PS of 1 or higher had a higher treatment-related mortality and serious adverse events.

Most adverse reactions are self-limiting and, usually, but not invariably, reverse or improve within 2 or 3 days of discontinuation of therapy. Examples of adverse reactions with permanent sequelae include: myocardial infarction, bowel perforation/infarction, and gangrene.

Immunogenicity

Serum samples from patients in the clinical studies were tested by enzyme-linked immunosorbent assay (ELISA) for anti-aldesleukin antibodies. Low titers of anti-aldesleukin antibodies were detected in 57 of 77 (74%) patients with metastatic renal cell carcinoma treated with an every 8-hour PROLEUKIN regimen and in 33 of 50 (66%) patients with metastatic melanoma treated with a variety of intravenous regimens. In a separate study, the effect of immunogenicity on the pharmacokinetics of aldesleukin was evaluated in 13 patients. Following the first cycle of therapy, comparing the geometric mean aldesleukin exposure (AUC) Day 15 to Day 1, there was an average 68% increase in 11 patients who developed anti-aldesleukin antibodies and no change was observed in the antibody-negative patients (n=2). Overall, neutralizing antibodies were detected in 1 patient. The impact of antialdesleukin antibody formation on clinical efficacy and safety of PROLEUKIN is unknown.

Immunogenicity assay results are highly dependent on several factors including assay sensitivity and specificity, assay methodology, sample handling, timing of sample collection, concomitant medications, and underlying disease. For these reasons, comparison of incidence of antibodies to PROLEUKIN with the incidence of antibodies to other products may be misleading.

Post Marketing Experience

The following adverse reactions have been identified during post-approval use of Proleukin. Because these reactions are reported voluntarily from a population of uncertain size, it is not

always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- Blood and lymphatic system: neutropenia, febrile neutropenia, eosinophilia, lymphocytopenia
- Cardiac: cardiomyopathy, cardiac tamponade
- Endocrine: hyperthyroidism
- Gastrointestinal: gastritis, intestinal obstruction, colitis
- General and administration site conditions: injection site necrosis
- Hepatobiliary: hepatitis, hepatosplenomegaly, cholecystitis
- Immune system: anaphylaxis, angioedema, urticaria
- Infections and infestations: pneumonia (bacterial, fungal, viral), fatal endocarditis, cellulitis
- Musculoskeletal and connective tissue: myopathy, myositis, rhabdomyolysis
- Nervous system: cerebral lesions, encephalopathy, extrapyramidal syndrome, neuralgia, neuritis, demyelinating neuropathy
- Psychiatric: insomnia
- Vascular: hypertension, fatal subdural and subarachnoid hemorrhage, cerebral hemorrhage, retroperitoneal hemorrhage

Exacerbation or initial presentation of a number of autoimmune and inflammatory disorders have been reported (See "**WARNINGS**" section, "**PRECAUTIONS**" section, "**Drug Interactions**" subsection). Persistent but nonprogressive vitiligo has been observed in malignant melanoma patients treated with interleukin-2. Synergistic, additive and novel toxicities have been reported with Proleukin used in combination with other drugs. Novel toxicities include delayed adverse reactions to iodinated contrast media and hypersensitivity reactions to antineoplastic agents (See "**PRECAUTIONS**" section, "**Drug Interactions**" subsection).

Experience has shown the following concomitant medications to be useful in the management of patients on Proleukin therapy: a) standard antipyretic therapy, including nonsteroidal anti-inflammatories (NSAIDs), started immediately prior to Proleukin to reduce fever. Renal function should be monitored as some NSAIDs may cause synergistic nephrotoxicity; b) meperidine used to control the rigors associated with fever; c) H_2 antagonists given for prophylaxis of gastrointestinal irritation and bleeding; d) antiemetics and antidiarrheals used as needed to treat other gastrointestinal side effects. Generally these medications were discontinued 12 hours after the last dose of Proleukin.

Patients with indwelling central lines have a higher risk of infection with gram positive organisms.[9-11] A reduced incidence of staphylococcal infections in Proleukin studies has been associated with the use of antibiotic prophylaxis which includes the use of oxacillin, nafcillin, ciprofloxacin, or vancomycin. Hydroxyzine or diphenhydramine has been used to control symptoms from pruritic rashes and continued until resolution of pruritus. Topical creams and ointments should be applied as needed for skin manifestations. Preparations containing a

steroid (e.g., hydrocortisone) should be avoided. **NOTE: Prior to the use of any product mentioned, the physician should refer to the package insert for the respective product.**

OVERDOSAGE

Side effects following the use of Proleukin® (aldesleukin) appear to be dose-related. Exceeding the recommended dose has been associated with a more rapid onset of expected dose-limiting toxicities. Symptoms which persist after cessation of Proleukin should be monitored and treated supportively. Life-threatening toxicities may be ameliorated by the intravenous administration of dexamethasone, which may also result in loss of the therapeutic effects of Proleukin.[12] **NOTE: Prior to the use of dexamethasone, the physician should refer to the package insert for this product.**

DOSAGE AND ADMINISTRATION

The recommended Proleukin® (aldesleukin) treatment regimen is administered by a 15-minute intravenous infusion every 8 hours. Before initiating treatment, carefully review the "**INDICATIONS AND USAGE**", "**CONTRAINDICATIONS**", "**WARNINGS**", "**PRECAUTIONS**", and "**ADVERSE REACTIONS**" sections, particularly regarding patient selection, possible serious adverse events, patient monitoring and withholding dosage. The following schedule has been used to treat adult patients with metastatic renal cell carcinoma (metastatic RCC) or metastatic melanoma. Each course of treatment consists of two 5-day treatment cycles separated by a rest period.

> 600,000 International Units/kg (0.037 mg/kg) dose administered every 8 hours by a 15-minute intravenous infusion for a maximum of 14 doses. Following 9 days of rest, the schedule is repeated for another 14 doses, for a maximum of 28 doses per course, as tolerated. During clinical trials, doses were frequently withheld for toxicity (See "**CLINICAL STUDIES**" section and "**Dose Modifications**" subsection). Metastatic RCC patients treated with this schedule received a median of 20 of the 28 doses during the first course of therapy. Metastatic melanoma patients received a median of 18 doses during the first course of therapy.

Retreatment

Patients should be evaluated for response approximately 4 weeks after completion of a course of therapy and again immediately prior to the scheduled start of the next treatment course. Additional courses of treatment should be given to patients only if there is some tumor shrinkage following the last course and retreatment is not contraindicated (See "**CONTRAINDICATIONS**" section). Each treatment course should be separated by a rest period of at least 7 weeks from the date of hospital discharge.

Dose Modifications

Dose modification for toxicity should be accomplished by withholding or interrupting a dose rather than reducing the dose to be given. Decisions to stop, hold, or restart Proleukin therapy must be made after a global assessment of the patient. With this in mind, the following guidelines should be used:

Retreatment with Proleukin is contraindicated in patients who have experienced the following toxicities:

Body System

Cardiovascular	Sustained ventricular tachycardia (≥5 beats)
	Cardiac rhythm disturbances not controlled or unresponsive to management
	Chest pain with ECG changes, consistent with angina or myocardial infarction
	Cardiac tamponade
Respiratory	Intubation for >72 hours
Urogenital	Renal failure requiring dialysis >72 hours
Nervous	Coma or toxic psychosis lasting >48 hours
	Repetitive or difficult to control seizures
Digestive	Bowel ischemia/perforation
	GI bleeding requiring surgery

Doses should be held and restarted according to the following:

Body System	Hold dose for	Subsequent doses may be given if
Cardiovascular	Atrial fibrillation, supraventricular tachycardia or bradycardia that requires treatment or is recurrent or persistent	Patient is asymptomatic with full recovery to normal sinus rhythm
	Systolic bp <90 mm Hg with increasing requirements for pressors	Systolic bp ≥90 mm Hg and stable or improving requirements for pressors
	Any ECG change consistent with MI, ischemia or myocarditis with or without chest pain; suspicion of cardiac ischemia	Patient is asymptomatic, MI and myocarditis have been ruled out, clinical suspicion of angina is low; there is no evidence of ventricular hypokinesia
Respiratory	O_2 saturation <90%	O_2 saturation >90%
Nervous	Mental status changes, including moderate confusion or agitation	Mental status changes completely resolved
Body as a Whole	Sepsis syndrome, patient is clinically unstable	Sepsis syndrome has resolved, patient is clinically stable, infection is under treatment
Urogenital	Serum creatinine >4.5 mg/dL or a serum creatinine of ≥4 mg/dL in the presence of severe volume overload, acidosis, or hyperkalemia	Serum creatinine <4 mg/dL and fluid and electrolyte status is stable
	Persistent oliguria, urine output of <10 mL/hour for 16 to 24 hours with rising serum creatinine	Urine output >10 mL/hour with a decrease of serum creatinine >1.5 mg/dL or normalization of serum creatinine
Digestive	Signs of hepatic failure including encephalopathy, increasing ascites, liver pain, hypoglycemia	All signs of hepatic failure have resolved*
	Stool guaiac repeatedly >3-4+	Stool guaiac negative
Skin	Bullous dermatitis or marked worsening of pre-existing skin condition, avoid	Resolution of all signs of bullous dermatitis

	topical steroid therapy	

* Discontinue all further treatment for that course. A new course of treatment, if warranted, should be initiated no sooner than 7 weeks after cessation of adverse event and hospital discharge.

Reconstitution and Dilution Directions: Reconstitution and dilution procedures other than those recommended may alter the delivery and/or pharmacology of Proleukin and thus should be avoided.

1. Proleukin® (aldesleukin) is a sterile, white to off-white, preservative-free, lyophilized powder suitable for IV infusion upon reconstitution and dilution. **EACH VIAL CONTAINS 22 MILLION International Units (1.3 mg) OF PROLEUKIN AND SHOULD BE RECONSTITUTED ASEPTICALLY WITH 1.2 mL OF STERILE WATER FOR INJECTION, USP. WHEN RECONSTITUTED AS DIRECTED, EACH mL CONTAINS 18 MILLION International Units (1.1 mg) OF PROLEUKIN.** The resulting solution should be a clear, colorless to slightly yellow liquid. The vial is for single-use only and any unused portion should be discarded.
2. During reconstitution, the Sterile Water for Injection, USP should be directed at the side of the vial and the contents gently swirled to avoid excess foaming. DO NOT SHAKE.
3. The dose of Proleukin, reconstituted with Sterile Water for Injection, USP (without preservative) should be diluted aseptically in 50 mL of 5% Dextrose Injection, USP (D5W) and infused over a 15-minute period.

   In cases where the total dose of Proleukin is 1.5 mg or less (e.g., a patient with a body weight of less than 40 kilograms), the dose of Proleukin should be diluted in a smaller volume of D5W. Concentrations of Proleukin below 0.03 mg/mL and above 0.07 mg/mL have shown increased variability in drug delivery. Dilution and delivery of Proleukin outside of this concentration range should be avoided.
4. Glass bottles and plastic (polyvinyl chloride) bags have been used in clinical trials with comparable results. It is recommended that plastic bags be used as the dilution container since experimental studies suggest that use of plastic containers results in more consistent drug delivery. **In-line filters should not be used when administering Proleukin.**
5. Before and after reconstitution and dilution, store in a refrigerator at 2° to 8°C (36° to 46°F). Do not freeze. Administer Proleukin within 48 hours of reconstitution. The solution should be brought to room temperature prior to infusion in the patient.
6. Reconstitution or dilution with Bacteriostatic Water for Injection, USP, or 0.9% Sodium Chloride Injection, USP should be avoided because of increased aggregation. Proleukin should not be coadministered with other drugs in the same container.
7. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

HOW SUPPLIED

Proleukin® (aldesleukin) is supplied in individually boxed single-use vials. Each vial contains 22 million International Units of Proleukin. Discard unused portion.

NDC 65483-116-07 Individually boxed single-use vial

Store vials of lyophilized Proleukin in a refrigerator at 2° to 8°C (36° to 46°F). PROTECT FROM LIGHT. Store in carton until time of use.

Reconstituted or diluted Proleukin is stable for up to 48 hours at refrigerated and room temperatures, 2° to 25°C (36° to 77°F). However, since this product contains no preservative, the reconstituted and diluted solutions should be stored in the refrigerator.

Do not use beyond the expiration date printed on the vial. **NOTE:** This product contains no preservative.

Rx Only

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