Innovation, Sciences et Développement économique Canada
Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3127997 A1 2020/08/06

(21) 3 127 997

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/01/28
(87) Date publication PCT/PCT Publication Date: 2020/08/06
(85) Entrée phase nationale/National Entry: 2021/07/27
(86) N° demande PCT/PCT Application No.: NL 2020/050043
(87) N° publication PCT/PCT Publication No.: 2020/159361
(30) Priorités/Priorities: 2019/01/28 (NL2022464); 2019/12/19 (NL2024515)

(51) Cl.Int./Int.Cl. *A61K 39/00* (2006.01), *A61P 35/00* (2006.01), *C07K 16/00* (2006.01)

(71) Demandeur/Applicant:
AMPHERA B.V., NL

(72) Inventeurs/Inventors:
AERTS, JOACHIM, NL;
DAMMEIJER, FLORIS, NL

(74) Agent: GOWLING WLG (CANADA) LLP

(54) Titre : COMPOSITION PHARMACEUTIQUE DESTINEE A ETRE UTILISEE DANS LE TRAITEMENT DU CANCER DU PANCREAS

(54) Title: PHARMACEUTICAL COMPOSITION FOR USE IN THE TREATMENT OF PANCREATIC CANCER

(57) Abrégé/Abstract:
The present invention relates to a tumour lysate and a pharmaceutical composition for use in the treatment of pancreatic cancer. In one aspect, the invention relates to pharmaceutical composition comprising dendritic cells, loaded with said lysate. In one aspect, the pharmaceutical composition is for use in the treatment of pancreatic cancer.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
06 August 2020 (06.08.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/159361 A1

(51) International Patent Classification:
A61K 39/00 (2006.01) *C07K 16/00* (2006.01)
A61P 35/00 (2006.01)

(21) International Application Number:
PCT/NL2020/050043

(22) International Filing Date:
28 January 2020 (28.01.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
2022464 28 January 2019 (28.01.2019) NL
2024515 19 December 2019 (19.12.2019) NL

(71) Applicant: **AMPHERA B.V.** [NL/NL]; Onderwijsboulevard 225, 5223 DE 's Hertogenbosch (NL).

(72) Inventors: **AERTS, Joachim**; Dr. Molewaterplein 40, 3015 GD Rotterdam (NL). **DAMMEIJER, Floris**; Dr. Molewaterplein 40, 3015 GD Rotterdam (NL).

(74) Agent: **NEDERLANDSCH OCTROOIBUREAU**; P.O.Box 29720, 2502 LS The Hague (NL).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*

WO 2020/159361 A1

(54) Title: PHARMACEUTICAL COMPOSITION FOR USE IN THE TREATMENT OF PANCREATIC CANCER

(57) Abstract: The present invention relates to a tumour lysate and a pharmaceutical composition for use in the treatment of pancreatic cancer. In one aspect, the invention relates to pharmaceutical composition comprising dendritic cells, loaded with said lysate. In one aspect, the pharmaceutical composition is for use in the treatment of pancreatic cancer.

PHARMACEUTICAL COMPOSITION FOR USE IN THE TREATMENT OF PANCREATIC CANCER

FIELD OF THE INVENTION

The present invention relates to a tumour lysate and a pharmaceutical composition for use in the treatment of pancreatic cancer. In one aspect, the invention relates to pharmaceutical composition comprising dendritic cells, loaded with said lysate, for use in the treatment of pancreatic cancer.

BACKGROUND OF THE INVENTION

The annual incidence of patients developing pancreatic cancer in the Netherlands is approximately 3500 (1). In 2020, pancreatic cancer is expected to be the second leading cause of cancer-related death (2). The 1-year overall survival (OS) for pancreatic cancer in the Netherlands is 20%; 5-year OS is only 3% (3). The vast majority of patients presents with either locally advanced or metastatic disease, which excludes them from curative surgery. Only 15-25% of all pancreatic cancer patients are eligible to undergo surgical resection (4). However, ten years after resection, OS is still only 4%, demonstrating that cure is rare (5). Apparently, the vast majority of patients with (borderline) resectable pancreatic cancer according to imaging techniques have occult metastatic disease, without further treatment the progression free period for such patients is only about 6 months (66). Even with the new regimens of chemotherapy, long-term survival is still exceptional. It was found that adjuvant chemotherapy with gemcitabine resulted in a disease free survival after resective surgery of 13.4 months and a median overall survival of 22.8 months (66). It has further found that treatment with folfirinox after surgical resection results in a median disease free survival of 21.6 months and an overall survival of 54.4 months (67).

Since recurrence rates are extremely high after resection, a need remains for new treatments in order to curb the progression of pancreatic cancer and increase the progression free survival and overall survival of patients suffering from pancreatic cancer.

The potential to harness the potency and the specificity of the immune system underlies the growing interest in cancer immunotherapy. One approach to activate the patient's immune system uses dendritic cell based immunotherapy. Dendritic cell based immunotherapy aims to boost the immune system of cancer patients by enhancing tumour

antigen recognition by activating cytotoxic T-cells and thus generating anti-tumour specific responses.

In this regard it is well known that dendritic cells are highly mobile and extremely potent antigen presenting cells located at strategic places where the body comes in contact with its environment. In these locations they pick up antigens and transport them to the secondary lymphoid organs where they instruct and control activation of natural killer cells, B and T-lymphocytes, and efficiently activate them against the antigens. This property makes them attractive candidates for use in therapeutic strategies against cancer. Furthermore, dendritic cells can be generated in large numbers *ex vivo*.

Cancer induces a highly immunosuppressive tumour microenvironment (TME) leading to the dysfunction of multiple immune effector cells (8, 9). For instance, cytokines related to anti-inflammatory Th2 phenotype and immune-suppressive regulatory T cells are elevated in peripheral blood in patients with pancreatic cancer compared to healthy controls (10, 11), whereas the accumulation of cytotoxic CD8 T cells is lagging behind (12). This causes a non-cytotoxic T-cell infiltrated tumour, and may explain the low response rate of immune checkpoint antibodies like PD-1/PD-L1 (13). In pancreatic cancer, early trials indeed show disappointing results with these antibodies, pointing to the need for a more basal activation of the immune system (14-16). The induction of robust immune effector cells could enhance CD8 T cell infiltration and shift the balance in favour of an anti-cancer response.

One approach to activate the patient's immune system and induce tumour directed cytotoxic T-cells is by using cancer vaccines. Cancer vaccines have yielded promising results in several preclinical and clinical studies (17). In complex immunological tumours, cellular therapies seem more effective than other types of vaccination (18). Various types of cellular vaccinations have been tested in pancreatic cancer in the setting of phase I or II trials. Below, we will discuss the most promising therapy types in pancreatic cancer (i.e. tumour cell-based vaccination, adoptive T-cell transfer and dendritic cell vaccination).

Tumour cell-based vaccines

In pancreatic cancer only two types of tumour cell-based vaccines (without adoptive cell transfer) are currently known. Their goal is to prime a robust immune response by activating different immune effector cells. Algenpantucel-L consists of two irradiated human pancreatic cancer cell lines (HAPa-1 and HAPa-2) which express the murine enzyme α-1,3-galactosyl transferase (α-GT)(19). While two phase III clinical trials with Algenpantucel-L are still ongoing, a recent press release announced failed improvement of OS of Algenpantucel-L versus standard of care in one of these phase III clinical trials. Median OS in the intervention

group was 27.3 months while the control group with standard of care showed a median OS of 30.4 months (20).

The second tumour cell-based vaccine tested in pancreatic cancer patients is GVAX. The GVAX vaccine is based on irradiated tumour cells modified to express granulocyte-macrophage colony-stimulating factor (GM-CSF) (21, 22). This is combined with CRS-207, *Listeria monocytogenes* engineered to express mesothelin. Some patients treated with GVAX/ CRS-207 and radiochemotherapy developed an immune response against mesothelin and showed an increase in progression free survival and OS (21, 23). However, the phase 2b trial ECLIPSE did not meet the primary endpoint of an improvement of OS for patients with pancreatic cancer (24).

Adoptive T cell transfer

Tumour-specific effector CD8+ T cells are considered to be the final, and vital, step in immune-mediated cancer eradication. Therefore, adoptive cell transfer (ACT) with effector T cells has been developed which includes tumour-infiltrating lymphocytes (TIL) therapy and receptor-engineered T cell therapy (25). However, widespread clinical use of TILs in solid tumours is limited due to practical barriers. Especially in pancreatic cancer harvesting of tumour cells is extremely challenging due to the prominent desmoplastic stroma present in pancreatic cancer (26, 27). To date, no clinical trial with TIL therapy has been performed in pancreatic cancer patients. Furthermore, lymphocytes can be engineered by introducing genes encoding for anti-tumour alpha-beta T cell receptors (TCRs) or chimeric antigen receptors (CARs) into mature T cells. (28). However, there are some concerns and weaknesses concerning TCR and CAR T-cell therapy. ACT with effector T cells bears the risk of toxicity when targeting antigens are shared by tumours and normal tissue, or when target antigens are highly similar to self-antigens. (29-31) Unexpected lethal toxicities have been observed in a number of trials due to previously unknown cross-reactivity (32 - 34). Furthermore, results in solid tumours are less encouraging due to the presence of an immune-suppressive micro-environment that may adversely affect recruitment and activation of adoptive CD8 T cells (35).

Dendritic cell vaccination

Dendritic cells (DCs) are the most potent activators of the immune system and play a fundamental role in the effectiveness of cancer vaccines (36). DCs can capture, process and present tumour associated antigens (TAAs) in context of a Major Histocompatibility Complex (MHC) Class I or II (37). Subsequently, DCs can prime naive T cells, memory T cells and B cells which are needed for the induction of a robust anti-cancer response (38, 39). DCs pulsed with TAAs have shown beneficial effect in tumour animal models (40, 41) where they

were shown to be essential in eliciting a vigorous anti-cancer response. Clinical studies have shown the safety and efficacy of DC immunotherapy (42, 43). Safety of DC-based immunotherapy in patients with pancreatic cancer was studied in several phase I and II studies. Until now, about 20 clinical DC immunotherapy trials in pancreatic cancer have been performed worldwide. DCs were pulsed with TAAs such as Wilms' tumour 1 (WT-1), MUC-1, carcino-embryonic antigen (CEA), survivin, human telomerase reverse transcriptase (hTERT) or autologous tumour material (44-51).

SUMMARY OF THE INVENTION

For at least some of the reasons set out above, a need remains for an efficient curative, palliative, or preventive treatment of cancer in general and pancreatic cancer in particular. The current invention provides such treatment for pancreatic cancer by means of treatment of such patients with lysate loaded dendritic cells.

A first aspect of the present invention relates to a method for the treatment of pancreatic cancer comprising administering to patients in need thereof dendritic cells loaded with a lysate, wherein the lysate is obtainable by a method comprising the steps of:

i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;

ii) inducing necrosis in said tumour cells; and

iii) lysing the necrotic tumour cells, such that a lysate is obtained; and

wherein said treatment extends the median progression free survival and/or overall survival of said patients.

It has surprisingly been found that dendritic cells loaded with a lysate of mesothelioma cells, previously successfully used in clinical trials for the treatment of mesothelioma (54), is also very useful in the treatment of pancreatic cancer.

A second aspect of the present invention relates to dendritic cells loaded with a lysate for use in the treatment of pancreatic cancer, wherein said dendritic cells are administered to a patient in need thereof and wherein the lysate is obtainable by a method comprising the steps of:

i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;

ii) inducing necrosis in said tumour cells; and

iii) lysing the necrotic tumour cells, such that a lysate is obtained; and wherein said treatment extends the median progression free survival and/or median overall survival of said patients.

A third aspect of the present invention relates a pharmaceutical composition for use in the treatment of pancreatic cancer, obtainable by a method comprising the steps of:

i) providing allogeneic mesothelioma tumour cells from at least two different cell lines, and preparing a lysate thereof;

ii) providing dendritic cells;

iii) loading the dendritic cells with the lysate of tumour cells and, optionally, providing and adding a pharmaceutically acceptable carrier. With said pharmaceutical composition it is possible to extend the median progression free survival and/or median overall survival of said patients.

DEFINITIONS

The term "*antigen*" as used herein has its conventional meaning and refers to a molecule capable of inducing an immune response. Within the context of the present invention the antigen may be a protein or a fragment thereof, such as a (poly)peptide representing an epitope of said protein. It is however also possible that the antigen used is an artificial peptide or a peptidomimetic, e.g., by incorporating rigid unnatural amino acids, such as 3-aminobenzoic acid, into peptides to make the peptide backbone rigid. The antigens used in the present invention are preferably proteins or parts thereof obtained or derived from a tumour-cell.

The term "*epitope*" as used herein has its conventional meaning and refers to the part of an antigen that is recognized by the immune system, in particular by antibodies, B cells, or T cells. Within the context of the present invention the antigen is a protein and the epitope is part thereof (i.e. a (poly)peptide, fragment or aggregate thereof).

The term "*cancer*" as used herein has its conventional meaning and refers to the broad class of disorders characterized by hyper-proliferative cell growth *in vivo*.

The term "*mesothelioma cancer cells*" or "mesothelioma tumour cells" as used herein has its conventional meaning and refers to cells from malignant mesothelioma.

The term "*pancreatic cancer cells*" or "pancreatic tumour cells" as used herein has its conventional meaning and refers to cells from a malignant pancreatic cancer.

The term "*for use in the treatment of pancreatic cancer*" as used herein has its conventional meaning and refers to the reduction of the size of a pancreatic tumour or number of pancreatic cancer cells, cause a pancreatic cancer to go into remission or prevent or delay further growth in size or cell number of pancreatic cancer cells.

The term "*cold tumour*" as used herein has its conventional meaning and refers to a tumour wherein there is no or minimal presence of infiltrating cytotoxic T-cells.

The term "*hot tumour*" as used herein has its conventional meaning and refers to a tumour wherein there is a considerable presence of cytotoxic T-cells either active or inactivated via for example the different immune checkpoints.

The term "*progression free survival*" (PFS) as used herein has its broad conventional meaning and refers to the time from diagnosis or start treatment or randomization to first disease progression or death. The assessment thereof is based on the so called RECIST 1.1 criteria as specified in Eisenhauer et al., 2009. In the context of the present invention, the progression free survival of the patients is calculated from the date the patients were subjected to surgical resection of said pancreatic cancer.

The term "*overall survival*" (OS) as used herein has its conventional meaning and refers to time from the date of diagnosis or start treatment or randomization of the patient to death. In the context of the present invention, the overall survival of patients is calculated from the date patients were subjected to surgical resection of said pancreatic cancer. The skilled person is well known with this term and in this regard reference is made to

The term "*surgical resection*" refers to a surgical operation wherein a pancreatic tumour has partly or completely been removed by surgery. Such tumour can be either the primary tumour or a metastatic secondary pancreatic tumour.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: Experimental setup Example 3. Immunocompetent C57bl/6 mice were treated with DC-vaccines consisting of monocyte-derived DCs loaded with either pancreatic cancer lysate (KPC-3) or with mesothelioma lysate (AE17). An untreated group was also included. Subsequently, a pancreatic tumour was induced with the KPC-3 tumour cell line and tumour growth was followed.

Figure 2: Tumour growth following DC vaccination. (A) Tumour size measured over time of untreated and treated mice. (B) Tumour growth curve per mouse. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.015. KPC-3 = pancreatic cancer lysate-DC therapy, AE17 = mesothelioma lysate-DC therapy.

Figure 3: End-stage analysis following DC vaccination. (A) $CD3^+$, $CD4^+$ and $CD8^+$ TILs as a percentage of $CD45^+$ alive subset of treated and untreated mice 27 days following DC vaccinations, determined by flow cytometry. **(B)** Percentages of CD44 or Ki67-positive $CD4^+$ and $CD8^+$ TILs of treated and untreated mice. **(C)** $CD3^+$, $CD4^+$ and $CD8^+$ T-cells as a percentage of $CD45^+$ alive subset in peripheral blood of treated and untreated mice. **(D)** $CD44^+CD62L^-$ subset or Ki67 positivity of $CD4^+$ and $CD8^+$ peripheral blood T-cells of treated and untreated mice. **(E)** Percentage of $PD\text{-}1^+TIM\text{-}3^-LAG^-$ within $CD8^+$ TILs. **(F)** Tregs ($CD4^+CD25^+FoxP3^+$) as a percentage of CD45 alive subset in tumours. All non-Treg $CD4^+$ subsets are $FoxP3^-$. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001.

Figure 4: Tumour-reactive T-cell responses following DC treatment. $CD8^+$ MACS-purified fresh splenocytes (assay performed at the day of sacrifice, day 27) were co-cultured with KPC-3 tumour cells. KPC-3 tumour cells were first stimulated overnight with INFγ (40U/ml), after which 100.000 cells were seeded together with $CD8^+$ T-cells at a ratio of 1:1 in a 96 wells flat bottom plate and incubated at 37 °C in a humidified atmosphere at 5% CO_2 for 5 hours together with 10µg/ml CD107a-FITC (BD Bioscience). After one hour, the protein transport inhibitor Golgi stop™ was added (BD Bioscience). For the markers granzyme B and TNFα splenocytes were stimulated with 50 ng/ml phorbol 12 myristate 13-acetate (PMA) and 500 ng/ml ionomycin (Sigma) for 5 hours. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. **P<0.01, ***P<0.001.

Figure 5: Experimental setup Example 4. KPC-3 C57Bl/6 mice were treated with either unloaded (i.e. in the absence of tumour lysate) but matured DCs (stimulated with CpG) or DCs that were matured and loaded with the mesothelioma AE17 lysate.

Figure 6: Tumour growth following DC vaccination. Tumour volume measured over time of mice treated with DCs pulsed with and without mesothelioma lysate. N=7 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.05 **P<0.01.

Figure 7: Schematic overview Example 5. Tumour and spleen from treated and untreated tumour-bearing mice from Example 4 were snap frozen and stored in single cell suspension respectively. Bone marrow was harvested from wild type non-tumour bearing mice for the culture of mature DCs.

Figure 8: Tumour-reactive T-cell responses following DC vaccination. Thawed splenocytes from pancreatic tumour-bearing mice were cocultured with GM-CSF cultured DCs that were loaded with 70ug autologous pancreatic tumour lysate or control lung lysate

(depicted on x-axis). 100.000 DCs were co-cultured with splenocytes of either untreated tumour bearing mice (first and fourth bar in each graph), tumour bearing mice treated with unloaded DCs (second and fifth bar in each graph), and tumour bearing mice treated with AE17 loaded DCs (third and sixth bar in each graph) at a ratio of 1:10 in a 96 wells round bottom plate and incubated at 37°C in a humidified atmosphere at 5% CO_2 for 24 hours. After 20 hours the protein transport inhibitor Golgi Stop™ was added (BD Bioscience) and after 23 hours 10µg/ml CD107a-FITC (BD Bioscience) was added per well. CD107, Granzyme B, IFNγ and TNFα were determined by flow cytometry. N=5-8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. **P<0.01, ***P<0.001.

Figure 9: Experimental setup Example 8. Immunocompetent C57bl/6 mice were subcutaneously injected with $1*10^5$ pancreatic tumour cells and treated with either DC vaccine, CD40 agonistic monoclonal antibody, or both as indicated in the Figure. On day 5, mice received $1*10^6$ DCs and on days 6 and 12 anti-CD40 agonistic monoclonal antibody or its isotype as indicated in the Figure.

Figure 10. Tumour growth. (A) Tumour size measured over time of untreated and treated mice with mesothelioma lysate-DC therapy, FGK45 or both. **(B)** Tumour volume on day 18 post-tumour injection. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.05, **P<0.01.

Figure 11. Peripheral blood analysis following DC vaccination and FGK injection. (A) CD69+ and Ki67+ cells as a percentage of $CD4^+$ and $CD8^+$ T cells in peripheral blood of treated and untreated mice. **(B)** $CD44^+CD62L^-$ and $CD44^-CD62L^+$ subsets as a percentage of $CD4^+$ and $CD8^+$ peripheral blood T-cells of treated and untreated mice. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001.

Figure 12. Endstage tumour analysis. $CD3^+$, $CD4^+$, $CD8^+$ and $CD4^+CD25^+$FoxP3+ TILs as a percentage of $CD45^+$ alive subset and absolute cell count per mg tumour of treated and untreated mice at end-stage disease, determined by flow cytometry. N=8 per group. Significance was determined using the non-parametric Mann–Whitney U test. Data presented as the mean ± s.e.m. *P<0.05, **P<0.01, ***P<0.001.

DETAILED DESCRIPTION OF THE INVENTION

A first aspect of the present invention relates to a method for the treatment of pancreatic cancer comprising administering to patients in need thereof dendritic cells loaded with a lysate, wherein the lysate is obtainable by a method comprising the steps of:

i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;

ii) inducing necrosis in said tumour cells; and

iii) lysing the necrotic tumour cells, such that a lysate is obtained; and

wherein said treatment extends the median progression free survival and/or median overall survival of said patients, in particular the median progression free survival.

A second aspect of the present invention relates to dendritic cells loaded with a lysate for use in the treatment of pancreatic cancer, wherein said dendritic cells are administered to a patient in need thereof and wherein the lysate is obtainable by a method comprising the steps of:

i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;

ii) inducing necrosis in said tumour cells; and

iii) lysing the necrotic tumour cells, such that a lysate is obtained; and

wherein said treatment extends the median progression free survival and/or median overall survival of said patients, in particular the median progression free survival.

A third aspect of the present invention relates a pharmaceutical composition for use in the treatment of pancreatic cancer, obtainable by a method comprising the steps of:

i) providing allogeneic mesothelioma tumour cells from at least two different cell lines, and preparing a lysate thereof;

ii) providing dendritic cells;

iii) loading the dendritic cells with the lysate of tumour cells and, optionally, providing and adding a pharmaceutically acceptable carrier. With said pharmaceutical composition it is possible to effectively extend the median progression free survival and/or median overall survival of said patients, in particular the median progression free survival.

It has surprisingly been found that dendritic cells loaded with a lysate of mesothelioma cells, previously successfully used in clinical trials for the treatment of mesothelioma (54), is also very useful in the treatment of pancreatic cancer. It has been

found in clinical trials that the progression free survival and therewith the overall survival of patients suffering from pancreatic cancer increased compared to patients that did not receive such dendritic cell based therapy. This was particularly the case for patients that were subjected to previous surgical resection of the pancreatic cancer and optional adjuvant chemotherapy, as will be discussed in more detail below.

It has specifically been found that with the present invention the median progression free survival of said patients is extended to at least 18 months after pancreatic cancer resection. Preferably the median progression free survival is even extended further to at least 20 months, at least 25 months, at least 30 months or at least 35 months after resection.

The increase of the progression free period achieved with the present invention is considerably longer then can be achieved with for example gemcitabine. In practice, after resection patients are offered adjuvant chemotherapy. This may (shortly) prolong the progression free period, however in many cases progression of the disease occurs quickly. As will be discussed in more detail below, it has been found that all the patients treated according to the present invention were still alive. Hence, the present treatment leads to a clinically relevant increase of the median progression free survival of pancreatic cancer patients.

In patients treated with gemcitabine after resection the median disease free survival was about 13 months after surgery (66). Other types of chemotherapy, such as folfirinox have reported a median disease free survival of about 21 months, but its use often leads to severe side effects, meaning that frail patients cannot use it.

In one aspect of the present invention the administration of the loaded dendritic cells to said patients leads to an increase of the median progression free survival when compared to adjuvant gemcitabine alone. More specifically, the median progression free survival is at least 3 months or alternatively at least 6 months more when compared to adjuvant chemotherapy with gemcitabine alone (66). The same applies for the median overall survival.

With the method, dendritic cells and pharmaceutical composition according to the present invention it has thus become possible to considerably extend the median progression free survival and the median overall survival of patients suffering from (resected) pancreatic cancer when compared to chemotherapy alone. Most importantly, this has been achieved without serious side effects.

The patients receiving the treatment according to the present invention may have received adjuvant chemotherapy after cancer resection. Preferably the patients have been administered gemcitabine. A suitable dosing schedule for administering gemcitabine would

be 6 cycles of gemcitabine every four weeks, consisting of 3 weekly infusions of gemcitabine (1000 mg/m^2), followed by a 1-week break.

Patients may also have received for folfirinox prior to the administration of the loaded dendritic cells (or a pharmaceutical composition thereof) (67).

It is preferred to first finish the cycles of adjuvant chemotherapy (such as gemcitabine) and start the administration of the loaded dendritic cells thereafter. However, it is also possible to start both the administration of the adjuvant chemotherapy together with the administration of the loaded dendritic cells shortly after cancer resection.

Nevertheless, it is also possible to only administer the loaded dendritic cells according to the present invention without prior chemotherapy.

The mesothelioma cell lysate

Because differential antigen expression takes place in tumours from different patients, it is not sufficient to provide a lysate derived from only one cell line to a group of patients.

With the present invention this is achieved by preparing a lysate of mesothelioma tumour cells from at least two different cell lines. By using different cell lines multiple antigens are thus present in the lysate, which lysate may be used to load dendritic cells. This way, the chances are reduced that a pancreatic tumour cell in a patient escapes, by down-regulating a specific antigen.

Furthermore, the use of a lysate of said tumour cells is essential for the present invention. Due to the use of this lysate, the different antigens from the different tumour cell lines are directly available to the dendritic antigen presenting cells. Besides the multitude repertoire of antigens, the advantage of using an allogeneic lysate is the off-the-shelf availability and a superior quality compared to autologous lysate.

A key problem associated with the use of autologous tumour cells is that the amount of tumour cells obtained from resected tumour material (either after surgery or through a biopsy) is limited in quantity and quality. Furthermore, the tumour material obtained from patients is, apart from total tumour amount, highly heterogeneous, which makes standardization difficult, and “contaminated” with normal cells (e.g., macrophages, lymphocytes). When this tumour material is then used for the treatment of pancreatic cancer, different outcomes of the phenotype and stimulatory capacity can be expected, with a potential negative impact on efficacy, but also complicating the development of a commercial product. For the reasons set out above, an allogenic approach is therefore used.

In the context of the present invention the term "allogeneic" has its normal scientific meaning and refers to tumour cells which are derived from an individual which is different from the individual to which the lysate resulting from the method according to the present invention shall be later administered. The use of tumour cell lysates from cell lines derived from allogeneic mesothelioma tumour cells provides a more standardized and easier approach, bypassing the need for an individually prepared autologous tumour lysate. It also creates opportunities to select the optimal source, dose and delivery onto dendritic cells or perform manipulations to increase the immunogenicity of the cells. The utilization of a robust and validated large scale manufacturing process also requires fewer product batches for quality control tests such as identity, purity, quantity and sterility/safety testing. A major advantage of the allogeneic approach over autologous is that the tumour cell lines can be selected and optimized, stored in bulk, and manufacturing / quality control timeliness shall not impact on the immediate disease progression of the patient as supply of lysate is off-the-shelf.

In accordance with the present invention the term "necrosis" has its normal scientific meaning and means morphological changes of cells. Necrosis is, inter alia, characterized for example by "leakiness" of the cell membrane, i.e. an increased permeability which also leads to an efflux of the cell's contents and an influx of substances perturbing homeostasis and ion equilibrium of the cell, DNA fragmentation and, finally, to the generation of granular structures originating from collapsed cells, i. e. cellular debris. Typically, necrosis results in the secretion of proteins into the surrounding which, when occurring *in vivo*, leads to a pro-inflammatory response.

Methods for the determination whether a cell is necrotic are known in the prior art. It is not important which method the person skilled in the art chooses since various methods are known. Necrosis can, e.g., be induced by freeze-thaw cycles, heat treatment, triton X-100, or H_2O_2.

Necrotic cells in accordance with the present invention can be determined, e. g., by light-, fluorescence or electron microscopy techniques, using, e. g., the classical staining with trypan blue, whereby the necrotic cells take up the dye and, thus, are stained blue, or distinguish necrotic cells via morphological changes including loss of membrane integrity, disintegration of organelles and/or flocculation of chromatin. Other methods include flow cytometry, e. g., by staining necrotic cells with propidium iodide.

In accordance with the present invention the term "apoptosis" has its normal scientific meaning and means programmed cell death. If cells are apoptotic various changes in the cell

occur, such as cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation.

Apoptotic cells can be determined, e. g. , via flow-cytometric methods, e. g. , attaining with Annexin V-FITC, with the fluorochrome : Flura-red, Quin-2, with 7-amino-actinomycin D (7-AAD), decrease of the accumulation of Rhodamine 123, detection of DNA fragmentation by endonucleases : TUNEL-method (terminal deoxynucleotidyl transferase caused X-UTP nick labelling), via light microscopy by staining with Hoechst 33258 dye, via Western blot analysis, e. g. , by detecting caspase 3 activity by labelling the 89 kDa product with a specific antibody or by detecting the efflux of cytochrome C by labelling with a specific antibody, or via agarose gel DNA-analysis detecting the characteristic DNA-fragmentation by a specific DNA-ladder.

In accordance with the present invention the term "lysing" relates to various methods known in the art for opening/destroying cells. In principle any method that can achieve lysis of the tumour cells may be employed. An appropriate one can be chosen by the person skilled in the art, e. g. opening/destruction of cells can be done enzymatically, chemically or physically. Examples of enzymes and enzyme cocktails that can be used for lysing the tumour cells are proteases, like proteinase K, lipases or glycosidases non-limiting examples for chemicals are ionophores, like nigromycin, detergents, like sodium dodecyl sulfate, acids or bases; and non-limiting examples of physical means are high pressure, like French pressing, osmolarity, temperature, like heat or cold. A preferred way of lysing cells is subjecting the cells to freezing and thawing cycles. Additionally, a method employing an appropriate combination of an enzyme other than the proteolytic enzyme, an acid, a base and the like may also be utilized.

According to the present invention the term "lysate" means an aqueous solution or suspension comprising the cellular proteins and factors produced by lysis of tumour cells. Such a lysate may comprise macromolecules, like DNA, RNA, proteins, peptides, carbohydrates, lipids and the like and/or smaller molecules, like amino acids, sugars, lipid acids and the like, or fractions from the lysed cells. The cellular fragments present in such a lysate may be of smooth or granular structure. Preferably, said aqueous medium is water, physiological saline, or a buffer solution.

The lysate used in the present invention is not limited to lysed necrotic cells. For example, due to the different sensitivity of the treated cells or due to the applied conditions, such as UVB radiation, also lysed apoptotic cells can form or be part of the lysate. It is preferred, however, that the lysate comprises at least 80%, more preferably at least 90%, more preferably at least 95%, most preferably at least 98% lysed necrotic cells. The

percentage of lysed necrotic cells can be influenced by the lysing method. Multiple snap-freezing in liquid nitrogen and thawing, for instance, leads to a relative high percentage of necrotic cells, whereas UVB radiation, for instance, leads to a relative high percentage of apoptotic cells. The skilled person is aware of methods for obtaining essentially necrotic cells.

The term lysate as used herein also encompasses preparations or fractions prepared or obtained from the above-mentioned lysates. These fractions can be obtained by methods known to those skilled in the art, e. g. , chromatography, including, e. g., affinity chromatography, ion-exchange chromatography, size-exclusion chromatography, reversed phase-chromatography, and chromatography with other chromatographic material in column or batch methods, other fractionation methods, e. g., filtration methods, e. g., ultrafiltration, dialysis, dialysis and concentration with size-exclusion in centrifugation, centrifugation in density-gradients or step matrices, precipitation, e. g. , affinity precipitations, salting-in or salting-out (ammonium sulfate-precipitation), alcoholic precipitations or other protein chemical, molecular biological, biochemical, immunological, chemical or physical methods to separate above components of the lysates. In a preferred embodiment those fractions which are more immunogenic than others are preferred. Those skilled in the art are able to choose a suitable method and determine its immunogenic potential by referring to the above general explanations and specific explanations in the examples herein, and appropriately modifying or altering those methods, if necessary.

In order to obtain a good immunogenic response it is preferred to use a mixture of allogeneic mesothelioma tumour cells, from at least two mesothelioma tumour cell lines, preferably at least three mesothelioma tumour cell lines, more preferably at least four mesothelioma tumour cell lines, for preparing the lysate. It is particularly preferred to use a mixture of at least five mesothelioma tumour cell lines for preparing the lysate.

Preferably, these at least two, at least three, at least four or at least five mesothelioma tumour cell lines are present in essentially equal cellular amounts at equal concentration preceding lysate preparation. The term “essentially equal cellular amounts” has its conventional meaning and preferably means that each of the cell lines are present in a cell ratio of between 1:2 – 2:1, relative to one another, more preferably of between 2:3 – 3:2, more preferably between 3:4 – 4:3, more preferably between 4:5 - 5:4, most preferably in a cell ratio of about 1:1.

As an example for five cell lines, the cells could be present in a cell ratio of 3:4:2:4:3, wherein cell line 1 has a ratio of 3:4 to cell line 2, a ratio of 3:2 to cell line 3, a ratio of 3:4 to cell line 4, and a ratio of 1:1 to cell line 5. Cell line 2 has a ratio of 4:3 to cell line 1, a ratio of

2:1 to cell line 3, a ratio of 1:1 to cell line 4, and a ratio of 4:3 to cell line 5. Cell ratios of cell lines 3, 4 and 5 with respect to the others are calculated the same and all fall within the preferred ratios defined above.

Using such mixtures of cell lines as a source of tumour lysate is advantageous in providing a broader antigenic repertoire of tumour associated antigens (wide variety of potential tumour antigens), which will increase the ability of immune responses to recognize and destroy tumour cells because the opportunities to escape immune surveillance by modulation of antigen expression are more limited.

The allogeneic mesothelioma tumour cells, used in the present invention are cultured in for example culture flasks. Due to the fact that these allogeneic cells have the ability to divide unlimitedly with minimal loss of their immunogenic properties, in contrast to non-cancerous cells, they are suitable to use for preparing the lysate. The cell lines that are used for preparing a lysate for use in the treatment of pancreas cancer in human subjects are derived from humans.

Presently five human mesothelioma cell lines have been developed that provide particularly good results. These cell lines have been deposited at “Deutsche Sammlung von Mikro-organismen und Zellkulturen” in Germany, hereinafter DSMZ. The cell lines were initially given the following codes and accession numbers: Thorr 01 (deposit No. DSM ACC3191), Thorr 02 (deposit No. DSM ACC3192), Thorr 03 (deposit No. DSM ACC3193), Thorr 04 (deposit No. DSM ACC3194), Thorr 05 (deposit No. DSM ACC3195).The deposit was made pursuant to the terms of the Budapest treaty on the international recognition of the deposit of micro-organisms for purposes of patent procedure. After the initial deposit, the cell lines were renamed as follows: Thorr 01 was renamed to Thorr 03, Thorr 02 was renamed to Thorr 01, Thorr 03 was renamed to Thorr 02, Thorr 04 was renamed to Thorr 05, and Thorr 05 was renamed to Thorr 06. Throughout the present patent application, the renamed designation are used, i.e.: Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

In a preferred embodiment, therefore, a lysate for use according to the invention is, therefore, provided, wherein the allogeneic mesothelioma tumour cells used are chosen from two or more of the following cell lines Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

Necrosis of the allogeneic mesothelioma tumour cells, can be achieved by methods commonly known in the prior art. However, subjecting the cells to freeze thawing cycles is

particularly preferred. Preferably, the cells are made necrotic and lysed by freezing at temperatures below -75 degrees Celsius and thawing at room temperature, particularly snap freezing in liquid nitrogen at temperatures below -170 degrees Celsius and thawing at room temperatures or more, e.g. in a water bath at about 37 degrees Celsius, is most preferred. It is also preferred that said freezing/thawing is repeated for at least 1 time, more preferably for at least 2 times, even more preferred for at least 3 times, particularly preferred for at least 4 times and most preferred for at least 5 times.

Preferably the tumour cells are treated with at least 50 Gy irradiation, preferably at least 100 Gy irradiation. This way it is avoided that any of the tumour cells remains viable. The irradiation treatment can be carried out before or after the tumour cells have been subjected to freezing and thawing.

In one preferred embodiment of the present invention the lysate comprises at least three mesothelioma cancer cell associated antigens. Preferably, the lysate comprises at least three, more preferably at least five, more preferably at least ten mesothelioma cancer cell associated antigens. In this regard it is further noted that the antigens may be derived from the same protein, i.e. the antigens may be different epitopes from the same protein. However, it is preferred to use antigens which are (or are based) on different tumour cell associated proteins. It is preferred that the at least three, more preferably at least five, more preferably at least ten mesothelioma cancer cell associated antigens are also expressed on pancreatic cancer cells, i.e. these antigens are shared between mesothelioma cancer cells and pancreatic cancer cells, at least in the majority of pancreatic cancer cells to be treated in a patient in need thereof.

It is particular beneficial that the lysate comprises various antigens that cover ideally all tumour cells of a tumour. After all, if a specific tumour cell does not have a specific antigen an immune response will not be triggered against such a cell. If other cells are attacked, but this cell is not, it will have an advantage and will be able to grow further resulting in a further growth of the tumour. The inventors have now been able to establish the most important antigens which can be used to load dendritic cells and target substantially all tumour cells in pancreatic cancer. This approach has allowed the present inventors to formulate lysate which is particularly useful for loading dendritic cells and inducing an immune response to pancreatic cancer cells.

Preferably at least three, more preferably at least five, more preferably at least six of the mesothelioma cancer cell associated antigens are chosen from the group of RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1. Further antigens which are of importance within the context of the present invention are RAB38/NY-MEL-1, BING4, MAGE

A12, HER-2/Neu, Glypican, LMP2. A mixture of at least three, preferably at least five, more preferably at least six, most preferably at least ten of the mentioned mesothelioma associated antigens is particularly effective against pancreatic cancer when used according to the invention.

In a preferred embodiment, a lysate for use according to the invention is provided, wherein the at least three, preferably at least five, more preferably at least six mesothelioma cancer cell associated antigens are chosen from the group of: RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1.

In another preferred embodiment, a lysate for use according to the invention is provided, wherein the at least three, preferably at least five, more preferably at least seven, more preferably at least nine, more preferably at least ten mesothelioma cancer cell associated antigens are chosen from the group of: RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1, RAB38/NY-MEL-1, BING4, MAGE A12, HER-2/Neu, Glypican, LMP2.

It has surprisingly been found that many of the antigens, present in mesothelioma cells, used to prepare a lysate of the invention, are shared with pancreatic cancer cells (Table 1). For example, the tumour associated antigen mesothelin, which is abundantly present in the lysate of the invention (further referred to as "PheraLys"), is also present in pancreatic cancers. The presence of mesothelin in pancreatic cancer has led to the initiation of clinical trials worldwide targeting mesothelin for this type of cancer. Combining *Listeria Monocytogenes*-expressing mesothelin and allogenic pancreatic cancer vaccination GVAX prolonged median survival of advanced pancreatic cancer patients from 3.9 months to 6.1 months (22). However, due to the mono-antigen approach the duration of the response is limited.

Rank[a]	Antigen	Gene ID	FPKM score[b]	PheraLys	Pancreatic cancer[c]
3	Mesothelin	10232	84.25	++	++
9	Survivin	332	38.71	++	++
18	HER-2/neu	2064	16.89	+	+
21	MUC1	4582	13.13	++	++
29	WT1	7490	10.28	+	+
30	KRAS	3845	9.26	+	+
36	LY6K	54742	6.84	+/-	+/-

Table 1. Antigens of interest for pancreatic cancer in PheraLys

[a]An extensive list (195) of over-expressed, differentiation and cancer germline antigens were checked for their frequency within each of the five malignant mesothelioma cell lines that are used to create PheraLys via RNA sequence analysis and ranked according to their average FPKM score
[b]FPKM = fragments per kilobase million mapped
[c]Antigen expression according to www.proteinatlas.org
++ = strong expression, + = medium expression, +/- = expression status differs between samples

In addition to the numerous antigens with relatively high expression, the antigens with relatively low expression may also induce a highly specific T-cell response in the patient. It was, e.g., shown that both dominant and subdominant neoantigens significantly increased the TCR-β repertoire upon DC vaccination (55). Therefore, all antigens may be of value in the patient and, whereas others have tried a single antigen, or a combination of a few antigens for dendritic cell loading, the magnitude of the number of antigens in PheraLys is clearly an advantage of the current approach.

It has, for instance, been demonstrated that efficacy of mono-antigen treatments is often of short duration in solid tumours (56). Tumours are able to relatively rapidly down regulate that specific antigen after which the treatment becomes ineffective. In contrast, immunotherapy with a multitude of tumour associated antigens decreases the possibility of tumour escape by eliciting a broad immune response and clinical response will be more durable. In one embodiment, the lysate is in the form of a pharmaceutical composition further comprising a pharmaceutically acceptable excipient or carrier, for use in the treatment of pancreatic cancer.

The lysate may also be loaded on dendritic cells *ex vivo* and formulated into a pharmaceutical composition as will be described in more detail below.

The dendritic cells

The term "*dendritic cells*" as used herein has its conventional meaning and refers to antigen-presenting cells (also known as accessory cells) of the mammalian immune system, which capture antigens and have the ability to migrate to the lymph nodes and spleen, where they are particularly active in presenting the processed antigen to T cells. The term dendritic cells also encompasses cells which have an activity and function similar to dendritic cells. Dendritic cells can be derived from either lymphoid or mononuclear phagocyte lineages. Such dendritic cells can be found in lymphatic and non-lymphatic tissue. The latter appear to induce a T cell response only when being activated and having migrated to lymphatic tissues.

Dendritic cells are known to be amongst the most potent activators and regulators of immune responses. One important feature is that they are presently the only antigen presenting cells known to stimulate naïve T cells. Immature dendritic cells are characterized by their ability to take-up and process antigens, a function that is dramatically reduced in mature dendritic cells, which in turn exhibit enhanced presentation of processed antigens on their surface, mainly bound to MHC Class I and Class II molecules. Maturation is also associated with upregulation of co-stimulatory molecules (such as CD40, CD80 and CD86), as well as certain other cell surface proteins (e. g. CD83 and DC-Sign). Dendritic cell maturation is also usually associated with enhanced migratory capacity, resulting (*in vivo*) in migration of dendritic cells to the regional lymph nodes, where the dendritic cells encounter T and B lymphocytes. In a preferred embodiment, the dendritic cells are immature when they are loaded with the lysate, but are matured and activated when administered to a patient in need thereof.

Dendritic cells can be obtained from humans, using methods known to those skilled in the art (57-59). After having obtained monocytes, these cells are differentiated *ex vivo* to immature dendritic cells, which are further maturated and activated. Preferably, the dendritic cells cultured are autologous dendritic cells. The advantage of using autologous dendritic cells is that immune reactions of the patients against these dendritic cells is avoided and that the immunological reaction is triggered against the antigens from the mesothelioma tumour cells, which were present in the lysate.

In a preferred embodiment, the dendritic cells are autologous to the subject having pancreatic cancer. Although using autologous dendritic cells provides many advantages, it may also be advantageous to use allogeneic dendritic cells. One of the major advantages of using allogeneic dendritic cells is that a medicament can be provided to patients that is ready to use. In other words one does not have to differentiate, load and activate the dendritic cells from an individual but one can immediately administer the loaded allogeneic dendritic cells. This saves patient's valuable time. In one preferred embodiment, therefore, the dendritic cells are allogeneic to the subject having pancreatic cancer.

Loading of the dendritic cells with the mesothelioma cell lysate

Dendritic cells or their precursors are differentiated using suitable growth factors and/or cytokines, e. g. GM-CSF and IL-4, the resulting immature dendritic cells are loaded with a lysate for use according to the invention. Immature dendritic cells, loaded with a lysate for use according to the invention, are further maturated to mature dendritic cells. In special cases also mature dendritic cells can be loaded (pulsed) with antigens or immunogens from the lysate.

Preferably, the dendritic cells are loaded with between 1 tumour cell equivalents per 100 dendritic cells to 10 tumour cell equivalents per 1 dendritic cell, preferably between 1 tumour cells per 10 dendritic cells to 1 tumour cell equivalent per 1 dendritic cell. Particularly preferred is about 1 tumour cell equivalent per 3 dendritic cells.

Preferably, a dosage of the composition administered to a patient comprises $1*10^6$ to $1*10^9$ loaded dendritic cells, preferably $2*10^6$ to $5*10^8$ loaded dendritic cells, more preferably $1*10^7$ to $1*10^8$ loaded dendritic cells, most preferably about $2.5*10^7$. Most preferably a dosage of the pharmaceutical composition comprises about $2.5*10^7$ dendritic cells loaded with about 1 tumour cell equivalent per 3 dendritic cells.

It is preferred to load the dendritic cells with more than one mesothelioma cancer cell associated antigen. Hence, preferably the composition for loading the dendritic cells comprises at least three, preferably at least five, more preferably at least ten mesothelioma cancer cell associated antigens. In this regard it is further noted that the antigens may be derived from the same protein, i.e. the antigens may be different epitopes from the same protein. However, it is preferred to use antigens which are (or are based) on different tumour cell associated proteins.

In order for the T-cells to be able to attack all tumour cells it is important to make sure that the dendritic cells are loaded with antigens that cover ideally all tumour cells of a tumour. After all, if a specific tumour cell does not have a specific antigen an immune response will not be triggered against such a cell. If other cells are attacked, but this cell is not, it will have an advantage and will be able to grow further resulting in a further growth of the tumour. The inventors have now been able to establish a lysate comprising the most important antigens which can be used to load dendritic cells and target pancreatic cancer. This approach has allowed the present inventors to formulate an antigen composition which is particularly useful for loading dendritic cells and inducing an immune response to pancreatic tumour cells.

The mesothelioma cancer cell associated antigens are preferably chosen from the group of RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1. It has been established for the first time that these antigens are able to induce by means of dendritic cell immunotherapy a strong immune reaction against pancreatic tumour cells. Further antigens which are of importance within the context of the present invention are RAB38/NY-MEL-1, BING4, MAGE A12, HER-2/Neu, Glypican, LMP2.

Furthermore, with respect to these tumour cell associated proteins it is noted that as antigens also parts of these proteins (i.e. epitopes thereof) may be used for loading the dendritic cells. In this regard it is further noted that also polypeptides or peptidomimetics of such epitopes may be used for loading the dendritic cells. In one embodiment, the antigen

composition comprises only antigens selected from the group of antigens depicted in Table 1. This is advantageous from a regulatory perspective.

In another embodiment the mesothelioma cancer cell associated antigens are obtained from a lysate of allogenic mesothelioma tumour cells from at least two different mesothelioma tumour cell lines, preferably at least three tumour cell lines, more preferably at least four tumour cell lines, most preferably at least five tumour cell lines. The advantage of the use of such a lysate is that many tumour associated antigens will be present in the lysate and that the dendritic cells are loaded with a considerable number of antigens, reducing the chances that a tumour cell will not be recognized and escapes the immune reaction.

The mesothelioma tumour cell lines used for preparing such a lysate are preferably chosen from Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

Said lysate is prepared from between $10*10^6$ and $200*10^6$ tumour cells/ml, preferably between $20*10^6$ and $100*10^6$, more preferably from between $30*10^6$ and $75*10^6$, more preferably from between $40*10^6$ and $60*10^6$ most preferably from about $50*10^6$ tumour cells/ml. Hence, the lysate according to the present invention comprises an equivalent of between $10*10^6$ and $200*10^6$, preferably of between $20*10^6$ and $100*10^6$, more preferably of between $30*10^6$ and $75*10^6$, more preferably of between $40*10^6$ and $60*10^6$, most preferably an equivalent of about $50*10^6$ tumour cells per ml. With equivalent in this context is meant the amount of tumour cells present in solution before lysis, as after lysis only fragments of cells are present.

It has further been found that the total protein content of the lysate for use according to the invention is of relevance, as this is directly related to the number of tumour cells used for preparing the composition. If the amount of protein (i.e. antigen) is too low the loading of dendritic cells will be poor and the induced immune response will be limited. If the protein concentration is too high, interactions between the different proteins will occur, making the antigens less available for absorption by the dendritic cells and causing stability problems. Hence, the total amount of protein in the antigen composition is preferably between 5 and 25 mg protein per ml, more preferably between 6 and 20 mg protein per ml, more preferably between 7 and 15 mg, most preferably between 7.9 and 11.8 mg protein per ml.

It is further preferred that only fragmented DNA is present in the lysate. First, the lysate is preferably subjected to freeze-thawing cycles (decreases the size of DNA) and preferably irradiated to an extremely high dose of 50 Gy, preferably 100 Gy of irradiation that leads to double strand breaks that cannot be repaired and thus leads to distorted and

illegible information (reduction of the oncogenic and infectious risk of residual DNA). Further, dendritic cells are preferably purified from non-incorporated lysate constituents by density gradient centrifugation, thereby removing residual small DNA-fragments. After removal of lysate from the dendritic cells, dendritic cells are preferably incubated *ex vivo* for at least 12 hours, preferably at least 24 hours, more preferably at least 48 hours before purification, thereby allowing free floating nucleic acid (RNA/DNA) to be degraded by natural nucleases. These measures lead to little complications in the downstream processing of both the lysate and the pharmaceutical composition, including the dendritic cells (no viscosity or complex formation, indicating the absence of sizeable DNA fragments). Although the DNA present in the lysate and/or the pharmaceutical composition is considered as cellular contaminant rather than a risk factor by the WHO Expert Committee on Biological Standardization, they set a dose limit of 10 ng/dose.

Therefore, the pharmaceutical composition according to the present invention preferably comprises less than 10 ng free DNA per dose, preferably less than 100pg, more preferably less than 1 pg, most preferably less than 0,01 pg free DNA per dose.

In a preferred embodiment, a lysate for use according to the invention is provided, wherein the lysate is loaded onto autologous dendritic cells before administering the lysate to a patient. Preferably, the dendritic cells are loaded with between 1 tumour cell equivalents per 100 dendritic cells to 10 tumour cell equivalents per 1 dendritic cell, more preferably between 1 tumour cell equivalent per 100 dendritic cells to 1 tumour cell equivalent per 1 dendritic cell, most preferably with about 3 dendritic cells to 1 tumour cell equivalent.

In order to induce a sufficiently large immune response it is advantageous to administer a patient in need thereof with between $1*10^6$ to $1*10^9$ loaded dendritic cells per administration, preferably $2*10^6$ to $5*10^8$ loaded dendritic cells, more preferably $1*10^7$ to $1*10^8$ loaded dendritic cells, most preferably about $2.5*10^7$ dendritic cells per administration.

The dendritic cells used may be autologous or allogenic. However, it is particularly preferred to use autologous dendritic cells. MHC class II molecules expressed on these autologous dendritic cells display peptides to the TCR expressed on T cells present in the treated patient. The ability of the TCR to discriminate foreign peptides from self-peptides presented by "self" MHC molecules is a requirement of an effective adaptive immune response. Use of allogenic dendritic cells, injected intra-tumoural has also been described, but it is unlikely that such allogeneic dendritic cells present the tumour antigens directly to the patient's T cells (60). Without being bound to theory it is believed that such allogeneic dendritic cells, when injected at the site of the tumour, may effectively recruit other immune cells to the site, e.g., NK cells, which ultimately kill the allogeneic dendritic cells, thereby

providing both the tumour antigens and a “danger signal” to intra-tumoural autologous dendritic cells that than induce a specific (T-cell) immune response towards the tumour antigens. In one preferred embodiment, therefore, a dendritic cell of the invention is allogeneic to the patient receiving it, wherein, preferably, the dendritic cell is administered intra-tumourally. Preferably, the lysate is provided as an off-the-shelve product, which can be used to load dendritic cells obtained from a patient suffering from pancreatic cancer. After loading and appropriate formulation for intravenous and/or intradermal administration, the loaded dendritic cells are administered to the patient.

The Pharmaceutical composition

The lysate as such and the loaded dendritic cells may be formulated as a pharmaceutical composition or kit. The skilled person will be able to prepare on the basis of his common general knowledge suitable pharmaceutical compositions.

The pharmaceutical composition according to the present invention may comprise or may be administered with a physiologically acceptable carrier to a patient, as described herein. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and buffers.

Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin. Such compositions will contain a therapeutically effective amount of the cell lysate, or loaded dendritic cells, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In an embodiment, the compositions are in a water-soluble form, such as pharmaceutical acceptable salts, which is meant to include both acid and base addition salts.

The compositions can be prepared in various forms, such as injection solutions, tablets, pills, suppositories, capsules, suspensions, and the like.

Pharmaceutical grade organic or inorganic carriers and/or diluents suitable for oral and topical use can be used to make up compositions containing the therapeutically active compounds. Diluents known to the art include aqueous media, vegetable and animal oils and fats. Stabilizing agents, wetting and emulsifying agents, salts for varying the osmotic pressure or buffers for securing an adequate pH value, and skin penetration enhancers can be used as auxiliary agents. The compositions may also include one or more of the following: carrier proteins such as serum albumin; buffers; fillers such as microcrystalline cellulose, lactose, corn and other starches; binding agents; sweeteners and other flavouring agents;

colouring agents; and polyethylene glycol. Additives are well known in the art, and are used in a variety of formulations.

The pharmaceutical composition according to the present invention may be formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous and/or intradermal administration to human beings. Typically, compositions for intravenous and/or intradermal administration are solutions in sterile isotonic aqueous buffer.

In an embodiment, a pharmaceutical composition that comprises the dendritic cells is formulated such that it is suitable for acting as a vaccine.

The forms or methods for manufacturing vaccine compositions according to the present invention are not particularly limited, and a composition in a desired form can be prepared by applying a single method available in the field of the art or methods in an appropriate combination. For the manufacture of a vaccine composition, aqueous media such as distilled water for injection and physiological saline, as well as one or more kinds of pharmaceutical additives available in the field of the art can be used. For example, buffering agents, pH adjusting agents, solubilizing aids, stabilizing agents, soothing agents, antiseptics and the like can be used, and specific ingredients thereof are well known to those skilled in the art. The composition can also be prepared as a solid preparation such as a lyophilized preparation, and then prepared as an injection by adding a solubilizing agent such as distilled water for injection before use. Depending upon the manner of introduction, the compounds may be formulated in a variety of ways as discussed below. The concentration of a therapeutically active compound in the formulation may vary from about 0.1-100 wt%, preferably between 0.1 – 10 wt%, more preferably between 0.1 and 1 wt%.

In a preferred embodiment, a pharmaceutical composition for use according to the invention is provided wherein the pharmaceutical composition is administered at least once, preferably at least twice, more preferably at least three times, and most preferably at least five times a dosage as defined above. It is most preferred that the pharmaceutical composition is administered in about two-weekly intervals for the first three doses and additional doses at about 3 and about 6 months after the last dose. With an “about two weekly” interval is meant, an interval between two doses of between 10 – 18 days, preferably of between 12 – 16 days, more preferably of between 13 – 15 days, most preferably of 14 days. With “about 3 months” is meant, an interval between the third and the fourth dose of between 10 – 16 weeks, preferably of between 11 – 15 weeks, more preferably of between 12 – 14 weeks, most preferably of 13 weeks. With “about 6 months” is meant, an interval between the third and the fifth dose of between 20 – 30 weeks, preferably of between 22 –

28 weeks, more preferably of between 24 – 26 weeks, most preferably of 25 weeks. The five doses are, for instance, administered in week 1, week 3, week 5, week 18, and week 30. Each of the doses may, independently from one another be administered intravenously and/or intradermally.

The present invention will be elucidated further by means of the following non-limiting examples.

Examples

Example 1

Description of PheraLys Manufacturing Process

PheraLys is considered a highly heterogeneous source of Tumour Associated Antigens (TAA) due to the inclusion of five highly heterogeneous MPM tumour cell lines.

Cell lines, named Thorr 01, Thorr 02, Thorr 03, Thorr 05 and Thorr06 (Thorr is the abbreviation for Thoracic Oncology Research Rotterdam) from 5 different patients with MM were selected for PheraLys preparation. These cell lines are deposited for patent purposes according to the Budapest Treaty at the Leibniz Institute DSMC-Collection of Microorganisms and Cell Cultures (DSMZ): Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

Individual Thorr cell lines are brought into culture and are incubated in a humidified atmosphere of 5% CO2, 95% air at 37°C overnight followed by a medium exchange and a PBS wash the following day. The cells are washed and expanded in fresh medium until a sufficient number of cells for each individual Thorr cell line are obtained. Cells are washed extensively with PBS, counted and stored at a concentration of $50x10^6$ cells per ml in PBS at <-70°C in a controlled environment until further use.
Equal cellular amounts of the different cell lines are mixed and stored at <-70°C. For preparation of the lysate, the intermediate product is thawed and aliquoted in 50 ml tubes, containing 30 ml of cell suspension. These 50 ml tubes are freeze-thawed 5 times by snap-freezing with liquid nitrogen. Thereafter, the 50 ml tubes are irradiated with 100 Gy by gamma irradiation with a Radioactive 137Cesium irradiation source (Cis Bio International). As of this point there are no more tumour cells present in the finalized lysate, therefore concentration is mentioned in Tumour Cell Equivalent (TCE). $50*10^6$ TCE equals the content of $50*10^6$ tumour cells.

Example 2

Tumour associated antigen expression in Thorr cell lines

The five tumour cell lines have been characterized by RNA sequencing with Affymetrix expression arrays. The expression profiles of the cell lines were evaluated against a list of 195 known antigens. This list of 195 antigens encompasses all differentiation/overexpressed antigens that are published in literature either as targets or prognostic markers. Furthermore it includes all cancer germline antigens that are currently listed as cancer-specific targets in the cancer/testis antigen database (www.cta.lncc.br). Cancer germline antigens are of specific interest as these have a bigger chance to trigger powerful immune responses since they are only expressed by cancer cells and not by healthy tissue.

FPKM (fragments per kilobase per million) approximates the relative abundance of transcripts in terms of fragments observed from an RNA-sequence experiment. Longer genes will have more fragments than shorter genes if transcript expression is the same. This is adjusted by dividing the FPM by the length of a gene, resulting in the metric fragments per kilobase of transcript per million mapped reads (FPKM).

The results show that the TAA of interest are heterogeneously expressed by the different Thorr cell lines (Table 2). This exemplifies the potential of the 5 selected Thorr cell lines to act as a broad, clinically relevant, TAA source.

Table 2: Most relevant antigens present in the model cell lines (RNA sequencing results)

Antigen	Gene ID	Amount of antigen expressed in Thorr 01*	Amount of antigen expressed in Thorr 02*	Amount of antigen expressed in Thorr 03*	Amount of antigen expressed in Thorr 05*	Amount of antigen expressed in Thorr 06*
RAGE-1/MOK	5891	1,91	10,7	50,73	310,14	48,91
Mesothelin	10232	42,89	50,9	69,36	143,6	114,49
EphA2	1969	32,65	97,77	24,82	62,6	162,78
Survivin	332	46,83	39,53	49,07	38,28	19,86
WT1	7490	6,47	29,49	0,45	0,28	14,71
MUC1	4582	10,31	12,91	11,11	18,72	12,58
RAB38/NY-MEL-1	23682	3,21	0,27	0,48	0	0,07
BING-4	9277	20,22	17,65	37,07	24,34	34,28
MAGE-A12	4111	0	0	51,8	0	0

HER-2/neu	2064	18,69	11,54	14,73	16,14	23,36
glypican-1	2817	128,93	29,62	43,47	92,31	66,31
LMP2	5698	29,77	148,59	4,14	111,89	158,2

*FPKM values (fragments per kilobase of exons per million fragments mapped).

Example 3

Immune response directed against pancreatic tumour by treatment with DCs loaded with either autologous pancreatic or allogeneic mesothelioma lysate

Immunocompetent C57bl/6 mice were treated with DC-vaccines consisting of monocyte-derived DCs loaded with either pancreatic cancer lysate (KPC-3) or with mesothelioma lysate (AE17). Loading was comparable to the human situation, i.e. 1 tumour cell equivalent per 3 DCs. An untreated group was also included. Subsequently, a pancreatic tumour was induced by subcutaneous injection with 100.000 cells of the pancreatic cancer KPC-3 cell line and tumour growth was followed (see for schematic setup: Figure 1). This experimental set-up corresponds to the situation of pancreatic cancer patients after surgery, with only micro-metastases left.

In this preclinical setting, $2x10^6$ DCs were injected subcutaneously and $1x10^6$ DCs intravenously seven days before tumour implantation. Since pancreatic cancer patients are intended to receive vaccination post-surgery, having no clinical signs of established tumour nor presence of desmoplastic stroma distinctive for established pancreatic cancer, vaccination prior to tumour establishment in our mouse model closely resembles the clinical setting. By treating mice before the establishment of macroscopic tumour formation and desmoplasia we mimic resected patients with potential presence of micrometastatic disease. DCs were stimulated overnight with CpG and loaded with either mesothelioma lysate (AE17 cell line; prof. Nelsons, Curtin University, Perth, Australia) or pancreatic cancer lysate (KPC-3). DCs were generated as previously described (54).

The systemic immune response was monitored 4 and 11 days following DC vaccination (interim analysis). At end-stage disease (27 days following DC vaccination), T cell phenotype (including activation, proliferation and exhaustion status) was analyzed in tumour, spleen and peripheral blood (end-stage analysis).

Tumour growth was significantly delayed in treated animals compared to untreated animals. The relative delay in tumour growth and tumour sizes at the different time points were comparable in the treated animals irrespective of the type of loading of the DCs, indicating that DC therapy with mesothelioma cell lysate is as effective as DC therapy with autologous pancreatic cell lysate (Figure 2).

Delay of tumour growth was accompanied by increased frequencies of tumour infiltrating lymphocytes (TILs) in both groups of DC treated mice compared to untreated mice (Figure 3A). Also, CD44 expression was higher on both CD4+ and CD8+ TILs in treated mice indicating a more prominent effector memory T cell phenotype. The proliferation marker Ki67 was also higher on CD8+ TILs in treated mice compared to untreated mice (Figure 3B). In addition, higher frequencies of PD-1+ LAG-3- TIM-3- CD8+ TILs were observed in treated mice, although with significant variation. This phenotype is associated with truly activated non-exhausted T cells needed for a robust anti-tumour response (Figure 3E).
There was no increase in suppressive intra-tumoural CD4+FoxP3+ Tregs after DC therapy (Figure 3F), which further substantiates an effective anti-tumour CD8+ T-cell response.
In peripheral blood, increased frequencies of T-cell subsets could be observed as early as four days after DC treatment. The increased frequencies of T-cells in peripheral blood and spleen (not shown) were still present 27 days after treatment, whereas the earlier observed enhanced values of CD44+CD62L- subsets and the Ki67 marker were restored to untreated baseline (Figure 3C, D).

To demonstrate the induction of a tumour-specific T-cell response, splenocytes were isolated on the day of sacrifice of the mice of Experiment I. $CD8^+$ MACS-purified splenocytes were *in vitro* stimulated with pancreatic tumour cells (KPC-3).
Upon stimulation with pancreatic tumour cells increased frequencies of various activation and degranulation markers were expressed by $CD8^+$ T-cells of treated mice compared to untreated mice.
Interferon-γ (IFNγ) and tumour necrosis factor α (TNFα) production was assessed by intracellular cytokine staining, and expressions of CD107a, CD69 and granzyme B were also assessed by flow cytometry. Notably, the frequencies of $IFN\gamma^+$ and $CD107a^+$ expressing $CD8^+$ T-cells were increased upon stimulation with tumour cells in all treated mice in comparison to untreated mice. In the case of CD69, granzyme B and TNFα, only higher frequencies could be observed in mice treated with mesothelioma-pulsed DCs (Figure 4).

Example 4

Loading of DCs with (shared) tumour associated antigens prerequisite for an effective anti-tumour response

It was investigated whether delayed tumour growth is dependent on the induction of a tumour-specific immune response induced by DCs loaded with tumour associated antigens

shared between mesothelioma and pancreatic cancer cell lysate or by the administration of matured DCs as such. To this end, KPC-3 C57Bl/6 mice were treated with either unloaded (i.e. in the absence of tumour lysate) but matured DCs (stimulated with CpG) or DCs that were matured and loaded with the mesothelioma AE17 lysate (see for schematic setup: Figure 5).

Unloaded, but matured DCs (referred to as unloaded DCs or DCs only) are not deliberately loaded with tumour-specific antigens. However, matured DCs will present peptides with which they came into contact and DCs will never express MHC molecules without bound peptide in the MHC groove. In this experiment DCs will have taken up peptides during the culturing process. These peptides/antigens will most likely not overlap with tumour associated antigens.
Mice treated with mesothelioma lysate loaded DCs had a significant delayed tumour growth, indicating that loading DCs with mesothelioma lysate induces a tumour-specific immune response directed against the pancreatic tumour (Figure 6).

Example 5

Induction of pancreas tumour-specific immune response

To monitor whether mesothelioma lysate loaded DCs induce a pancreas tumour-specific immune response, splenocytes and tumours from treated and untreated tumour-bearing mice from Example 4 were isolated on the day of sacrifice. Bone marrow was harvested from wild type non-tumour bearing mice for the culture of mature DCs.
DCs were cultured from mouse bone marrow with GM-CSF and loaded with autologous pancreas tumour lysate or with healthy lung lysate as a control. Autologous pancreatic lysate and healthy lung lysate were made from snap frozen end stage tumours or lung tissue, respectively, by bead mediated homogenisation. DCs loaded with autologous pancreatic tumour- or control lung lysate were co-cultured with thawed splenocytes for 24 hours. A schematic overview of this (potency) assay is depicted in Figure 7.

Upon co-culture of autologous pancreatic tumour lysate loaded DCs with splenocytes from mice treated with mesothelioma loaded DCs, we found an increased expression of the cytotoxic markers CD107, Granzyme B, and pro-inflammatory cytokines IFNγ and TNFα in CD8+ T-cells, as compared to splenocytes from untreated mice or from mice treated with unloaded DCs (Figure 8).

The increase in these cytotoxic markers and pro-inflammatory cytokines was not observed when DCs loaded with control lung lysate were co-cultured with splenocytes from treated or untreated mice.

Example 6

Description of Manufacturing Process of MesoPher Drug Substance for clinical use (Dendritic cells, loaded with a tumour lysate).

The apheresis product is the cellular starting material, it is generated by standard 9L leukapheresis procedure to collect mononuclear cells using an apheresis unit according to hospital procedures. After the procedure, the product is transferred to the cleanroom and prepared for CliniMACS procedure by labeling with CD14+ Microbeads. The CD14+ monocyte cell product is transferred to 200 ml conical tubes, centrifuged, and resuspended in X-VIVO15 medium supplemented with 2% Human serum/HS(= culture medium) into a final concentration of $100*10^6$ /30 ml. This cell suspension is seeded into 225 cm^2 culture flasks, 30 ml per flask. The flasks are incubated overnight in a 37°C, 5% CO_2 incubator. The remaining cells are cryopreserved in 10% DMSO.

At day 2, 15 ml of culture medium is replaced with 15 ml fresh culture medium supplemented with cytokines GM-CSF and IL-4 for each culture flask. The final concentration of the cytokines is 800 IU/ml GM-CSF and 500 IU/ml IL-4. The monocytes are cultured at 37°C, 5% CO_2 for 4 days.

At day 5, cells are harvested from the flasks into 200 ml tubes and centrifuged. The cell product is diluted to $0.5x10^6$/ml using culture medium in an end volume of maximum 840 ml ($420*10^6$ DC) and minimum 200 ml ($100*10^6$ DC). This suspension is supplemented with 800 IU/ml GM-CSF, 500 IU/ml IL-4, 1:3 TCE PheraLys product /DC (TCE: tumour cell equivalent), and 10 ug/ml endotoxin-free Keyhole Limpet Hemocyanin (KLH). This cell suspension is plated into 6-wells plates. The 6-well tissue culture plates are incubated for 2 additional days in a 37°C, 5% CO_2 incubator.

At day 8, DC are matured through the addition of fresh culture medium supplemented with maturation factors to a final concentration of 5 ng/ml IL-1β, 15 ng/ml IL-6, 20 ng/ml TNF-α and 10 µg/ml PGE2. The 6-well tissue culture plates are incubated for 2 additional days in a 37°C, 5% CO_2 incubator.

At day 10, the mature DC are harvested and centrifuged. After centrifugation, culture supernatant is collected separately. Cells are resuspended and pooled in 50 ml PBS. On this suspension a density gradient centrifugation (Lymphoprep) step is performed in 2x50ml tubes to remove excess PheraLys. Cells are collected from the interface of the gradient (the

DC) and washed in PBS by centrifugation. End volume of this suspension is 50 ml in a 50 ml tube. Total cell numbers are defined by a cell counting.
The cell suspension generated in Step 10 is defined as MesoPher Drug Substance (DS).

Example 7

Clinical use of a lysate or pharmaceutical composition according to the invention for the treatment of pancreatic cancer.

A phase II study with MesoPher in patients with pancreatic cancer has been carried out. The study synopsis is as follows:

Objectives: To investigate feasibility, safety and toxicity as well as the induced immune response upon vaccination with an allogeneic tumour cell lysate loaded onto autologous dendritic cells in resected pancreatic cancer patients who received standard of care treatment.

Study design: An open-label, single centre phase II study

Study Population: Patients older than 18 years with surgically resected pancreatic cancer who received standard of care treatment

Sample size: 10 patients

Main objective of the trial: To investigate feasibility of an allogeneic tumor cell lysate (PheraLys) loaded onto autologous dendritic cells (MesoPher) in resected pancreatic cancer patients who received adjuvant standard of care treatment

Secondary objectives of the trial: To investigate safety and toxicity as well as immune-response of an allogeneic tumor cell lysate (PheraLys) loaded onto autologous dendritic cells (MesoPher) in resected pancreatic cancer patients who received standard of care treatment.

Investigational treatment:

Formulation: MesoPher: autologous monocyte-derived dendritic cells loaded with PheraLys
Dose: 25 million loaded DCs
Route of administration: 1/3 intradermal injection in the forearm and 2/3 intravenously
Number of doses: Five vaccinations in total.
Schedule of doses: 3 biweekly doses and 2 additional gifts (3 and 6 months after last dose)
Inclusion criteria:

- Surgically resected pancreatic cancer.
- Completed post-operative standard treatment. Standard of care treatment includes the choice of adjuvant chemotherapy. Patients who did not complete adjuvant chemotherapy due to toxicity or who are not able to start standard of care due to

specific reasons are allowed to participate in the study after approval of the coordinating investigator.

- No disease activity as assessed by radiological imaging.
- Patients must be at least 18 years old and must be able to give written informed consent.
- Patients must be ambulatory (WHO-ECOG performance status 0,1 or 2) and in stable medical condition.
- Patients must have normal organ function and adequate bone marrow reserve: absolute neutrophil count > 1.0 x 109/l, platelet count > 100 x 109/l, and Hb > 6.0 mmol/l (as determined during screening).
- Positive DTH skin test (induration > 2mm after 48 hrs) against at least one positive control antigen tetanus toxoid (see section 8.3 for DTH skin test procedure).
- Women of childbearing potential must have a negative serum pregnancy test at screening and a negative urine pregnancy test just prior to the first study drug administration on Day 1, and must be willing to use an effective contraceptive method (intrauterine devices, hormonal contraceptives, contraceptive pill, implants, transdermal patches, hormonal vaginal devices, infusions with prolonged release) or true abstinence (when this is in line with the preferred and usual lifestyle)* during the study and for at least 12 months after the last study drug administration.

*True abstinence is acceptable when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (such as calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception. Men must be willing to use an effective contraceptive method (e.g. condom, vasectomy) during the study and for at least 12 months after the last study drug administration.

- Ability to return to the hospital for adequate follow-up as required by this protocol.
- Written informed consent according to ICH-GCP.

Exclusion criteria:

- Medical or psychological impediment to probable compliance with the protocol.
- Current or previous treatment with immunotherapeutic agents.
- Current use of steroids (or other immunosuppressive agents). Patients must have had 6 weeks of discontinuation and must stop any such treatment during the time of the study. Prophylactic usage of dexamethasone during chemotherapy is excluded from this 6 weeks interval.
- Prior malignancy except adequately treated basal cell or squamous cell skin cancer, superficial or in-situ cancer of the bladder or other cancer for which the patient has been disease-free for five years.

- Serious concomitant disease, or active infections.
- History of autoimmune disease or organ allografts (or with active acute or chronic infection, including HIV and viral hepatitis).
- Serious intercurrent chronic or acute illness such as pulmonary disease (asthma or COPD), cardiac disease (NYHA class III or IV), hepatic disease or other illness considered by the study coordinator to constitute an unwarranted high risk for investigational DC treatment.
- Known allergy to shell fish (may contain keyhole limpet hemocyanin (KLH)).
- Pregnant or lactating women.
- Inadequate peripheral vein access to perform leukapheresis.
- Concomitant participation in another clinical intervention trial (except participation in a biobank study).
- An organic brain syndrome or other significant psychiatric abnormality which would compromise the ability to give informed consent, and preclude participation in the full protocol and follow-up.
- Absence of assurance of compliance with the protocol. Lack of availability for follow-up assessment.

Results

- The preliminary results of the above mentioned clinical trial have shown that none of the patients that were enrolled in the present study suffered from progression of the disease. The patients that were sufficiently long in the study (6 out of 10) had a mean progression free survival of 23 months and no patients have experienced progression of the disease or has died, the overall survival has thus also been extended to at least 23 months (calculated from the date of surgical resection). In this regard it is also noted that none of the other patients) suffered from progression of the disease.

- The results further show that none of the patients suffered from severe adverse effects that related to the treatment. The most reported side adverse effect was a mild injection side reaction. Hence, the present invention is also very suitable for relatively weak patients.

- These remarkable results show that with the present invention it has become possible to considerably extend the life expectancy of patients suffering from pancreatic cancer and avoid serious adverse effects. The preliminary results of this study are shown in the table below. With respect to the present results it is again noted that

none of the patients have yet died, meaning that the progression free survival of all patients treated according the invention is at least more than 23.8 months. Furthermore, the overall survival is also expected to be longer than reported with chemotherapy alone.

Patient ID	PFS	OS
1	23.5	23.5
2	22.1	22.1
3	38.7	38.7
4	15.9	15.9
5	18.4	18.4
6	20.6	20.6

References

1. Fest J, Ruiter R, van Rooij FJ, van der Geest LG, Lemmens VE, Ikram MA, et al. Underestimation of pancreatic cancer in the national cancer registry - Reconsidering the incidence and survival rates. Eur J Cancer. 2017;72:186-91.

2. Rahib L, Smith BD, Aizenberg R, Rosenzweig AB, Fleshman JM, Matrisian LM. Projecting cancer incidence and deaths to 2030: the unexpected burden of thyroid, liver, and pancreas cancers in the United States. Cancer Res. 2014;74(11):2913-21.

3. (IKNL), N.C.C.N., Pancreas- en periampullair carcinoom. - Kankerzorg in beeld. 2014.

4. Cleary SP, Gryfe R, Guindi M, Greig P, Smith L, Mackenzie R, et al. Prognostic factors in resected pancreatic adenocarcinoma: analysis of actual 5-year survivors. J Am Coll Surg. 2004;198(5):722-31.

5. Paniccia A, Hosokawa P, Henderson W, Schulick RD, Edil BH, McCarter MD, et al. Characteristics of 10-Year Survivors of Pancreatic Ductal Adenocarcinoma. JAMA Surg. 2015;150(8):701-10.

6. Neoptolemos JP, Palmer DH, Ghaneh P, Psarelli EE, Valle JW, Halloran CM, et al. Comparison of adjuvant gemcitabine and capecitabine with gemcitabine monotherapy in patients with resected pancreatic cancer (ESPAC-4): a multicentre, open-label, randomised, phase 3 trial. Lancet (London, England). 2017;389(10073):1011-24.

7. Loehrer P PM, Cardenes HR, et al. A randomized phase III study of gemcitabine in combination with radiation therapy versus gemcitabine alone in patients with localized, unresectable pancreatic cancer. E4201 J Clin Oncol Suppl(abstract 4506). 2008.

8. Chen J, Guo XZ, Li HY, Liu X, Ren LN, Wang D, et al. Generation of CTL responses against pancreatic cancer in vitro using dendritic cells co-transfected with MUC4 and survivin RNA. Vaccine. 2013;31(41):4585-90.

9. Lepisto AJ, Moser AJ, Zeh H, Lee K, Bartlett D, McKolanis JR, et al. A phase I/II study of a MUC1 peptide pulsed autologous dendritic cell vaccine as adjuvant therapy in patients with resected pancreatic and biliary tumors. Cancer Ther. 2008;6(B):955-64.

10. Poch B, Lotspeich E, Ramadani M, Gansauge S, Beger HG, Gansauge F. Systemic immune dysfunction in pancreatic cancer patients. Langenbecks Arch Surg. 2007;392(3):353-8.

11. Ikemoto T, Yamaguchi T, Morine Y, Imura S, Soejima Y, Fujii M, et al. Clinical roles of increased populations of Foxp3+CD4+ T cells in peripheral blood from advanced pancreatic cancer patients. Pancreas. 2006;33(4):386-90.

12. Carstens JL, Correa de Sampaio P, Yang D, Barua S, Wang H, Rao A, et al. Spatial computation of intratumoral T cells correlates with survival of patients with pancreatic cancer. Nat Commun. 2017;8:15095.

13. Aerts JG, Hegmans JP. Tumor-specific cytotoxic T cells are crucial for efficacy of immunomodulatory antibodies in patients with lung cancer. Cancer Res. 2013;73(8):2381-8.

14. Royal RE, Levy C, Turner K, Mathur A, Hughes M, Kammula US, et al. Phase 2 trial of single agent Ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. J Immunother. 2010;33(8):828-33.

15. Aglietta M, Barone C, Sawyer MB, Moore MJ, Miller WH, Jr., Bagala C, et al. A phase I dose escalation trial of tremelimumab (CP-675,206) in combination with gemcitabine in chemotherapy-naive patients with metastatic pancreatic cancer. Ann Oncol. 2014;25(9):1750-5.

16. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med. 2012;366(26):2455-65.

17. Papaioannou NE, Beniata OV, Vitsos P, Tsitsilonis O, Samara P. Harnessing the immune system to improve cancer therapy. Ann Transl Med. 2016;4(14):261.

18. Dammeijer F, Lievense LA, Veerman GD, Hoogsteden HC, Hegmans JP, Arends LR, et al. Efficacy of Tumor Vaccines and Cellular Immunotherapies in Non-Small- Cell Lung Cancer: A Systematic Review and Meta-Analysis. J Clin Oncol. 2016;34(26):3204-

19. Oyasiji T, Ma WW. Novel adjuvant therapies for pancreatic adenocarcinoma. Journal of Gastrointestinal Oncology. 2015;6(4):430-5.

20. D. C. NewLink Genetics Announces Results from Phase 3 IMPRESS Trial of Algenpantucel-L for Patients with Resected Pancreatic Cancer. Press Release: Globe Newswire: NewLink Genetics Corporation. 2016.

21. Lutz E, Yeo CJ, Lillemoe KD, Biedrzycki B, Kobrin B, Herman J, et al. A lethally irradiated allogeneic granulocyte-macrophage colony stimulating factor-secreting tumor vaccine for pancreatic adenocarcinoma. A Phase II trial of safety, efficacy, and immune activation. Ann Surg. 2011;253(2):328-35.

22. Le DT, Wang-Gillam A, Picozzi V, Greten TF, Crocenzi T, Springett G, et al. Safety and survival with GVAX pancreas prime and Listeria Monocytogenes-expressing mesothelin (CRS-207) boost vaccines for metastatic pancreatic cancer. J Clin Oncol. 2015;33(12):1325-33.

23. Jaffee EM, Hruban RH, Biedrzycki B, Laheru D, Schepers K, Sauter PR, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. J Clin Oncol. 2001;19(1):145-56.

24. Dung L, et al (2017). Results from a phase 2b, randomized, multicenter study of GVAX pancreas and CRS-207 compared to chemotherapy in adults with previously-treated metastatic pancreatic adenocarcinoma (ECLIPSE Study). Journal of Clinical Oncology. 35. 345-345.

25. Rosenberg SA, Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science. 2015;348(6230):62-8.

26. Ryschich E, Notzel T, Hinz U, Autschbach F, Ferguson J, Simon I, et al. Control of T-cell-mediated immune response by HLA class I in human pancreatic carcinoma. Clin Cancer Res. 2005;11(2 Pt 1):498-504.

27. von Bernstorff W, Voss M, Freichel S, Schmid A, Vogel I, Johnk C, et al. Systemic and local immunosuppression in pancreatic cancer patients. Clin Cancer Res. 2001;7(3 Suppl):925s-32s.

28. Klebanoff CA, Rosenberg SA, Restifo NP. Prospects for gene-engineered T cell immunotherapy for solid cancers. Nat Med. 2016;22(1):26-36.
immunotherapeutics. Immunotherapy. 2011;3(4):517-37.

29. Johnson LA, Morgan RA, Dudley ME, Cassard L, Yang JC, Hughes MS, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009;114(3):535-46.

30. Lamers CH, Sleijfer S, van Steenbergen S, van Elzakker P, van Krimpen B, Groot C, et al. Treatment of metastatic renal cell carcinoma with CAIX CAR-engineered T cells: clinical evaluation and management of on-target toxicity. Mol Ther. 2013;21(4):904-12.

31. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2011;19(3):620-6.

32. Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res. 2014;2(2):112-20.
33. Linette GP, Stadtmauer EA, Maus MV, Rapoport AP, Levine BL, Emery L, et al. Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. Blood. 2013;122(6):863-71.
34. Morgan RA, Chinnasamy N, Abate-Daga D, Gros A, Robbins PF, Zheng Z, et al. Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy. J Immunother. 2013;36(2):133-51.
35. Debets R, Donnadieu E, Chouaib S, Coukos G. TCR-engineered T cells to treat tumors: Seeing but not touching? Semin Immunol. 2016;28(1):10-21.
36. Banchereau J, Steinman RM. Dendritic cells and the control of immunity. Nature. 1998;392(6673):245-52.
37. Hansen SG, Wu HL, Burwitz BJ, Hughes CM, Hammond KB, Ventura AB, et al. Broadly targeted CD8(+) T cell responses restricted by major histocompatibility complex E. Science. 2016;351(6274):714-20.
38. Heuvers ME, Aerts JG, Cornelissen R, Groen H, Hoogsteden HC, Hegmans JP. Patient-tailored modulation of the immune system may revolutionize future lung cancer treatment. BMC Cancer. 2012;12:580.
39. Cornelissen R, Lievense LA, Heuvers ME, Maat AP, Hendriks RW, Hoogsteden HC, et al. Dendritic cell-based immunotherapy in mesothelioma. Immunotherapy. 2012;4(10):1011-22.
40. DeMatos P, Abdel-Wahab Z, Vervaert C, Seigler HF. Vaccination with dendritic cells inhibits the growth of hepatic metastases in B6 mice. Cell Immunol. 1998;185(1):65-74.
41. Fields RC, Shimizu K, Mule JJ. Murine dendritic cells pulsed with whole tumor lysates mediate potent antitumor immune responses in vitro and in vivo. Proc Natl Acad Sci U S A. 1998;95(16):9482-7.
42. Palucka AK, Ueno H, Connolly J, Kerneis-Norvell F, Blanck JP, Johnston DA, et al. Dendritic cells loaded with killed allogeneic melanoma cells can induce objective clinical responses and MART-1 specific CD8+ T-cell immunity. J Immunother. 2006;29(5):545-57.
43. Thomas-Kaskel AK, Zeiser R, Jochim R, Robbel C, Schultze-Seemann W, Waller CF, et al. Vaccination of advanced prostate cancer patients with PSCA and PSA peptide-loaded dendritic cells induces DTH responses that correlate with superior overall survival. Int J Cancer. 2006;119(10):2428-34.
44. Pecher G, Haring A, Kaiser L, Thiel E. Mucin gene (MUC1) transfected dendritic cells as vaccine: results of a phase I/II clinical trial. Cancer Immunol Immunother. 2002;51(11-12):669-73.

45. Stift A, Friedl J, Dubsky P, Bachleitner-Hofmann T, Schueller G, Zontsich T, et al. Dendritic cell-based vaccination in solid cancer. J Clin Oncol. 2003;21(1):135-42.
46. Kondo H, Hazama S, Kawaoka T, Yoshino S, Yoshida S, Tokuno K, et al. Adoptive immunotherapy for pancreatic cancer using MUC1 peptide-pulsed dendritic cells and activated T lymphocytes. Anticancer Res. 2008;28(1B):379-87.
47. Koido S, Homma S, Okamoto M, Takakura K, Mori M, Yoshizaki S, et al. Treatment with chemotherapy and dendritic cells pulsed with multiple Wilms' tumor 1 (WT1)- specific MHC class I/II-restricted epitopes for pancreatic cancer. Clin Cancer Res. 2014;20(16):4228-39.
48. Mayanagi S, Kitago M, Sakurai T, Matsuda T, Fujita T, Higuchi H, et al. Phase I pilot study of Wilms tumor gene 1 peptide-pulsed dendritic cell vaccination combined with gemcitabine in pancreatic cancer. Cancer Sci. 2015;106(4):397-406.
49. Mehrotra S, Britten CD, Chin S, Garrett-Mayer E, Cloud CA, Li M, et al. Vaccination with poly(IC:LC) and peptide-pulsed autologous dendritic cells in patients with pancreatic cancer. J Hematol Oncol. 2017;10(1):82.
50. Bauer C, Dauer M, Saraj S, Schnurr M, Bauernfeind F, Sterzik A, et al. Dendritic cell-based vaccination of patients with advanced pancreatic carcinoma: results of a pilot study. Cancer Immunol Immunother. 2011;60(8):1097-107.
51. Suso EM, Dueland S, Rasmussen AM, Vetrhus T, Aamdal S, Kvalheim G, et al. hTERT mRNA dendritic cell vaccination: complete response in a pancreatic cancer patient associated with response against several hTERT epitopes. Cancer Immunol Immunother. 2011;60(6):809-18.
52. Amedei A, Niccolai E, Prisco D. Pancreatic cancer: role of the immune system in cancer progression and vaccine-based immunotherapy. Hum Vaccin Immunother. 2014;10(11):3354-68.
53. Okamoto M, Kobayashi M, Yonemitsu Y, Koido S, Homma S. Dendritic cellbased vaccine for pancreatic cancer in Japan. World J Gastrointest Pharmacol Ther. 2016;7(1):133-8.
54. Aerts JGJV, de Goeje PL, Cornelissen R, Kaijen-Lambers MEH, Bezemer K, van der Leest CH, Mahaweni NM, Kunert A, Eskens FALM, Waasdorp C, Braakman E, van der Holt B, Vulto AG, Hendriks RW, Hegmans JPJJ, Hoogsteden HC. Autologous Dendritic Cells Pulsed with Allogeneic Tumor Cell Lysate in Mesothelioma: From Mouse to Human. Clin Cancer Res. 2018 Feb 15;24(4):766-776
55. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA, Ly A, Lie WR, Hildebrand WH, Mardis ER, Linette GP. Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science. 201;348(6236):803-8
56. Ho MY, Tang SJ, Sun KH, Yang W. Immunotherapy for lung cancers. J Biomed Biotechnol. 2011;2011:250860

57. Hegmans JP, Veltman JD, Lambers ME, de Vries IJ, Figdor CG, Hendriks RW, Hoogsteden HC, Lambrecht BN, Aerts JG. Consolidative dendritic cell-based immunotherapy elicits cytotoxicity against malignant mesothelioma. Am J Respir Crit Care Med. 2010 Jun 15;181(12):1383-90.

58. Banchereau J, Palucka AK. Dendritic cells as therapeutic vaccines against cancer. Nat Rev Immunol. 2005 Apr;5(4):296-306.

59. Berger TG, Strasser E, Smith R, Carste C, Schuler-Thurner B, Kaempgen E, Schuler G. Efficient elutriation of monocytes within a closed system (Elutra) for clinical-scale generation of dendritic cells. J Immunol Methods. 2005 Mar;298(1-2):61-72.

60. Magnusson A, Laurell A, Lonnemark M, Brekkan E, Adamson L, Tolf A, Andersson B, Wallgren A, KiesslingR, and Karlsson-Parra A. Intratumoral vaccination with activated allogeneic dendritic cells in patients with newly diagnosed metastatic renal cell carcinoma (mRCC). J Clin Oncol. 2014;32:15_suppl:3085-3085

61. Vonderheide RH, Burg JM, Mick R, et al. Phase I study of the CD40 agonist antibody CP-870,893 combined with carboplatin and paclitaxel in patients with advanced solid tumors. Oncoimmunology. 2013;2(1):e23033

62. Vitale LA, Thomas LJ, He LZ, O'Neill T, Widger J, Crocker A, Sundarapandiyan K, Storey JR, Forsberg EM, Weidlick J, Baronas AR, Gergel LE, Boyer JM, Sisson C, Goldstein J, Marsh HC Jr, Keler T. Development of CDX-1140, an agonist CD40 antibody for cancer immunotherapy. Cancer Immunol Immunother. 2018 Oct 31. doi: 10.1007/s00262-018-2267-0. [Epub ahead of print]

63. Habib M, Noval Rivas M, Chamekh M, Wieckowski S, Sun W, Bianco A, Trouche N, Chaloin O, Dumortier H, Goldman M, Guichard G, Fournel S, Vray B. Cutting edge: small molecule CD40 ligand mimetics promote control of parasitemia and enhance T cells producing IFN-gamma during experimental Trypanosoma cruzi infection. J Immunol. 2007 Jun 1;178(11):6700-4.)

64. Morrison AH, Byrne KT, Vonderheide RH. Immunotherapy and Prevention of Pancreatic Cancer. Trends Cancer. 2018 Jun;4(6):418-428.

65. Vonderheide RH, Bajor DL, Winograd R, Evans RA, Bayne LJ, Beatty GL. CD40 immunotherapy for pancreatic cancer. Cancer Immunol Immunother. 2013 May;62(5):949-54.

66. Oettle et al., Adjuvant chemotherapy with gemcitabine and long-term outcomes among patients with resected pancreatic-cancer, the CONKO-001 randomized trial, JAMA vol. 310; no. 14, 2013.

67. Conroy et al., Folfirinox or Gemcitabine as adjuvant therapy for pancreatic cancer, New England Journal of Medicine, vol. 379; no. 25, 2018.

CLAIMS

1. Method for the treatment of pancreatic cancer comprising administering to patients in need thereof dendritic cells loaded with a lysate, wherein the lysate is obtainable by a method comprising the steps of:
i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;
ii) inducing necrosis in said tumour cells; and
iii) lysing the necrotic tumour cells, such that a lysate is obtained; and
wherein said treatment extends the median progression free survival and/or median overall survival of said patients.

2. Method according to claim 1, wherein said patients have been subjected to surgical resection of said pancreatic cancer.

3. Method according to any of the previous claims, wherein the median progression free survival of said patients is extended to at least 18 months, at least 20 months, at least 25 months, at least 30 months or at least 35 months after pancreatic cancer resection.

4. Method according to any of the previous claims, wherein said patients receive adjuvant chemotherapy after pancreatic cancer resection, preferably said patients are administered the loaded dendritic cells after resection and adjuvant chemotherapy.

5. Method according to any of the previous claims, wherein the median progression free survival of said patients is extended compared to such patients treated with chemotherapy alone.

6. Method according to any of the previous claims, wherein said adjuvant chemotherapy comprises treatment with gemcitabine or folferinox.

7. Method according to the previous claim, wherein a patient in need thereof is administered 6 cycles of gemcitabine every four weeks, consisting of 3 weekly infusions of gemcitabine (1000 mg/m^2), followed by a 1-week break.

8. Method according to any of the previous claims, wherein the median progression free survival is at least 3 months more than, in particular at least 6 months more than the median progression free survival of patients that only received adjuvant chemotherapy

with gemcitabine.

9. Method according to any of the previous claims wherein induction of necrosis of the mesothelioma tumour cells is achieved by subjecting the cells to freeze-thawing cycles.

10. Method according to any of the previous claims, wherein after inducing necrosis and lysing of the tumour cells, the lysate obtained is subjected to at least 50 Gy, preferably at least 100 Gy irradiation.

11. Method according to any of the previous claims, wherein the mesothelioma tumour cells provided comprise tumour cells from at least three, preferably at least four, most preferably at least five mesothelioma tumour cell lines.

12. Method according to any of the previous claims, wherein the mesothelioma tumour cells from the at least two, at least three, at least four, or at least five mesothelioma tumour cell lines are provided in essentially equal amounts.

13. Method according to any of the previous claims, wherein the allogeneic mesothelioma tumour cells used are chosen from two or more of the following cell lines Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

14. Method according to any of the previous claims, wherein the lysate comprises at least three, preferably at least five, more preferably at least ten, mesothelioma cancer cell associated antigens.

15. Method according to the previous claim, wherein the at least three, preferably at least five, more preferably at least ten mesothelioma cancer cell associated antigens are chosen from the group of: RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1, RAB38/NY-MEL-1, BING4, MAGE A12, HER-2/Neu, Glypican, LMP2.

16. Method according to any of the previous claims, wherein the lysate is loaded onto autologous dendritic cells of said patient.

17. Method according to any of the previous claims, wherein the dendritic cells are loaded with between 1 tumour cell equivalents per 100 dendritic cells to 10 tumour cell equivalents per 1 dendritic cell.

18. Method according to any of the previous claims, wherein a patient in need thereof is administered $1*10^6$ to $1*10^9$ loaded dendritic cells, preferably $1*10^7$ to $1*10^8$ loaded dendritic cells, most preferably about $2.5*10^7$ loaded dendritic cells per dose.

19. Dendritic cells loaded with a lysate for use in the treatment of pancreatic cancer, wherein said dendritic cells are administered to a patient in need thereof and wherein the lysate is obtainable by a method comprising the steps of:
i) providing human mesothelioma cells from at least two different mesothelioma tumour cell lines;
ii) inducing necrosis in said tumour cells; and
iii) lysing the necrotic tumour cells, such that a lysate is obtained; and
wherein said treatment extends the median progression free survival and/or median overall survival of said patients.

20. Dendritic cells for use according to claim 1, wherein said patients have been subjected to surgical resection of said pancreatic cancer.

21. Method according to any of the previous claims, wherein the progression free survival of said patients is extended to at least 18 months, at least 20, at least 25 months, at least 30 months or at least 35 months after pancreatic cancer resection.

22. Dendritic cells for use according to any of the previous claims, wherein said patients receive adjuvant chemotherapy after pancreatic cancer resection, preferably said patients are administered the loaded dendritic cells after resection and adjuvant chemotherapy.

23. Dendritic cells for use according to any of the previous claims, wherein the median progression free survival of said patients is extended compared to such patients treated with chemotherapy alone.

24. Dendritic cells for use according to any of the previous claims, wherein said adjuvant chemotherapy comprises treatment with gemcitabine or folferinox.

25. Dendritic cells for use according to any of the previous claims, wherein a patient in need thereof is administered 6 cycles of gemcitabine every four weeks, consisting of 3 weekly infusions of gemcitabine (1000 mg/m^2), followed by a 1-week break.

26. Dendritic cells according to any of the previous claims, wherein the median progression free survival is at least 3 months more than, in particular at least 6 months more than the median progression free survival of patients that only received adjuvant chemotherapy with gemcitabine.

27. Dendritic cells for use according to any of the previous claims. wherein induction of necrosis of the mesothelioma tumour cells is achieved by subjecting the cells to freeze-thawing cycles.

28. Dendritic cells for use according to any of the previous claims, wherein after inducing necrosis and lysing of the tumour cells, the lysate obtained is subjected to at least 50 Gy, preferably at least 100 Gy irradiation.

29. Dendritic cells for use according to any of the previous claims, wherein the mesothelioma tumour cells provided comprise tumour cells from at least three, preferably at least four, most preferably at least five mesothelioma tumour cell lines.

30. Dendritic cells for use according to any of the previous claims, wherein the mesothelioma tumour cells from the at least two, at least three, at least four, or at least five mesothelioma tumour cell lines are provided in essentially equal amounts.

31. Dendritic cells for use according to any of the previous claims, wherein the allogeneic mesothelioma tumour cells used are chosen from two or more of the following cell lines Thorr 01 (deposit No. DSM ACC3192), Thorr 02 (deposit No. DSM ACC3193), Thorr 03 (deposit No. DSM ACC3191), Thorr 05 (deposit No. DSM ACC3194), Thorr 06 (deposit No. DSM ACC3195).

32. Dendritic cells for use according to any of the previous claims, wherein the lysate comprises at least three, preferably at least five, more preferably at least ten, mesothelioma cancer cell associated antigens.

33. Dendritic cells for use according to the previous claim, wherein the at least three, preferably at least five, more preferably at least ten mesothelioma cancer cell associated

antigens are chosen from the group of: RAGE1/MOK, Mesothelin, EphA2, Survivin, WT1, MUC1, RAB38/NY-MEL-1, BING4, MAGE A12, HER-2/Neu, Glypican, LMP2.

34. Dendritic cells for use according to any of the previous claims, wherein the lysate is loaded onto autologous dendritic cells of said patient.

35. Dendritic cells for use according to any of the previous claims, wherein the dendritic cells are loaded with between 1 tumour cell equivalents per 100 dendritic cells to 10 tumour cell equivalents per 1 dendritic cell.

36. Dendritic cells for use according to any of the previous claims, wherein a patient in need thereof is administered $1*10^6$ to $1*10^9$ loaded dendritic cells, preferably $1*10^7$ to $1*10^8$ loaded dendritic cells, most preferably about $2.5 *10^7$ loaded dendritic cells per dose.

37. Pharmaceutical composition for use in the treatment of pancreatic cancer, obtainable by a method comprising the steps of:
    i) providing allogeneic mesothelioma tumour cells from at least two different cell lines, and preparing a lysate thereof;
    ii) providing dendritic cells;
    iii) loading the dendritic cells with the lysate of tumour cells and, optionally, providing and adding a pharmaceutically acceptable carrier.

38. Pharmaceutical composition for use according to the previous claim, wherein said treatment extends the median progression free survival and/or median overall survival of patients suffering from pancreatic cancer.

39. Pharmaceutical composition for use according to any of the previous claims, wherein said patients have been subjected to surgical resection of said pancreatic cancer.

40. Method according to any of the previous claims, wherein the progression free survival of said patients is extended to at least 18 months, at least 20 months, at least 25 months, at least 30 months or at least 35 months after pancreatic cancer resection.

41. Pharmaceutical composition for use according to any of the previous claims, wherein said patients receive adjuvant chemotherapy after pancreatic cancer resection, preferably said patients are administered the loaded dendritic cells after resection and adjuvant

chemotherapy.

42. Pharmaceutical composition according to any of the previous claims, wherein the median progression free survival of said patients is extended compared to such patients treated with chemotherapy alone.

43. Pharmaceutical composition according to any of the previous claims, wherein said adjuvant chemotherapy comprises treatment with gemcitabine or folferinox.

44. Pharmaceutical composition for use according to the previous claim, wherein a patient in need thereof is administered 6 cycles of gemcitabine every four weeks, consisting of 3 weekly infusions of gemcitabine (1000 mg/m^2), followed by a 1-week break.

45. Pharmaceutical composition according to any of the previous claims, wherein the median progression free survival is at least 3 months more than, in particular at least 6 months more than the median progression free survival of patients that only received adjuvant chemotherapy with gemcitabine.

46. Pharmaceutical composition for use according to any of the previous claims, wherein a dosage of the composition administered to a patient comprises $1*10^6$ to $1*10^9$ dendritic cells, preferably $1*10^7$ to $1*10^8$ dendritic cells, most preferably about 2.5 $*10^7$ dendritic cells.

47. Pharmaceutical composition for use according to any of the previous claims, wherein the dendritic cells are loaded with between 1 tumour cell equivalents per 100 dendritic cells to 10 tumour cell equivalents per 1 dendritic cell.

48. Pharmaceutical composition for use according to any of the previous claims, wherein a dosage as defined in the previous claim is administered at least once, preferably at least twice, more preferably at least three times, most preferably at least five times.

49. Pharmaceutical composition for use according to claim 43 or 44, wherein the at least five doses are to be administered in about two-weekly intervals for the first three doses and additional doses at about 3 and about 6 months after the last dose.

50. Pharmaceutical composition for use according to any of the previous claims, wherein the composition comprises an adjuvant.

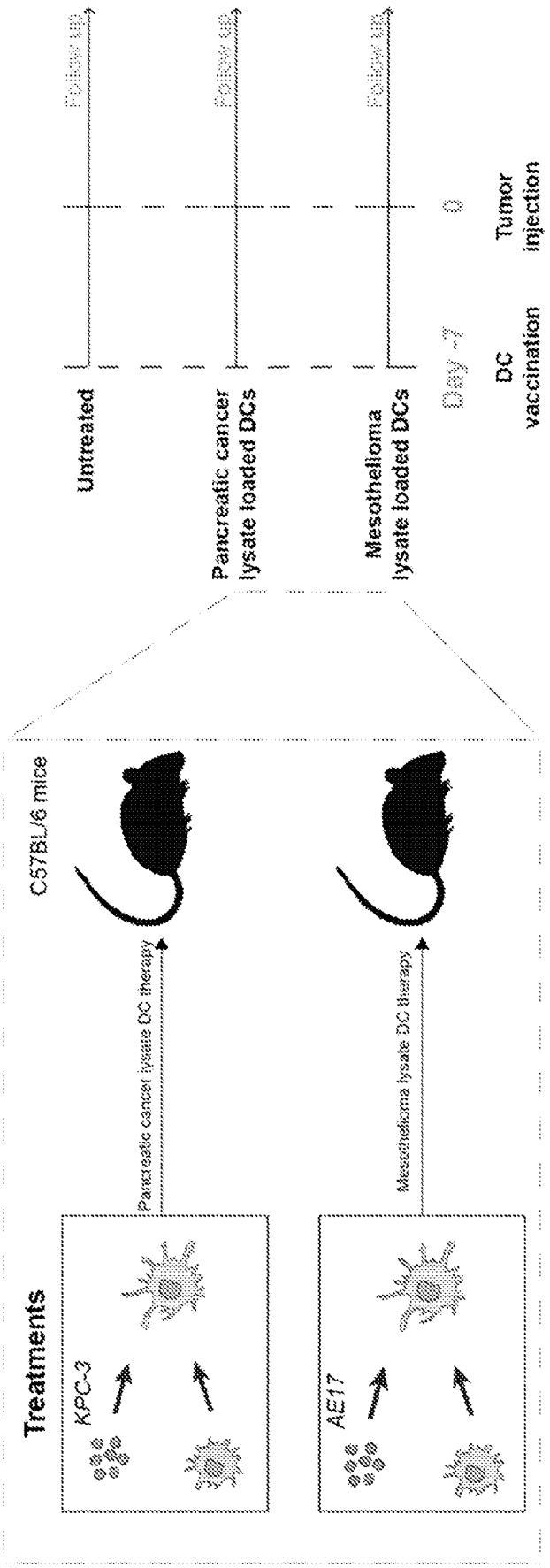
Treatments
KPC-3
AE17
Pancreatic cancer lysate DC therapy
Mesothelioma lysate DC therapy
C57BL/6 mice
Untreated
Pancreatic cancer lysate loaded DCs
Mesothelioma lysate loaded DCs
Follow up
Follow up
Follow up
Day -7
DC vaccination
0
Tumor injection

Figure 1

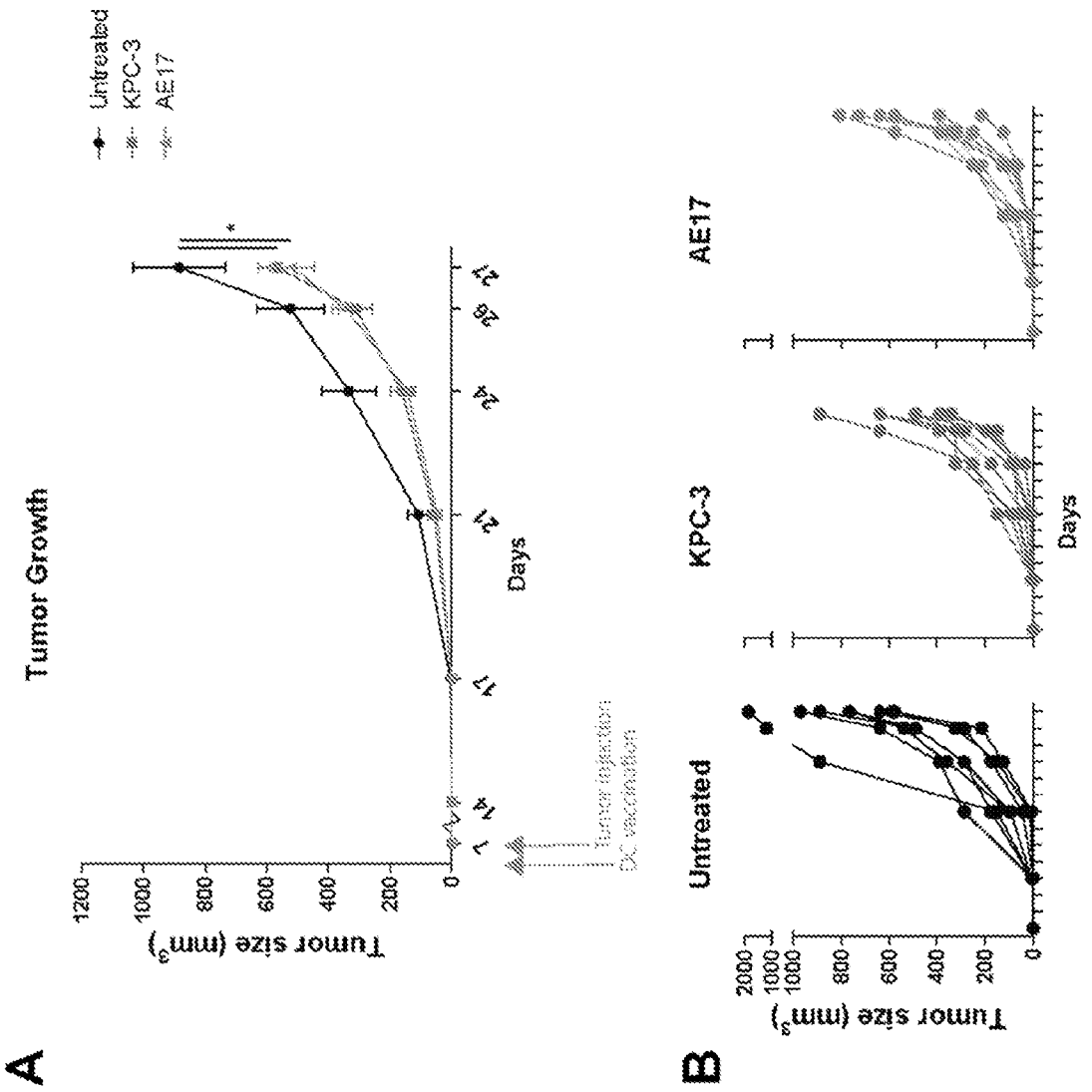
A
Tumor Growth
Untreated
KPC-3
AE17
Tumor size (mm³)
1200
1000
800
600
400
200
0
*
Days
Tumor injection
DC vaccination
B
Untreated
KPC-3
AE17
Tumor size (mm³)
2000
1000
800
600
400
200
0
Days

Figure 2

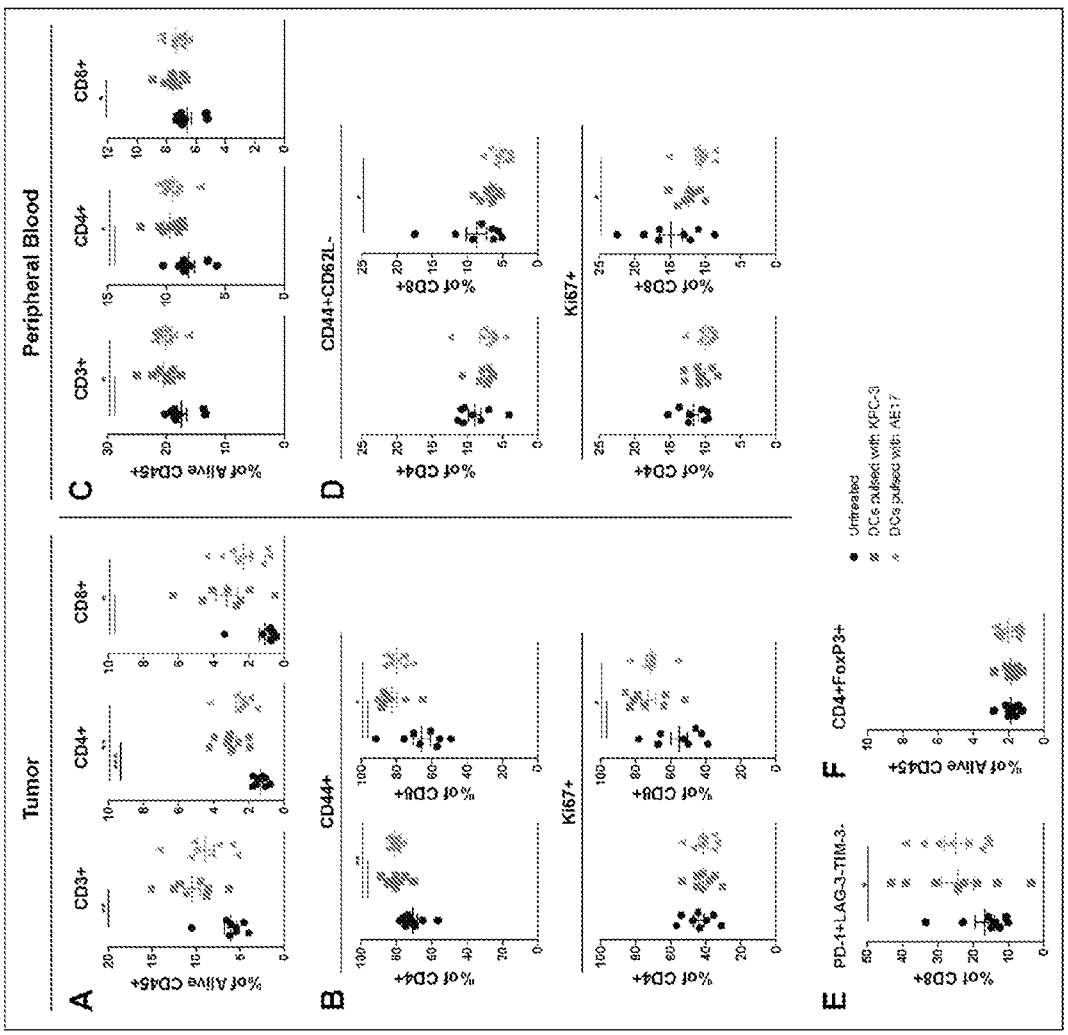
Tumor
Peripheral Blood
A
B
C
D
E
F
CD3+
CD4+
CD8+
CD44+
Ki67+
CD44+CD62L-
PD-1+LAG-3-TIM-3-
CD4+FoxP3+
%of Alive CD45+
%of CD4+
%of CD8+
Untreated
DCs pulsed with KPC-3
DCs pulsed with AE17

Figure 3

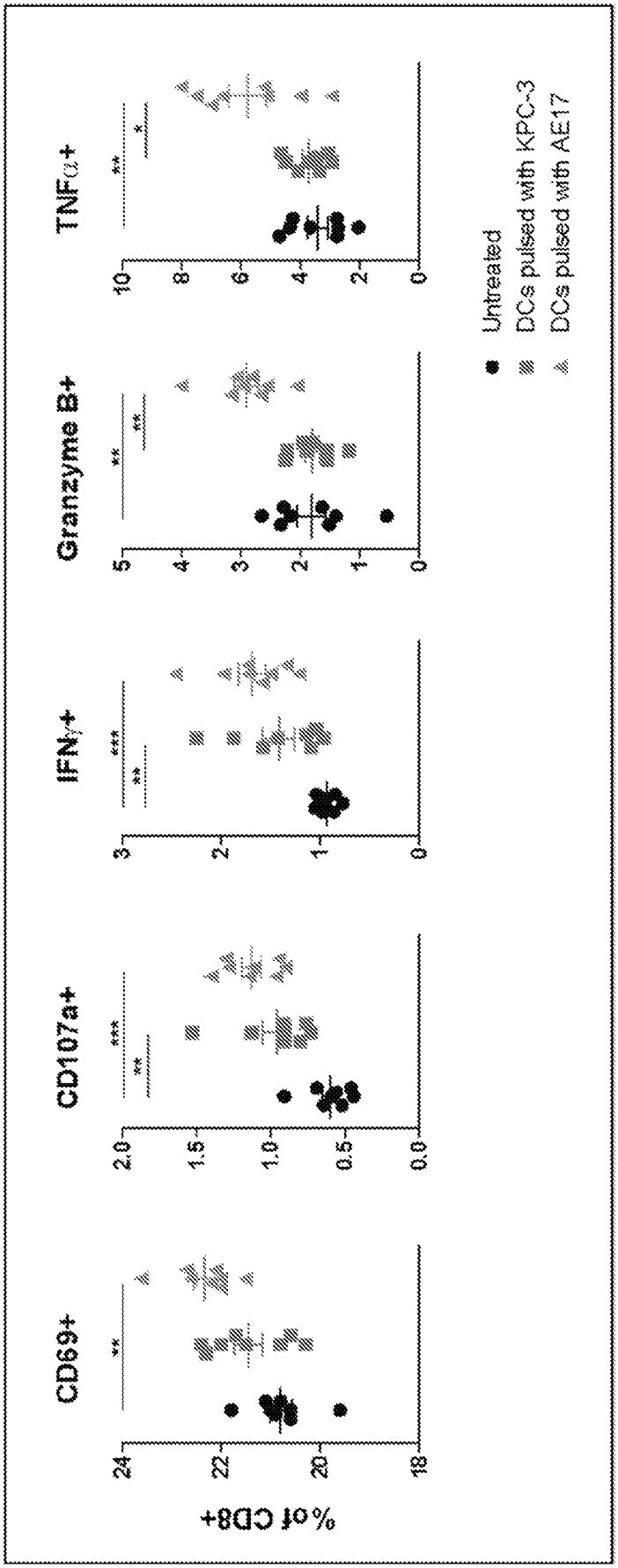
CD69+
CD107a+
IFNγ+
Granzyme B+
TNFα+
% of CD8+
Untreated
DCs pulsed with KPC-3
DCs pulsed with AE17

Figure 4

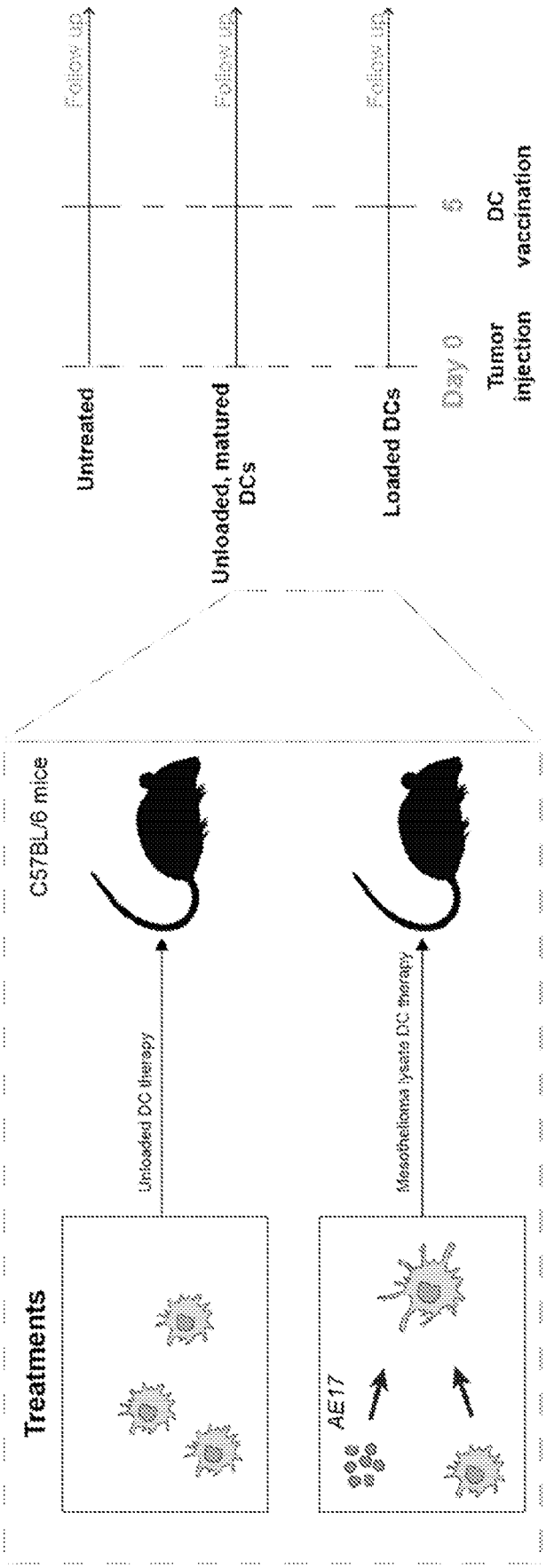
Treatments
C57BL/6 mice
Unloaded DC therapy
Mesothelioma lysate DC therapy
AE17
Untreated
Unloaded, matured DCs
Loaded DCs
Day 0
Tumor injection
6
DC vaccination
Follow up
Follow up
Follow up

Figure 5

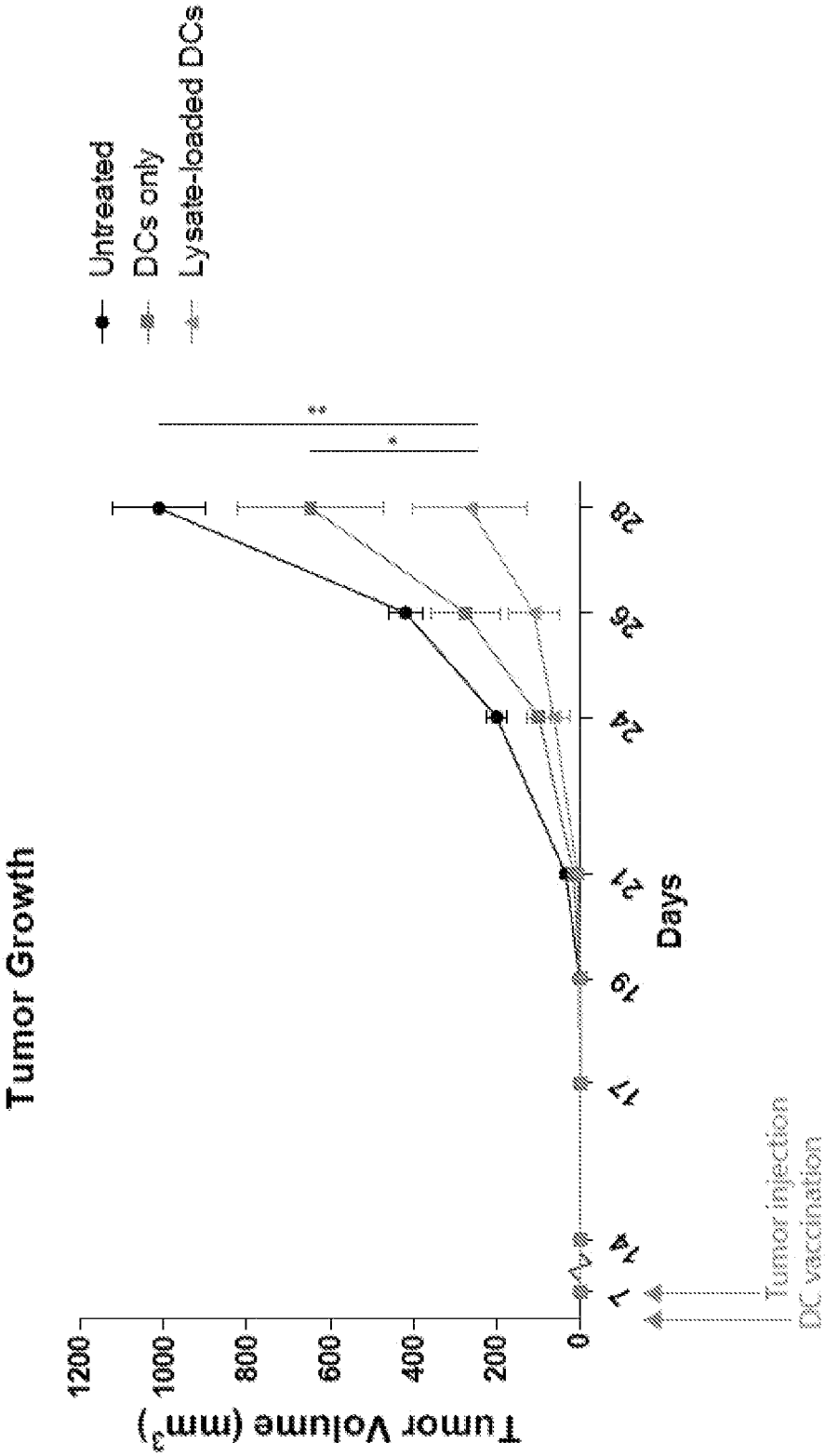
Tumor Growth
Tumor Volume (mm³)
1200
1000
800
600
400
200
0
Days
1
14
17
19
21
24
26
28
Tumor injection
DC vaccination
Untreated
DCs only
Lysate-loaded DCs
*
**

Figure 6

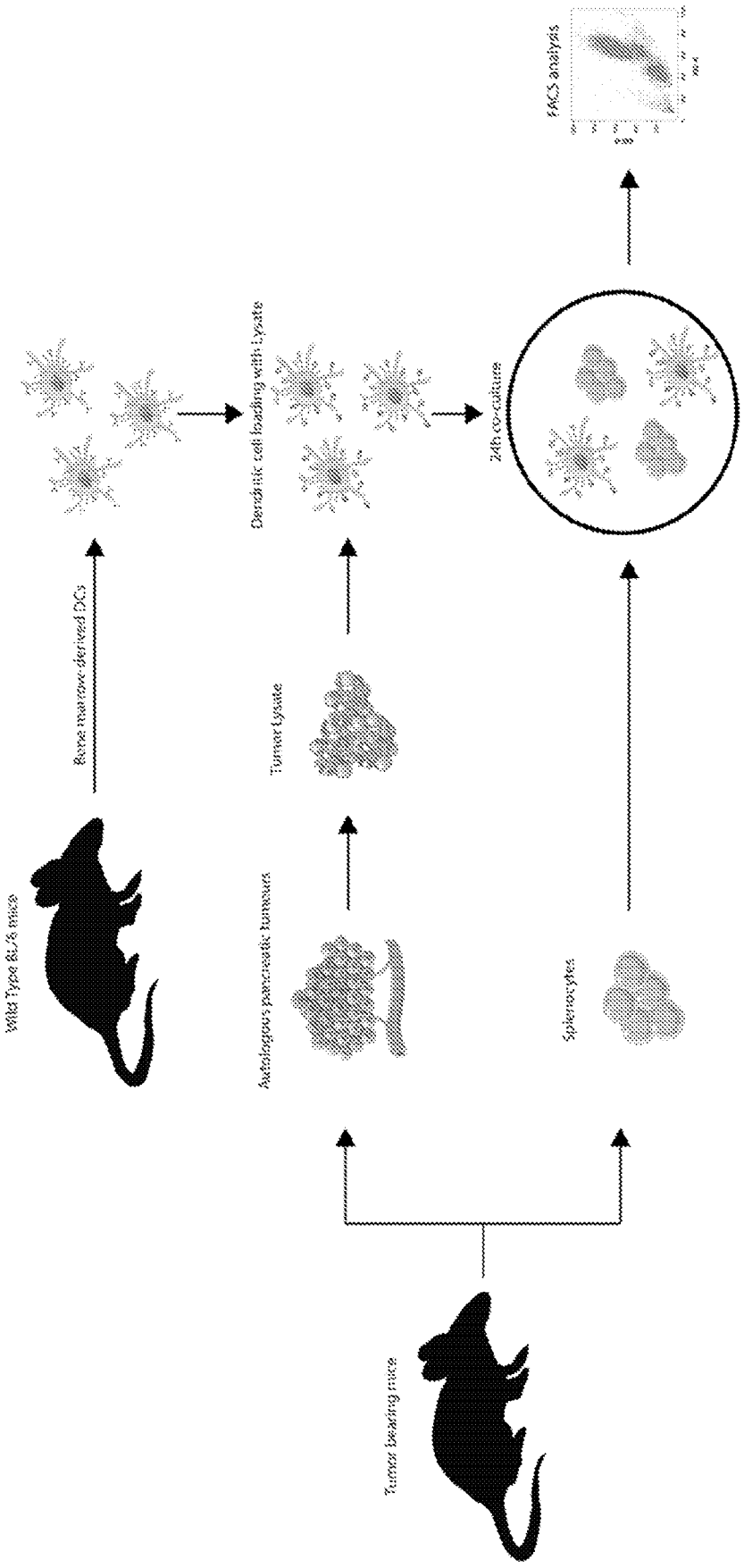
Tumor bearing mice
Wild Type BL/6 mice
Bone marrow derived DCs
Autologous pancreatic tumours
Tumor Lysate
Dendritic cell loading with Lysate
Splenocytes
24h co-culture
FACS analysis

Figure 7

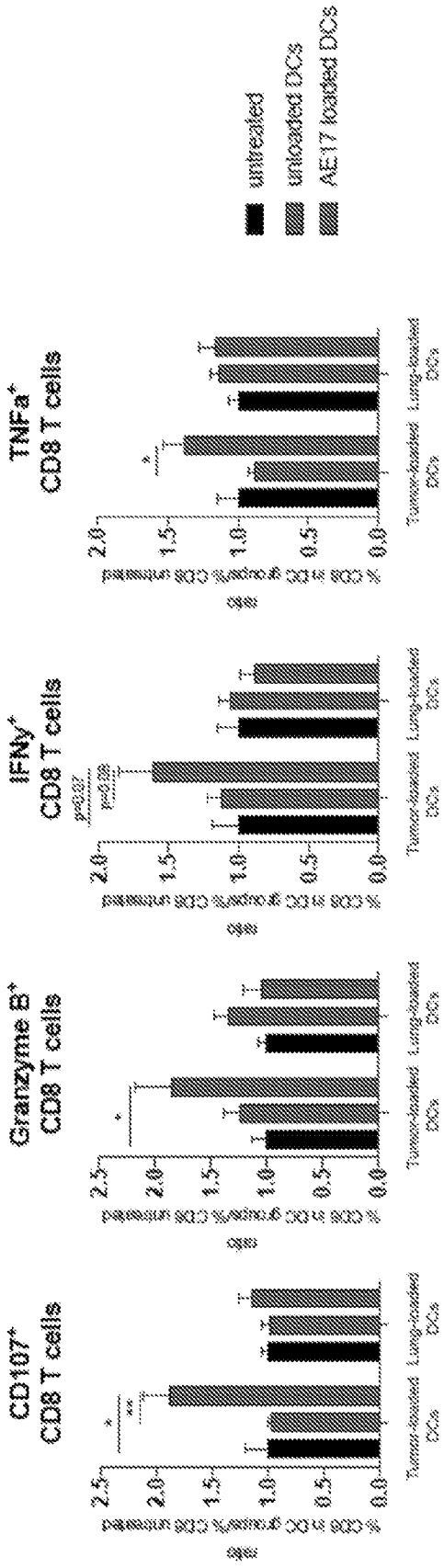
untreated
unloaded DCs
AE17 loaded DCs
CD107+ CD8 T cells
Granzyme B+ CD8 T cells
IFNγ+ CD8 T cells
TNFa+ CD8 T cells
% CD8 specific
Tumor-loaded DCs
Lung-loaded DCs

Figure 8

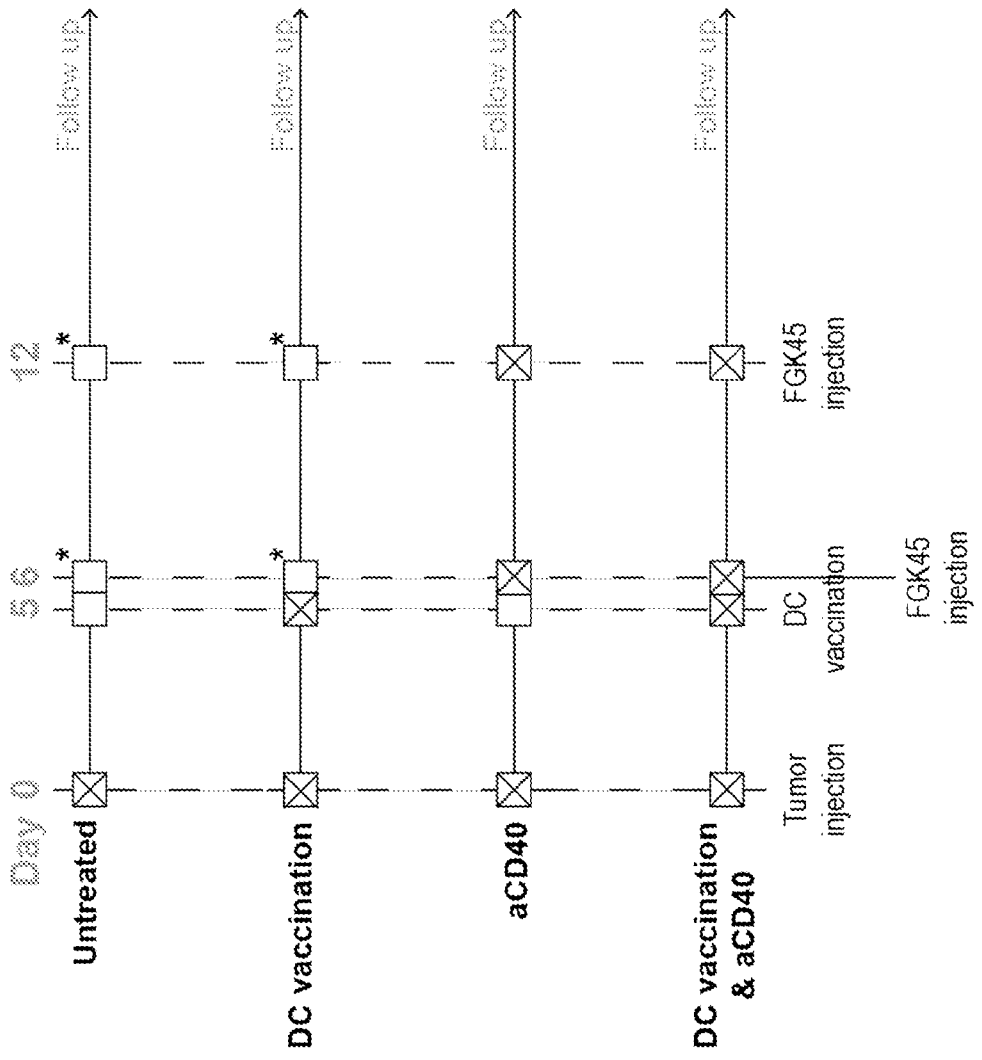
Day 0
5 6
12
Follow up
Untreated
DC vaccination
aCD40
DC vaccination & aCD40
Tumor injection
DC vaccination
FGK45 injection
FGK45 injection
* Isotype control IgG2a mAb (clone 2A3)

Figure 9

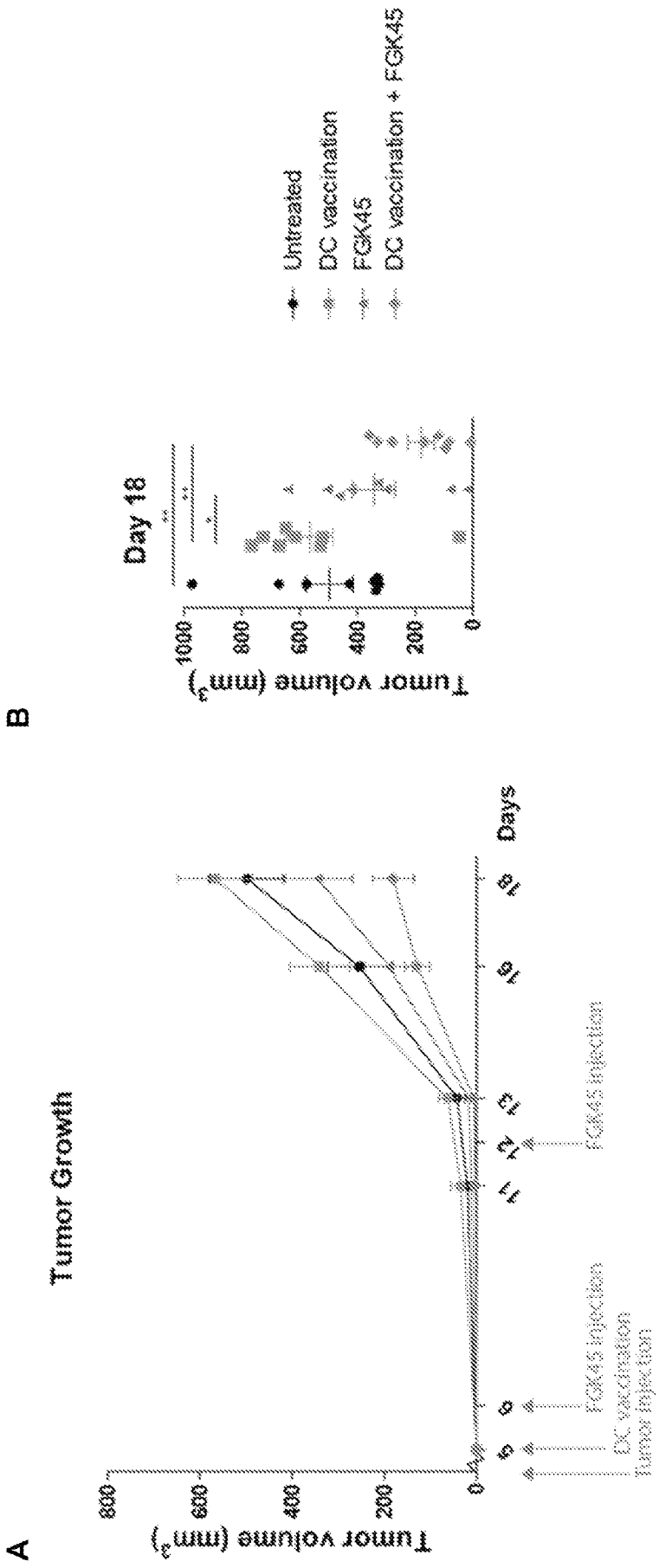
A
Tumor Growth
Tumor volume (mm³)
800
600
400
200
0
Days
0
6
14
17
21
28
DC vaccination
Tumor injection
FGK45 injection
FGK45 injection
B
Day 18
Tumor volume (mm³)
1000
800
600
400
200
0
Untreated
DC vaccination
FGK45
DC vaccination + FGK45

Figure 10

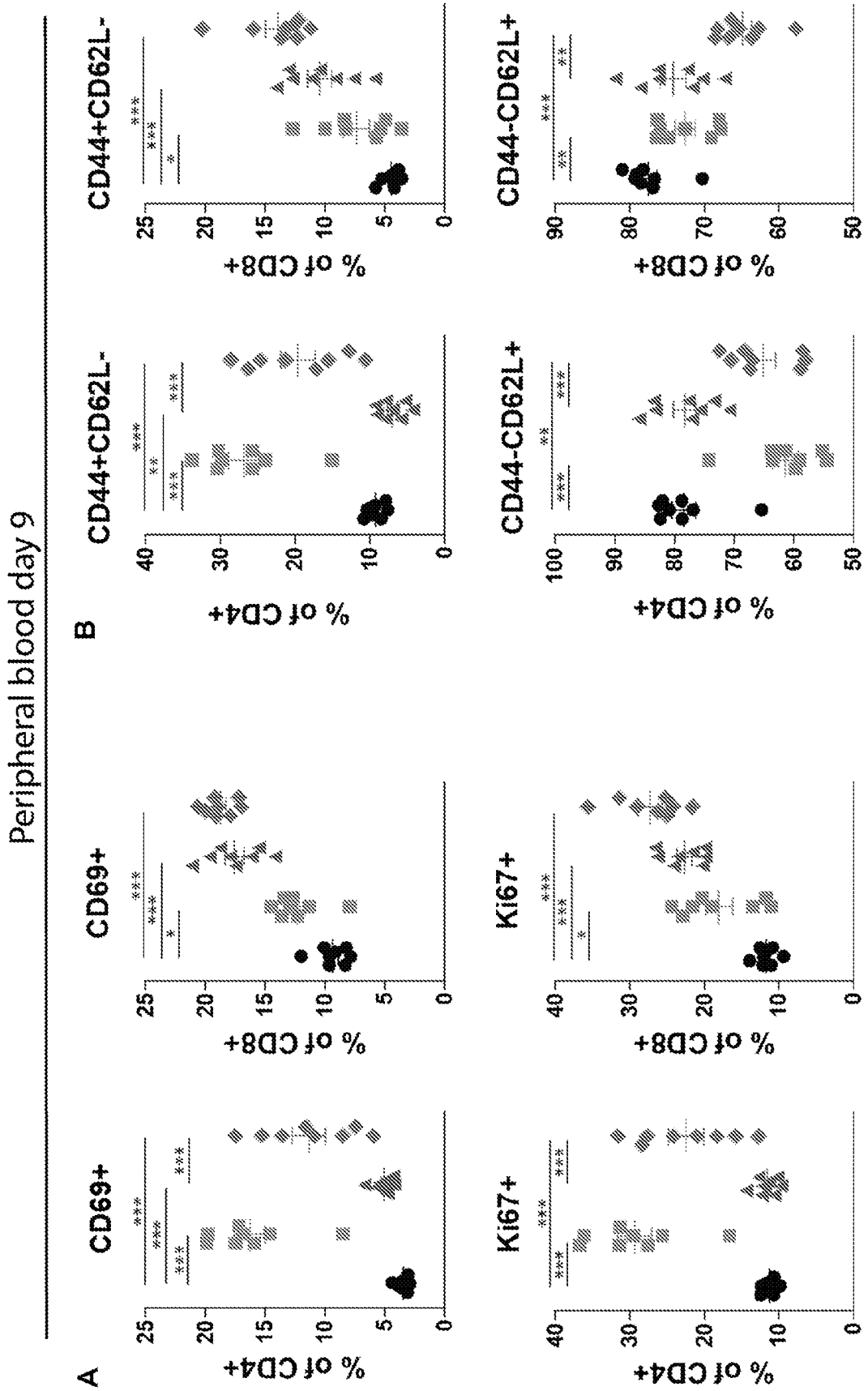
Peripheral blood day 9
A
CD69+
% of CD4+
Ki67+
% of CD4+
B
CD44+CD62L-
% of CD4+
CD44-CD62L+
% of CD4+
CD69+
% of CD8+
Ki67+
% of CD8+
CD44+CD62L-
% of CD8+
CD44-CD62L+
% of CD8+

Figure 11

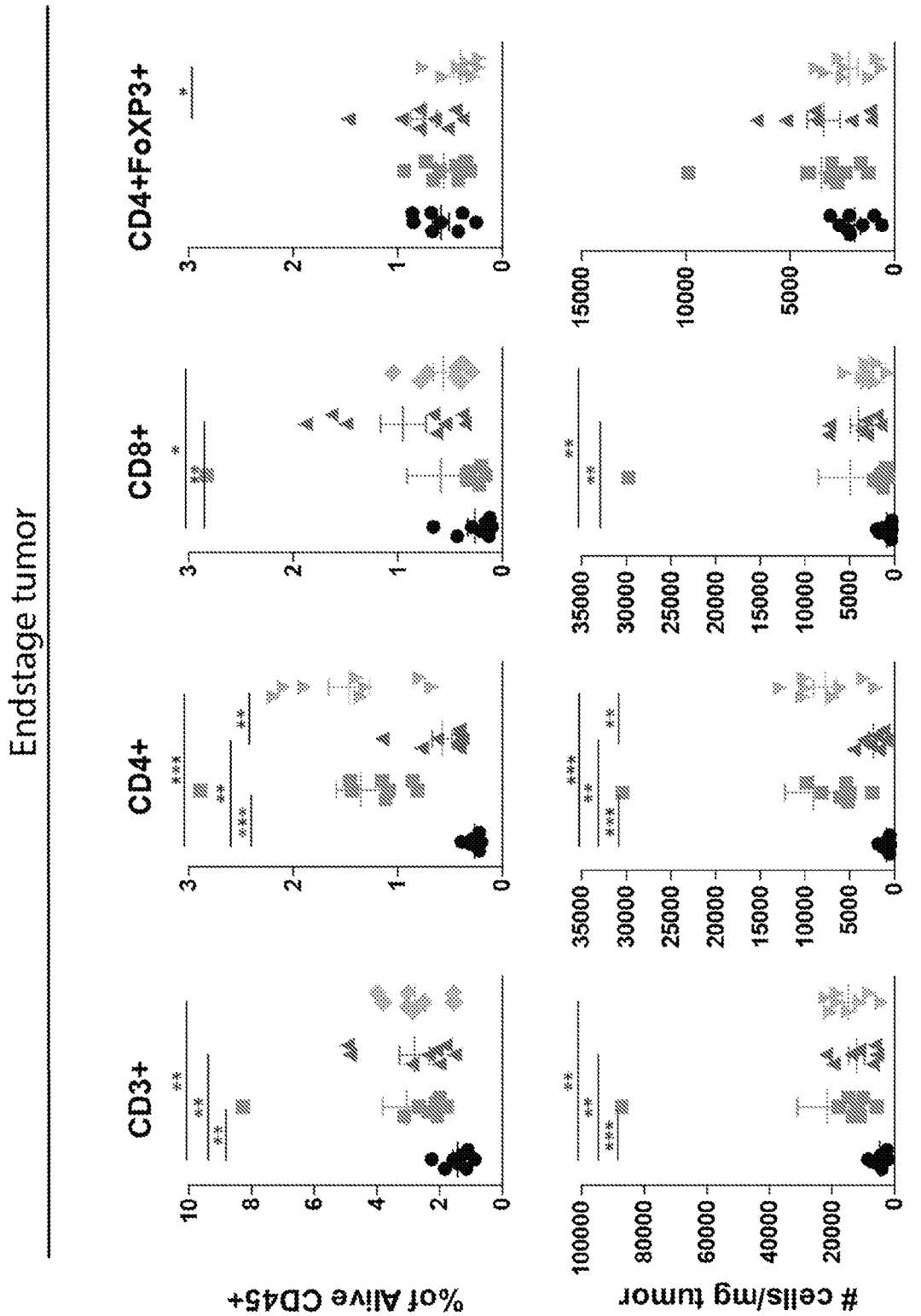
Endstage tumor
CD3+
CD4+
CD8+
CD4+FoXP3+
%of Alive CD45+
cells/mg tumor

Figure 12