Effectivity of Long Antigen Exposition Dendritic Cell Therapy (LANEX-DC®) in the Palliative Treatment of Pancreatic Cancer

F. Gansauge*[,1], B. Poch[1], R. Kleef[2] and M. Schwarz[1]

[1]*Center for oncologic, endocrine and minimal-invasive surgery, Silcherstr. 36, 89231 Neu-Ulm, Germany;* [2]*Institute for integrative oncology, Auhofstr. 1-3, 1130 Vienna, Austria*

Abstract: *Purpose:* In pancreatic cancer median survival times range around 6, 6 to 6,9 months. Here we retrospectively analyzed the outcome of immunotherapy in the additional palliative treatment of pancreatic cancer with long antigen exposition dendritic cell therapy (LANEX-DC®) in 138 patients who were treated at our institution. *Patients:* Data were available of 134 patients (97.1%). The median interval between first diagnosis and start of treatment was 1.4 months. *Results:* Therapy was well tolerated and no serious side effects were observed. The survival rate after 6 months was 72.2 % and afters 9 month 50.4%. The median survival time according to Kaplan- Meier regression analysis was 8.9 months. Median survival was significantly higher in the group of patients who started immunotherapy within 2 months following diagnosis (p=0.029) or repeated immunotherapy (p=0.027). Interestingly, younger patients <= 60 years of age lived significantly longer as patients > 60 years of age (p = 0.022). *Conclusion:* We were able to demonstrate in a large retrospective analysis that additional treatment with dendritic cells (LANEX-DC®) is highly effective and extends the median survival times up to 8.9 months. Furthermore we were able to demonstrate that median survival can be increased by early beginning and repetition of LANEX-DC® treatment.

Keywords: Cancer, dendritic cells, immunotherapy, LANEX-DC®, pancreas.

INTRODUCTION

Cancer of the Pancreas is a common type of cancer and despite improved diagnostic and therapeutic facilities still has a poor prognosis. Even in patients with totally resected tumors, the median survival is mostly under 2 years. At diagnosis of pancreatic cancer only one-third of the tumors are resectable [1]. For chemotherapy gemcitabine was shown to improve survival and has a positive influence on the quality of life [2]. Protocols of combinations of gemcitabine and erlotinib led to a median survival of up to 6.2 months as compared to 5.9 months with gemcitabine monotherapy [3]. A further marginal improvement concerning median survival times (6.5 months) was shown by Kulke and coworkers using capecitabine in combination with erlotinib [4]. These data were also shown by Boeck and coworkers when using capecitabine plus erlotinib. In this study it was also shown that no difference exists between capecitabine/erlotinib and gemcitabine/erlotinib concerning median survival times (6.9 months versus 6.6 months)[5]. Furthermore it was shown, that patients who develop skin rash following erlotinib treatment have a higher live expectancy than patients without skin rash [3, 6] Nevertheless there is need for new therapies also as additional concepts to conventional treatments especially with regard to the high toxicities like skin rash, edema or nausea of several protocols used.

*Address correspondence to this author at the Center for oncologic, endocrine and minimal-invasive surgery, Silcherstr. 36, 89231 Neu-Ulm, Germany; Tel: 49 731 71576 0; Fax: 49 731 71576 251;
E-mail: frank.gansauge@eurosurgery.de

One of the natural defence mechanisms against malignancies is the immune system. Though, in the last decade, many efforts were undertaken to induce tumor specific immunity with active immunotherapy with antigen loaded dendritic cells [7-10]. Dendritic cells are powerful antigen presenting cells (APCs), and so far the only known APCs priming an immune response by native T-cells [11, 12]. They are responsible for uptake of antigens, which they process and present to T-cells, also in HLA class I dependent cellular immunity against tumor cells.

In clinical phase I and II studies treatment with antigen loaded dendritic cells led to promising results in patients with different malignancies [13-16]. Also in pancreatic carcinoma, Morse and coworkers reported of three patients with resected pancreatic carcinoma, who received autologous, monocyte-derived dendritic cells and were alive and without evidence of recurrence 2 ½ years after diagnosis [17]. In this study, cells were loaded with the mRNA of the tumor associated antigen CEA and this molecule seems to elicit an immune response. In another study of Nakamura *et al.* 17 patients were treated with dendritic cell therapy and the median survival was 9.0 months. Out of these patients 11 patients received additional chemotherapy, 6 patients did not receive chemotherapy. No difference between these groups was observed [18]. Similar results were found by several other groups using different substances for pulsing the dendritic cells or using different treatment protocols [19-22]. Nevertheless all studies so far showed positive effects of dendritic cell therapy concerning median survival times.

Herein, we report of 134 pancreatic carcinoma patients who have been vaccinated with dendritic cells pulsed either with tumor lysate or CA 19-9.

PATIENTS & METHODS

Patients

138 patients suffering from metastatic pancreatic carcinoma have been vaccinated with autologous antigen pulsed dendritic cells on the basis of compassionate treatments between December 2001 and October 2010 at our institution. In this retrospective analysis data of 134 Patients were available. The mean age was 63.9 years (33 to 87 years). 26 patients showed UICC stage IVa, 108 patients UICC stage IVb. 38 of the patients showed recurrence of the disease following resection of the tumor. 84 patients suffered from hepatic, 30 from peritoneal, 5 from lymph nodes, 5 from bone and 16 from lung metastases. Three patients had undergone palliative resection of the pancreatic tumor (R1/R2 resection). The majority of the patients received gemcitabine therapy (n=110), 6 patients received gemcitabine/oxaliplatin chemotherapy, 4 patients received 5-FU chemotherapy, 2 patients received gemcitabine plus 5-FU, one patient received 5-FU plus oxaliplatin and 1 patient received mitomycin chemotherapy.10 patients refused conventional chemotherapy. The median time between diagnosis and immunotherapy was 1.4 months in a range from 0.1 to 24.3 months. All patients gave a written informed consent.

Generation of Mature Antigen-loaded Monocyte-derived Dendritic Cells

The whole procedure for gaining the mature dendritic cells was performed according to Good Manufacturing Practice standards. LANEX-DC® (long antigen exposition dendritic cells) were produced as follows:

Peripheral blood mononuclear cells (PBMCs) were isolated from 250 ml of heparinized venous blood of the patient by density gradient centrifugation (Biocoll®, Biochrom, Germany). PBMCs were seeded in 6-well-plates (BD Falcon, Heidelberg, Germany), and after 2 hours the non-adherent cells were removed. Adherent cells were cultured in RPMI 1640 (Sigma-Aldrich, Munich, Germany) supplemented with 10% of the patient's serum, 50 IU/ml penicillin, 50μg/ml streptomycin and 2mM L-glutamine (all Sigma-Aldrich, Munich, Germany) in the presence of 750 U/ml rh-GM-CSF and 500 U/ml rh-IL-4 (both CellGenix, Freiburg, Germany) for 7 days. On day 1, moDCs were pulsed with serum containing at least 100 U/ml Ca19-9 (n=125) (Fitzgerald, Concord MA, USA) or with tumor lysate (n=9). On day 4, medium was removed and non-adherent cells were collected from the old medium by centrifugation and resuspended in fresh RPMI 1640 supplemented with 10% serum. Again, 750 U/ml rh-GM-CSF, 500 U/ml rh-IL-4 and 100 U/ml Ca19-9 were added. Maturation of moDCs was induced by adding 20 ng/ml rh-IL-1β, 20 ng/ml rh-TNF-α and 60 ng/ml rh-IL-6 (all CellGenix, Freiburg, Germany). At day 7, moDCs were harvested, washed twice in sterile PBS, and an aliquot of the cells was removed for phenotypic analysis and sterility testing. The rest of the cells were divided in two vaccination preparations. moDCs for immediate vaccination were resuspended in 1ml sterile saline solution containing 10% autologous serum and administered by subcutaneous injection in the abdominal subcutis near the inguinal lymph nodes (500

μl of cell suspension each side). moDCs for the later revaccination were frozen in autologous serum containing 10% DMSO using a cell freezer (BV-65, Consarctic, Schöllkrippen, Germany). On the day of revaccination (between day 35 and 49) the cells were thawed, washed twice in PBS and resuspended in 1ml sterile saline solution containing 10% autologous serum. The vaccination was performed like the first vaccination.

Flowcytometric Analysis

For purity control of the dendritic cells flow cytometric analysis of the DCs was performed using an Epics XL (Beckman-Coulter, Germany). Data were evaluated with the Expo 32 software. The following antibodies were used (all Beckman-Coulter, Krefeld, Germany): HLA-DR-FITC (clone Immu-357), CD1a-PE (clone BL6), CD80-FITC (clone MAB104), CD83-PE (clone HB15a).

For immunphenotyping the following antibodies were used (all Beckman-Coulter, Krefeld, Germany): CD3-PC5 (clone UCHT1), CD14-PC5 (clone RMO52), CD16-PC5 (clone 3G8), CD19-PC5 (clone J4.119), CD20-PC5 (clone B9E9), CD56-PC5 (clone N901), IgG1-FITC-isotype (clone 679.1Mc7), IgG2a-PE-isotype (clone 7T4-1F5), IgG1-PC5-isotype (clone 679.1Mc7) and IgG2a-PC5-isotype (clone 7T4-1F5).

Functional Analyses

Dendritic cells were generated out of the blood of 5 healthy volunteers. The dendritic cells were either incubated in the own plasma (DC-EP) or in plasma of a pancreatic cancer patient (DC-CP) containing 1000 U/ml CA19-9. At day seven lymphocytes from the healthy volunteers were isolated again and were added to the dendritic cell cultures in a ratio of 4:1 (lymphocytes, dendritic cells). After an incubation period of 72 hours lymphocytes were used for cell cycle analysis or for lysis assays.

For cell cycle analysis cells were stained with Propidiumiodid (Beckman-Coulter, Krefeld, Germany) and analysed by flow cytometric analysis. As negative control lymphocyte monoculture cultured in plasma served (L), as positive control a lymphocyte monoculture stimulated with 5μg/ml PHA (Phytohaemagglutinine-L, Biochrome, Berlin, Germany) served (L-PHA). For lysis assays the lymphocytes were gained from the coculture with dendritic cells and cocultivated with cells of the pancreatic cancer cell line AsPC-1, which expresses high levels of CA19-9 on its surface, in a ratio of 4:1 (lymphocytes : AsPC-1) for five hours. Lactate dehydrogenase was determined by the cytotoxicity detection kit (LDH, Roche Molecular Biochemicals) in the supernatant of the cultures. As negative control the supernatant of AsPC-1 monoculture served (AsPC-M), as positive control AsPC-1 cells were lysed by adding 2% of Triton X-100 (Sigma-Aldrich, München, Germany) to the culture (AsPC-MAX).

Statistical Analysis

Significance was defined as $p \leq 0.05$. Student's t-test, Mann Whitney U-test, Kaplan-Meier estimates and Chi-Square test were computed using the MedCalc® or Prism® software packages.

RESULTS

Characteristics of the Gained Dendritic Cells

After 7 days of culturing the costimulatory molecules were determined by flow cytometry. Maturation process of the dendritic cells was evident by the upregulation of CD80 (> 90%), CD 83 (> 80%) and HLA-DR (> 90%). In addition, the cells were lineage negative as determined by the detection of CD3, CD14, CD16, CD19, CD20 and CD 56 (< 2%) (Fig. **1**).

Functional Analyses – Proliferation Assays

In lymphocyte cultures from healthy volunteers which were cocultured with dendritic cells matured and grown in plasma containing 1000 U/ml CA-19-9 (L-DC-TP) 33.1% (SD: 12.5%) of the cells were in S/G2 phase, whereas in lymphocyte cultures from healthy volunteers which were cocultured with dendritic cells matured and grown in the own plasma (L-DC-EP) only 17.3% (SD: 2.7%) of the cells were in S/G2 phase (p < 0.001). The results are shown in (Fig. **2**).

Functional Analyses – Lysis Assays

The LDH release in AsPC-1 cultures cocultivated with L-DC-TP (AsPC-L-TP) was significant higher (94.6 U/ml, SD: 13.9) than in AsPC-1 cultures cocultivated with L-DC-EP (AsPC-L-EP) (43.6 U/ml, SD: 8.0, p < 0.001) suggesting a specific priming of the dendritic cells by CA19-9 containing plasma. The results are shown in (Fig. **3**).

Patients Characteristics

A total of 138 patients were treated. Data were available from 134 patients (97.1 %) The mean age was 63.9 years in a range from 33 to 87 years. The characteristics are listed in table **1**. As the dendritic cell vaccination was applied supportive to common treatment regimens, 124/134 patients (92.5%) received also palliative chemotherapy. 10 patients refused conventional chemotherapy (7.5%). The median interval between first diagnosis and start of treatment was 1.4 months (0.1 – 24.3 months). Two patients died prior to the second vaccination with LANEX-DC®.

Side Effects

The therapy was well tolerated and no serious side effects were observed. Some patients developed mild fever which was scored as grade II according to the toxicity criteria of the WHO. We never saw allergic or autoimmune reactions or other hematologic, hepatic or renal toxicity.

Influence of the Therapy on the Distribution of Peripheral Immune Cells

To examine the influence of dendritic cell vaccination on the immune system, we determined the amount of T-lymphocytes, CD 4 positive cells, CD 8 positive cells, natural killer cells and B-cells before and after therapy by flow cytometry in 25 patients (Fig. **4**). During therapy, the absolute number of T-lymphocytes increased significantly (p≤0.03). There was also a tendency to increased cell numbers in the pool of the CD 4 positive cells, the NK cells and the B cells, albeit this didn't reach significance. The cell
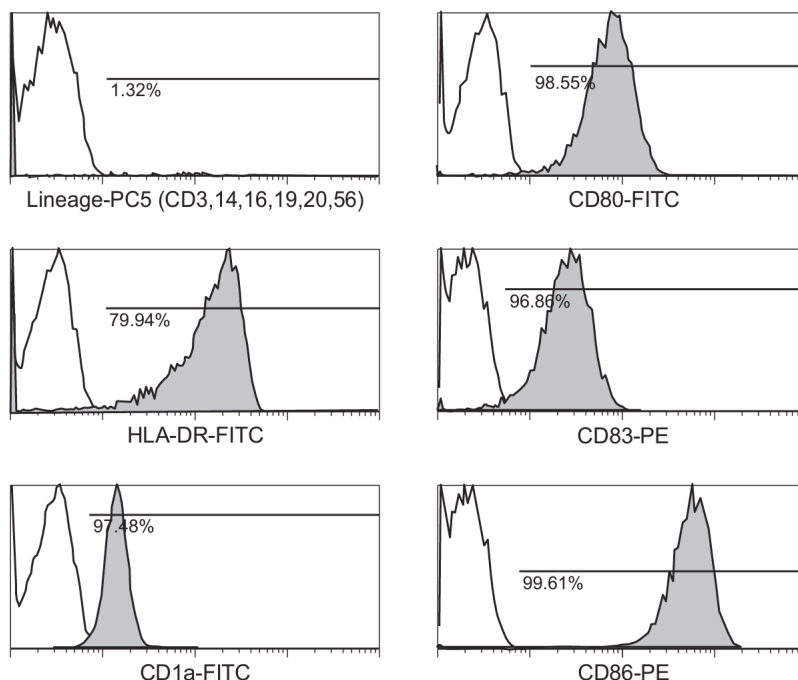

Fig. (1). Phenotype of a representative culture of dendritic cells, which was cultured for 7 days and matured in the presence of Il-1β, TNF-α and IL-6. The cells were analysed for expression of surface antigens by flow cytometry.
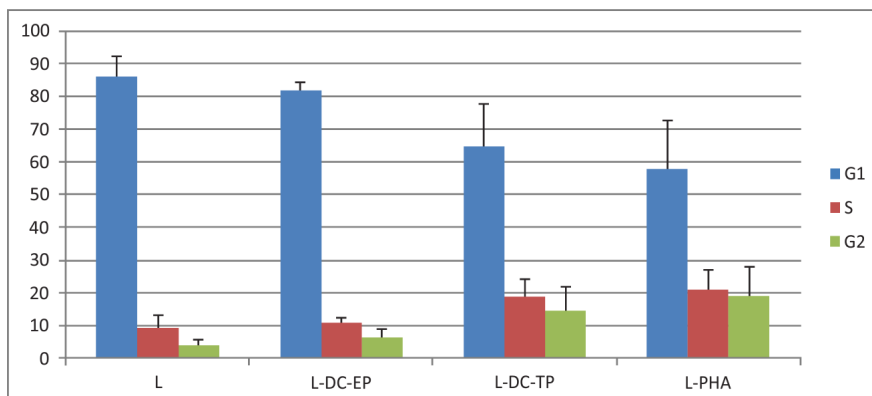
Fig. (2). A significant increase in proliferation rates of the lymphocytes was seen in lymphocytes cocultured with dendritic cells matured and grown in plasma of a cancer patient containing 1000 U/ml CA19-9 (L-DC-TP) as compared to those cocultured with dendritic cells matured and grown in the own plasma (L-DC-EP) (P < 0.001). The negative control consisted of lymphocyte monoculture (L), the positive control consisted of lymphocytes stimulated with PHA (L-PHA).
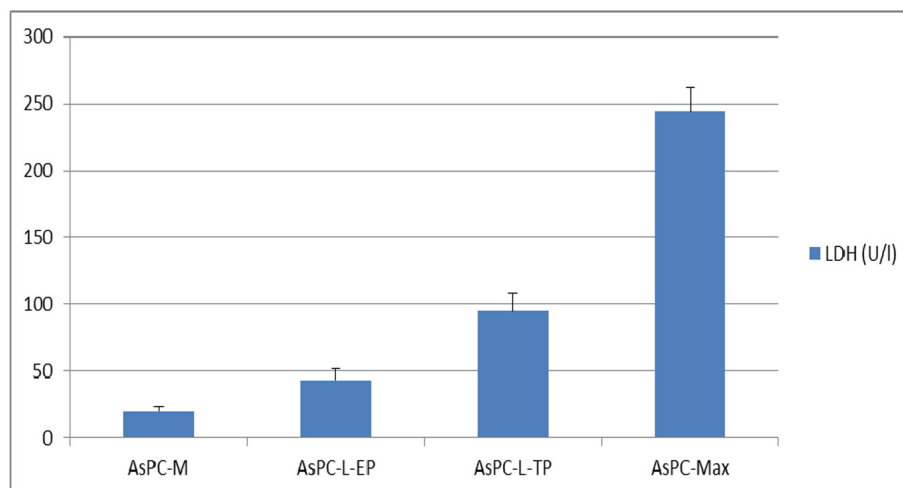

Fig. (3). A significant increase in LDH-release from AsPC-1 cultures was seen in AsPC-1 cultures cocultivated with L-DC-TP (AsPC-L-TP) as compared to AsPC-1 cultures cocultivated with L-DC-EP (AsPC-L-EP) (p < 0.001). The negative control consisted of AsPC-1 monoculture (AsPC-M), tho positive control consisted of As-PC-1 monoculture lysed by addition of Triton X-100 (AsPC-MAX).

population of the CD 8 positive cells showed a significant increase (p≤ 0.001) after therapy with the dendritic cells, suggesting that the responsible cells for the seen clinical effects of the therapy are within the CD 8 positive lymphocytes (Fig. **4**). Nevertheless, no statistical significant difference was seen in correlation-analysis between survival and increase of T-cells or CD8+ cells under therapy which may be due to the limited number of patients.

Clinical Effects of DC Vaccination in Pancreatic Cancer Patients

Response: 3 month after therapy 63 among the 134 patients (47%) showed stable disease. In 18 patients partial response was achieved (13.4%) and 53 patients had progressive disease (39.6%). No case of complete response was seen.

Survival: Survival was determined from the beginning of LANEX-DC® therapy. The survival rate after 6 months was 72.2 % and afters 9 month 50.4%. After 12 months 30.0 % of the patients and after 18 months 14.4 % of the patients were still alive. The results are listed in table **2**.

The median survival time according to Kaplan- Meier regression analysis was 8.9 months (Fig. **5**; Table **2**).

Additional Chemotherapy increases median survival: 10 patients refused chemotherapy whereas 124 patients received chemotherapy (110 patients gemcitabine monotherapy). The median survival in the group receiving additional chemotherapy was 9.0 months whereas the small group of patients who refused chemotherapy had a median survival of 5.9 months (Chi square = 4.126, p = 0.044).

Early beginning of LANEX-DC® treatment following diagnosis increases median survival significantly: Patients

(n=85) who started LANEX-DC® treatment within 2 months following diagnosis lived significantly longer than patients (n=49) who underwent LANEX-DC® treatment later than 2 months following diagnosis (10.4 months versus 7.6 months, Chi square = 4.791, p = 0.029). The Kaplan-Meier-Analysis is shown in (Fig. **6**).

Table 1.

	Patients Characteristics
Number of patients	134
(years)	
Range	33-87
Median	63.9
Sex	
Male	76
Female	58
UICC Stage	
Stage IV a	26
Stage IV b	108
Recurrence	38
Chemotherapy	
Gemcitabine	110
Gemcitabine + 5-FU	2
Gemcitabine + Oxaliplatin	6
Mitomycin Monotherapy	1
5-FU Monotherapy	4
5-FU + Oxaliplatin	1
None	10
Metastatic site	
Hepatic	84
Peritoneal	30
Lymph nodes	5
Bone	5
Lung	16

Repetition of LANEX-DC® treatment increases survival significantly: For this analysis all patients who survived at least 4 months after start of LANEX-DC® therapy were involved (n=112). 33 patients repeated up to 5 times LANEX-DC® therapy (mean=2.36 cycles of LANEX-DC® therapy), whereas 79 patients only had a single cycle of LANEX-DC® therapy. The median survival in the group with a single cycle of dendritic cell therapy was 9.0 months as compared to 13.4 months in the group with repeated cycles of LANEX-DC® therapy (Chi-square = 4.921, p = 0.027). The Kaplan-Meier analysis is shown in figure **7**.

Material used for pulsing of LANEX-DC®: In 9 patients native tumor material was achievable and the chilled tumor material was used for pulsing the dendritic cells. The median survival time in these patients was 8.2 months as compared to 9.0 months in the group of patients where serum containing at least 100 U/ml CA 19-9 was used for pulsing (n=125) (Chi-square = 0.848, p = 0.361).

Influence of UICC stage, metastases and relapse-status: 26 patients were classified UICC stage 4a, 108 patients were classified UICC stage 4b. There was no statistical significant difference in the median survival between UICC stage 4a (10.0 months) and UICC stage 4b (8.4 months) (Chi-square = 2.518, p = 0.117). The existence of peritoneal metastases did not influence the median survival times (Chi-square = 0.0076, p = 0.935) whereas the existence of liver metastases was a negative predictive factor. Patients with liver metastases (n=84) had a median survival time of 7.8 months as compared to a median survival time of 12.0 months in patients without liver metastases (n=50) (Chi-square = 19.514, p < 0.0005). Patients with recurrence following resection of pancreatic cancer (n=38) had a median survival of 11.8 months as compared to a median survival time of 8.0 months in patients without prior resection of pancreatic cancer (n=96) (Chi-square = 5.546, p = 0.02).

Influence of gender and age: In total 58 of the patients were female, 76 of the patients were male. Although it did not reach statistical significance, there was a tendency, that females lived longer (10.5 months) as compared to males (8.0 months) (Chi-square = 3.538, p = 0.063). This observation may be influenced by the fact, that the median interval between first diagnosis and start of treatment was lower in the female group (1.2 months) as compared to the male group (1.5 months). Interestingly, younger patients <= 60 years of age (n=46, median survival time: 10.3 months) lived significantly longer as patients > 60 years of age (n=88, median survival time: 8.0 months) (chi-square = 5.299, p = 0.022).

DISCUSSION

In this retrospective analysis we evaluated the clinical results of 134 patients suffering from pancreatic carcinoma, who were treated with antigen loaded dendritic cells. As many tumors do not elicit a sufficient immune response, which may be due to the reduced function of the immune system or especially to the absence of functional dendritic cells, vaccination with ex vivo generated dendritic cells may overcome this lack [23, 24]. Large numbers of dendritic cells can be achieved from peripheral CD 14+ monocytes using GM-CSF and IL-4 [25]. As maturation stimuli we used TNF-α, IL-1β and IL-6. Viewed under the phase contrast, the obtained cells showed the typical shape of dendritic cells with many thin processes or veils, which fit to the function of these APCs for antigen capturing and antigen presentation [26]. As we could demonstrate by flow cytometry, the contamination with NK-, T- and B-cells was negligible. The dendritic cells expressed high levels of CD83, CD80 and HLA-DR, suggesting the yielded cells were of a mature phenotype [27]. Mature dendritic cells can stimulate different T-cells including CD8+ CTLs which then proliferate vigorously. We were able to demonstrate that pulsing of the
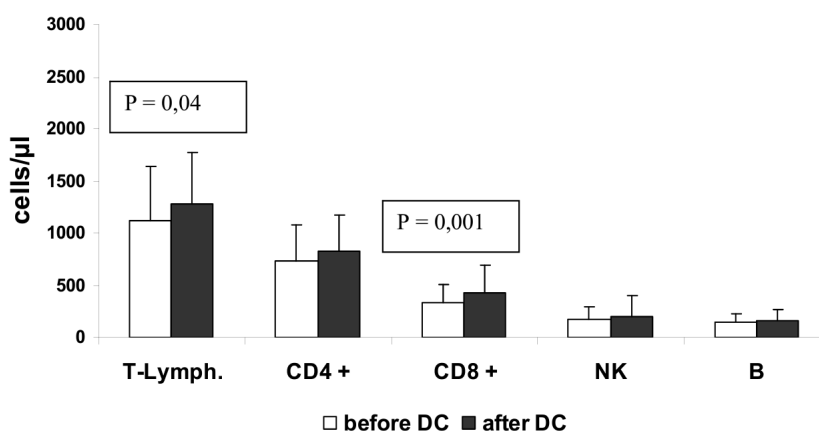
Fig. (4). Distribution of peripheral immune cells before and after therapy with autologous dendritic cells in patients suffering of pancreatic carcinoma. The cells were determined by flow cytometry using the Cytostat tetra Chrome-System of the Beckmann Coulter GmbH.

Table 2.

Response and survival after therapy with autologous, antigen loaded dendritic cells in palliatively treated pancreatic cancer patients	
Response after 3 months	
Complete response	0.0 %
Partial response	13.4 %
Stable disease	47.0 %
Progressive disease	39.6 %
Survival	
Survival rate (6 months)	72.2 %
Survival rate (9 months)	50.4 %
Survival rate (12 months)	30.0 %
Survival rate (18 months)	14.4 %
Median survival (months)	8.9

dendritic cells with CA19-9 led to increased proliferation rates of the lymphocytes added and that the lytic activity against AsPC-1 cells of these lymphocytes was significantly higher when lymphocytes were cocultured with dendritic cells which were pulsed with plasma containing high levels of CA19-9. In our study, the population of the CD8+ cells significantly increased after dendritic cell vaccination reflecting the activating influence of the immunisation on these CTLs. Heiser and colleagues used dendritic cells transfected with PSA in therapy of prostate carcinoma and could show responses, which were predominantly mediated by MHC class I-restricted CD8 CTLs. This was proven by the addition of a CD8 or MHC class I mAB resulting in the inhibition of the PSA-specific cytolytic activity of the effector cells *in vitro* [28]. From our results, we presume, that the produced dendritic cells are mature and able to elicit an immune response *in vivo*. To afford a specific immune reaction a lot of different tumor antigens are described. In melanoma

there are a lot of MHC restricted peptides used in different trials, resulting in clear clinical effects [29]. In other malignancies, for example in prostate carcinoma dendritic cells were pulsed with proteins like PSA or PAP, also inducing measurable clinical results [30, 14]. Another source of tumor antigens is tumor lysate, which is often used in clinical studies for pulsing autologous dendritic cells, and is also capable in eliciting tumor specific immune reactions [15, 31]. For dendritic cell therapy in advanced pancreatic cancer different sources of antigens like tumor-lysates, CEA, CA125, Her-2, CA19-9 or MUC1 peptides have been used for pulsing [17-22, 32, 33] showing all positive effects concerning median survival times. To increase the specifity we pulsed the cells with CA 19-9 in most cases, since it was shown by Ziske and coworkers, that resistance of pancreatic carcinoma cells is reversed by dendritic cells pulsed with CA 19-9 [32]. In 9 out of 134 patients tumor lysate was achievable and was used for pulsing the dendritic cells, showing no significant

difference concerning median survival time to those patients where CA19-9 was used for pulsing.
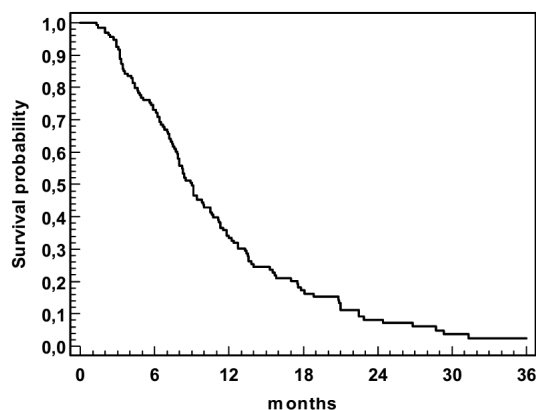

Fig. (5). Kaplan-Meier survival curve of advanced pancreatic cancer patients treated with autologous antigen-pulsed dendritic cells. The median survival time was 8.9 months.
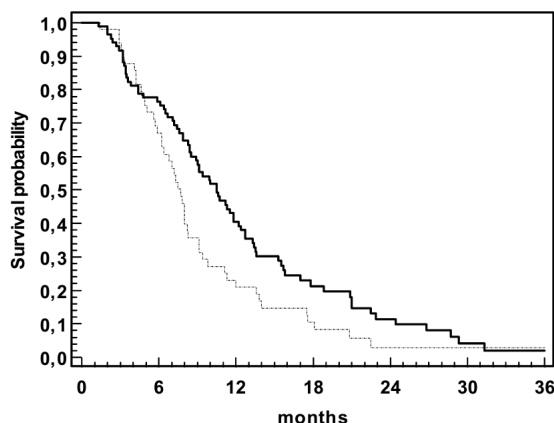

Fig. (6). Kaplan-Meier survival curve of patients treated with LANEX-DC®. Patients who started LANEX-DC® therapy within 2 months following diagnosis (n=85) lived significantly longer than patients (n=49) who underwent LANEX-DC® treatment later than 2 months following diagnosis (10.4 months versus 7.6 months, Chi square = 4.791, p = 0.029).

Clinically we could observe 13.4% partial responses and 47% of the patients developed stable disease. The survival rates were 72.2% after 6 months and 14.4 % after 18 months. The median survival according to Kaplan-Meier regression analysis was 8.9 months following beginning of dendritic cell therapy. Considering the advanced stages of the disease and the relatively long interval between diagnosis and start of dentritic cell therapy (mean 1,4 months) in the 134 patients we evaluated and taking into consideration, that the vast majority of our patients received gemcitabine monotherapy, the median survival rates seem to be superior to those achieved with gemcitabine or combinations of erlotinib with

gemcitabine or capecitabine [3-6]. Although all studies so far showed evidence for an improved survival in the treatment of advanced pancreatic cancer with dendritic cells, the question whether an additional chemotherapy (mostly gemcitabine) improves survival even more is unclear. Nakamura and coworkers saw no additional effect of chemotherapy to dendritic cell therapy [18], whereas Kaneko and coworkers as well as our group found a slight advantage of additional chemotherapy using gemcitabine in combination with dendritic cell therapy [19]. Anyhow, in all the studies mentioned the numbers of patients were rather small and this therapeutic question should be issued in a clinical controlled study. Anyhow, more than 260 patients with advanced pancreatic cancer treated with dendritic cell therapy have been reported, showing superior results than chemotherapy alone (Table **3**).

In addition the quality of life was very high, without serious side effects, making it necessary to stay in hospital. Interestingly the younger patients (<= 60 years) had even more benefit than older patients (> 60 years) although it is well known from the clinical experience that younger patients have lower median survival times than older patients [34]. A possible explanation for this phenomenon could be that immunological responses are higher in younger patients than in older ones, underlying the effectivity of LANEX-DC® treatment. In conclusion we were able to demonstrate in a large retrospective analysis that additional treatment with dendritic cells (LANEX-DC®) is highly effective and extends the median survival times up to 8.9 months. Furthermore we were able to demonstrate that median survival can be increased by early beginning and repetition of LANEX-DC® treatment.
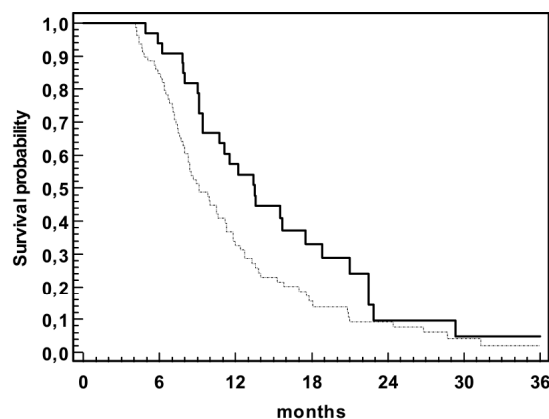

Fig. (7). Kaplan-Meier survival curve of patients who had one cycle of LANEX-DC® (n=79, dotted line) and patients who had repeated LANEX-DC® treatment (n=33, solid line). Only patients who survived at least 4 months were analysed (n=112). Patients who repeated LANEX-DC® treatment lived significantly longer than those with a single cycle of LANEX-DC® (13.4 months versus 9.0 months (Chi-square = 4.921, p = 0.027).

CONFLICT OF INTEREST

Due to legal regulations Frank Gansauge is the production manager and joint owner of the laboratory Dr. Gansauge, where dendritic cells (LANEX-DC®) were produced.

Table 3.

Author	Years	Num.of Patients	Med. Survival (Months)
Kaneko *et al.*[19]	2005	18	14.5
	2005	28	15.8
Nakamura *et al.*[18]	2009	17	9
Hirooka *et al.*[20]	2009	5	n.d.
Bauer *et al.*[21]	2011	12	10.5
Kimura *et al.*[22]	2011	49	11.9
Gansauge *et al.*	2013	134	8.9

ACKNOWLEDGEMENTS

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