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ORIGINAL ARTICLE

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# Vaccination with autologous tumour antigen-pulsed dendritic cells in advanced gynaecological malignancies: clinical and immunological evaluation of a phase I trial

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**Abstract** Dendritic cell (DC)-based therapy has proven to be effective in patients with malignant lymphoma, melanoma, and renal and prostate carcinoma. In this phase I clinical trial, we have shown that patients with advanced gynaecological malignancies can be effectively vaccinated with DC pulsed with keyhole limpet haemocyanin (KLH) and autologous tumour antigens. Two patients with uterine sarcoma and six subjects with ovarian carcinoma received three to 23 intracutaneous injections of antigen-pulsed DC at 10-day or 4-week intervals. Three patients showed stable disease lasting 25 to 45 weeks, and five experienced tumour progression within the first 14 weeks. KLH- and tumour lysate-specific delayed-type hypersensitivity (DTH) reactions were observed in six and one patient, respectively. Lymphoproliferative responses to KLH and to tumour lysate stimulation were recorded in six patients and in two patients respectively. Tumour antigen-stimulated interferon-gamma (IFN-$\gamma$) secretion by peripheral blood mononuclear cells (PBMC) in one patient was consistent with a $T_H$ type 1 cytokine bias. The treatment was safe, well tolerated, immunologically active and except for local cutaneous hypersensitivity devoid of significant adverse effects.

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## Introduction

Ovarian carcinoma is the main cause of death in patients with gynaecological malignancies, its poor clinical outcome being ascribed to detection at an already advanced stage. Standard treatment includes surgery, combined chemotherapy and radiation, with 90% of the patients developing recurrence [35]. Uterine sarcoma comprises less than 1% of all gynaecological malignancies. The 5-year survival rate for patients with stage I disease is approximately 50% versus 0–20% for the remaining stages. After surgery, no standard therapy is currently available for patients with residual disease [19]. Treatment of recurrent disease has been challenged by drug-resistance and remains a major clinical problem, underlining the need for novel intervention strategies that maintain activity against refractory tumours without compromising the host's immune response. The capacity of the cellular immune response to specifically recognise tumour-associated antigens (TAA) and even eradicate established tumours has provided a firm basis for novel immunotherapeutic approaches to cancer treatment [16, 20]. Substantial evidence has further demonstrated that the mode of antigen delivery is of paramount importance in eliciting effective antitumour immunity [31].

Dendritic cells (DC) represent a heterogeneous population of cells which by means of specialised features such as uptake, processing and presentation of antigen, migration to lymph nodes and expression of co-stimulatory molecules, play a pivotal role in the generation of specific effector and memory T cells [1, 9]. DC have thus been used as potent adjuvants in vaccination trials for triggering or amplifying an inadequate immune response [7, 30]. The capacity of antigen-pulsed DC to generate CTL-mediated protective immunity and even regression of established tumours has been documented in animal models [3, 6, 15]

and in various human malignancies including B cell lymphoma [11], malignant melanoma [4, 18, 30], prostate [14] and renal cell carcinoma [10, 12]. Strategies aimed at arming DC with target antigens for use in clinical trials have included recombinant or fusion proteins [11, 32], HLA-restricted, TAA-derived CTL-epitopes [2, 14, 18, 29], DC-tumour cell hybrids [12] and autologous antigens from tumour cell lysates [4, 8, 10, 18]. Based on these findings, we initiated a phase I trial on advanced gynaecological malignancies using DC pulsed with autologous tumour cell lysate and keyhole limpet haemocyanin (KLH), a foreign helper protein that reportedly enhances anti-tumour immunity [10, 18, 25]. We present here the immunological and clinical results on the treated patients.

## Materials and methods

### Patients and treatment

Eight patients were entered into the study. Eligible patients had histologically confirmed progressive or recurrent ovarian carcinoma (six cases) or uterine sarcoma (two cases) with no possibility of further conventional treatment, and adequate haematological, hepatic and renal function. The last cycle of chemo- radio- or potentially immunosuppressive therapy terminated at least 4 weeks prior to collecting cells for vaccination. All participants gave written informed consent and every third month underwent a complete clinical examination as well as a radiological evaluation of all measurable tumour sites by computed tomography (CT) or magnetic resonance imaging (MRI). Physical examination was performed and patient history was noted at each visit to assess toxicity and tumour development. Patient characteristics have been listed in Table 1. On an outpatient basis and depending on clinical response, patients received three to 23 injections of KLH- and tumour antigen-pulsed DC at 10-day or 4-week intervals, according to the generation of DC from 100 ml freshly drawn blood or from leukapheresis products, respectively. Treatment was continued for as long as the disease remained stable. Patients in whom tumour progression was observed were excluded from one vaccination after this had been detected. On an individual basis and upon her own request, patient No. 3 received six further vaccinations after surgery for tumour progression. Clinical response was determined by gynaecological examination, radiological evaluation or assessment of CA-125 tumour marker serum levels according to World Health Organisation (WHO) criteria. Blood samples were collected for laboratory and immunological monitoring before each vaccination.

### Tumour cell lysates

Tumour specimens were processed immediately after surgery or stored at –80°C until use. Fatty and necrotic material was discarded, erythrocytes were removed by lysis and the tissue specimens were extensively washed in ice-cold phosphate-buffered saline (PBS). Single-cell suspensions were obtained by grinding samples in a Medi-Machine (Dako, Copenhagen, Denmark) and subjected to six freeze (liquid nitrogen) –thaw (37°C) cycles, with cell disruption verified by trypan blue staining. The crude lysate was sonicated for 10 min and cleared by centrifugation (30 min, 2,000×*g*, 4°C). The supernatant was recovered after removal of the top lipid layer, sterile-filtered (0.2 μm), protein contents determined and aliquots stored at –80°C.

### Generation of monocyte-derived DC

DC were generated from monocyte-enriched cell fractions as described previously [23, 28]. Briefly, peripheral blood mononuclear

**Table 1** Characteristics and clinical evaluation of the eight patients included in the trial (*L* local, *D* distant, *S* surgery, *ChT* chemotherapy, *IT* immunotherapy with HER2/neu peptide-loaded DC; *RT* radiotherapy, *MRI* magnetic resonance imaging, *CT* computed tomography; *PD* progressive disease, *AWD* alive with disease, *DOD* died of disease)

| Patient No. | Age[a] | Diagnosis | FIGO stage/grade | Interval between diagnosis-recurrence (months) | Site of recurrence and treatment before vaccination[b] | Tumour evaluation | Progression-free interval under vaccination (months) | Follow-up |
|---|---|---|---|---|---|---|---|---|
| 1 | 63 | Uterine sarcoma | Ib/GIII | 8 | L; S | MRI | 3 | DOD |
| 2 | 67 | Uterine sarcoma | III | 12 | 1st L; S, ChT<br>2nd L; S | MRI | 6 | DOD |
| 3 | 35 | Ovarian cancer | IIIc/GI | 13 | 1st L; S, ChT<br>2nd L; S, IT<br>3rd D; S | MRI | 45 + 23 | AWD |
| 4 | 39 | Ovarian cancer | IIIc/GII–III | 13 | L; S, ChT | CT | 25 | AWD |
| 5 | 46 | Ovarian cancer | III/GIII | 7 | 1st L; S, ChT<br>2nd L, D; S, ChT | CT | 11 | DOD |
| 6 | 44 | Ovarian cancer | III/GII–III | 26 | 1st L; S, ChT<br>2nd D; S, ChT, RT | CT | 8 | AWD |
| 7 | 46 | Ovarian cancer | IIIc/GII–III | 48 | 1st L; S, ChT<br>2nd L; S, ChT | CT | 14 | AWD |
| 8 | 46 | Ovarian cancer | IIIc/GIII | 35 | 1st D; S | CT | 27 | AWD |

[a]Patient age in years at diagnosis
[b]Last treatment at least 4 weeks prior to obtaining blood for vaccination

cells (PBMC) were isolated by Lymphoprep (Nycomed, Oslo, Norway) density gradient either from 100 ml heparinised venous blood and used immediately, or from leukapheresis products and then cryopreserved in 20% GMP-quality human serum albumin (Centeon, Marburg, Germany) and 10% DMSO (Sigma, Deideshofen, Germany) using an automatic freezing unit (Biosafe-200, Messer, Euteneuen, Germany). Adherent cells were cultured for 6 days (leukapheresis products) or 7 days (fresh blood) in serum-free CellGro medium (CellGenix, Freiburg, Germany) supplemented with rhGM-CSF (800 U/ml; Novartis, Basel, Switzerland) and rh-IL-4 (1,000 U/ml; Strathmann, Hamburg, Germany). Non-adherent immature DC were harvested on days 6 or 7 respectively and pulsed with high-purity, endotoxin-free KLH (50 μg/ml; Calbiochem, Bad Soden, Germany) and autologous tumour cell lysate (100 μg/ml) in the presence of GM-CSF (800 U/ml), IL-4 (1,000 U/ml) and tumour necrosis factor-alpha (TNF-α; CellGenix, 20 ng/ml) to incorporate $T_H$- and CTL-epitopes. Cells were harvested on days 7 or 10 respectively, extensively washed, suspended in 700 μl PBS and administered intracutaneously in close proximity to the axillary lymph nodes. The vaccine was routinely analysed for estimation of DC purity and tested for infectious agents.

### Delayed-type hypersensitivity skin test

Delayed-type hypersensitivity (DTH) testing to assess immunocompetence to common recall antigens (Multitest Immignost, Fellbach, Germany) was performed on the ventral surface of one forearm. Antigen-specific memory cells triggering a local inflammatory response to the vaccine were determined by intradermal application of KLH (5 μg), tumour cell lysate (10 μg) and DC pulsed with KLH or tumour lysate on the other forearm. DTH responses were evaluated after 48 h.

### Flow cytometric analysis

DC surface marker analysis was performed using fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies (mAbs) to CD1a, CD3, CD11a, CD14, CD16/56, CD19, CD40, CD45, CD50, CD54, CD58, CD80, CD83, CD86 and HLA-DR, as well as isotype-matched control mAbs (all from PharMingen, Hamburg, Germany). Samples were analysed on a FACScalibur (Becton Dickinson, Mountain View, Calif.) using CellQuest software; 20,000 events per sample were acquired as list mode data.

### Analysis of antigen-specific proliferation

PBMC were seeded at $1.5\times10^5$ cells/well of a 96-well flat-bottomed plate (Becton Dickinson, Heidelberg, Germany) in 100 μl RPMI 1640 with 10% foetal calf serum (FCS; Biochrom, Berlin, Germany) and cultured in six replicate wells in the presence or absence of graded doses of antigens. Antigens and doses were as follows: KLH, 0–100 μg/ml; tumour cell lysate, 0–200 μg/ml; PHA, 1 μg/ml. Proliferation was determined after 5 days stimulation and 17 h bromodeoxyuridine (BrdU) incorporation by adding anti-BrdU-POD and TMB substrate and measuring the absorbance of the reaction product at 450–690 nm in a multiwell spectrophotometer (Tecan, Cologne, Germany).

### ELISPOT assays for interferon-gamma

PBMC were seeded at $4\times10^4$ to $2.5\times10^5$ cells/well of a 96-well Multiscreen HA plate (Millipore, Eschborn, Germany) coated with anti-interferon-gamma (IFN-γ) mAbs (1-D1K; Mabtech, Nacha, Sweden) and incubated in six replicates for 48 h at 37°C in the presence or absence of antigen. Antigens were as follows: KLH, 0–50 μg/ml; tumour cell lysate, 0–100 μg/ml; PHA, 1 μg/ml. IFN-γ-secreting cells were detected using biotinylated anti-IFN-γ mAbs (7-B6-1; Mabtech). Spot-forming cells (SFC) were determined using a computer-assisted video image analyser (Bioreader-2000, Biosys, Karben, Germany) customized to meet objective criteria for size, chromatic density, shape and colour. Specific spots were calculated by subtracting the mean number of spots $+2\times$SD of the medium control from the mean spot number of the experimental wells. Antigen-specific cells were considered to have increased compared to non-responders when numbers were ≥1/10,000 PBMC.

## Results

### Preparation and characterisation of the DC vaccine

DC were generated using serum-free GMP-quality CellGro medium. Fully differentiated DC were thus obtained, avoiding the risks of potentially immunosuppressive autologous plasma, as well as the latent hazardous immunostimulatory effects of FCS. DC were obtained in patients Nos. 4, 7 and 8 from frozen aliquots of leukapheresis products and injected at 4-week intervals [28]. One apheresis (ca. $5\times10^9$ PBMC) was sufficient for five vaccinations, DC counts ranged from $1.5\times10^7$ to $9\times10^7$, respectively. In patients Nos. 1, 2, 3, 5 and 6, impaired vascular access by double antecubital venipuncture due to extensive chemotherapy precluded leukapheresis. DC were then alternatively isolated from 100 ml freshly drawn blood ($0.8\times10^8$ to $1\times10^8$ PBMC) and administered at 10-day intervals [28]. By augmenting the vaccination frequencies we thus sought to counterbalance the lower cell counts in the initial blood sample and administer an increased number of DC to establish antitumour immunity. DC numbers showed wide patient-to-patient variability, depending critically on the monocyte counts in the blood sample. Of note, while the number of mature $CD83^+$ DC [36] in patient No. 3 (8% monocytes) ranged from $2.4\times10^7$ to $7.4\times10^7$, in patients Nos. 1, 2 and 6, who appeared to be highly immunocompromised (3% monocytes; data not shown) it only reached $1\times10^6$ to $2.5\times10^6$. These results are illustrated in Table 2. On average, 60% of the cells harvested at day 7 or 10 were large with an indistinct morphology, and ca. 55% were strongly positive for CD83/CD86, expressing in addition high levels of adhesion (CD11a, CD50, CD54, CD58), co-stimulatory (CD40, CD80, CD86), CD1a and MHC class II molecules. Functionally, these DC were 20 times more potent than monocytes and B cells at stimulating the proliferation of allogeneic T cells (data not shown). Routine sterility testing did not detect microbial contamination in any of the 61 vaccines.

### Clinical response

Clinical response was evaluated by CT or MRI in all patients with measurable residual disease after cytoreductive chemotherapy or debulking surgery. Follow-up was routinely performed by measurement of CA-125

**Table 2** Immunological evaluation of treated patients

| Patient no. | No. of vaccinations | Vaccination interval | DC dose ($\times 10^6$) | Effects | | DTH | | | Specific proliferation | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Local | General | Multitest score[a] | Specific reaction under vaccination | | KLH | Tumour lysate |
| | | | | | | | KLH | Tumour lysate | | |
| 1 | 3 | 10 d | 1.5–2 | – | – | 5.5 | ND | ND | – | – |
| 2 | 6 | 10 d | 1–2 | + | – | 10.5 | + | – | + | – |
| 3 | 23 | 10 d[b] | 24–74 | + | + | 10.75 | + | + | + | + |
| 4 | 6 | 4 weeks | 16–86 | + | – | 11.5 | + | – | + | + |
| 5 | 8 | 10 d | 4–7.6 | + | – | 9 | + | – | + | – |
| 6 | 4 | 10 d | 1–2.5 | – | – | 6.6 | ND | ND | – | – |
| 7 | 6 | 4 weeks | 15–25 | + | – | 6 | + | – | + | – |
| 8 | 5 | 4 weeks | 28–90 | + | + | 7.7 | + | – | + | – |

[a]Score: (a + b/2) + (c + d/2) + (e + f/2) of all indurations
[b]Vaccinations 1–14 at 10-day-intervals; vaccinations15–23 at 4-week intervals
ND: not determined

serum levels. These findings are listed in Table 1. Both patients with uterine sarcoma (Nos. 1 and 2) and one patient with an ovarian carcinoma (No. 5) showed disease progression and died shortly after receiving the third, sixth and eighth vaccination, respectively. Patient No. 6 showed tumour progression after the fourth vaccination and received third-line chemotherapy. Patient No. 3 (ovarian carcinoma) had an extended period (45 weeks) of disease stabilisation. CA-125 levels decreased from 34 U/ml before immunisation to 23 U/ml (c.v. 10%) after the fourth vaccination, remained constant up to the seventeenth and increased to 75 U/ml 3 weeks later (data not shown). MRI staging revealed local and distant metastases. After reductive surgery, this patient requested on an individual basis and outside the study design to be given immunotherapy using cell antigens from the recently excised tumour. She has received six additional vaccinations and to date has remained progression-free for 23 further weeks. In patient No. 4 (ovarian cancer), disease remained stable for 25 weeks. The CA-125 level prior to therapy was 115 U/ml and stayed constant following four vaccinations. Shortly after the fifth vaccination it increased to 206 U/ml; tumour progression was evidenced by CT and the patient underwent surgery. In patient No. 7, tumour progression was confirmed by CT-scan and increased CA-125 levels after 14 progression-free weeks. In patient No. 8, CT-scan after five vaccinations revealed disease stabilisation which continued for 27 weeks. This patient is currently under immunisation.

The treatment was safe, feasible and well tolerated. No major toxic (grade 2) or severe adverse effects related to the vaccine were observed. Minor general effects in two patients (Nos. 3 and 8) included mild transient fatigue, chills and low-grade fever for which no treatment was required. Slight lymph node enlargement was observed in patient No. 8. Five patients (Nos. 3, 4, 5, 7, 8) showed repeated mild hypersensitivity skin reaction in the form of temporary erythema at the vaccination site. None of the trial participants experienced rash or lymphadenopathy or showed clinical signs of autoimmunity.

## Immunological response

DTH skin tests for common recall antigens served to determine cellular immunocompetence prior to immunisation. Vaccine-induced antigen-specific immunity was determined in vivo by separately assessing DTH response to KLH and autologous tumour cell lysate. No DTH reactivity to the vaccine antigens was detected in any of the patients before vaccination, illustrating the suitability of this approach for eliciting and boosting T cell immunity. Four patients displaying a high score to recall antigens (Nos. 2, 3, 4 and 5) and two subjects with moderate to low scores (Nos. 7 and 8) demonstrated under vaccination strong DTH reactivity towards KLH, indicating the efficient generation of KLH-specific memory $CD4^+$ $T_H$ cells. In contrast, a detectable tumour lysate-specific immune response was observed only in patient No. 3. These results are summarised in Table 2.

Priming of T cell immunity to KLH and tumour antigens was further analysed by measuring in vitro the proliferative capacity of PBMC upon antigen stimulation in a 6-day BrdU incorporation assay. None of the patients displayed a pre-existing proliferative response to the vaccine antigens. Strong responses to KLH were detected after a single vaccination in the PBMC of six patients showing significant and moderate DTH reactivity to recall antigens (Nos. 2, 3, 4, 5, 7 and 8). This response consistently increased after subsequent vaccinations, indicating the boosting of KLH-directed immunity (Figs. 1, 2, 3). Tumour cell lysate-specific proliferation was significantly increased in two of the five patients who showed a vigorous lymphoproliferative response to KLH (Nos. 3 and 4), suggesting the induction of memory T cells capable of responding to epitopes

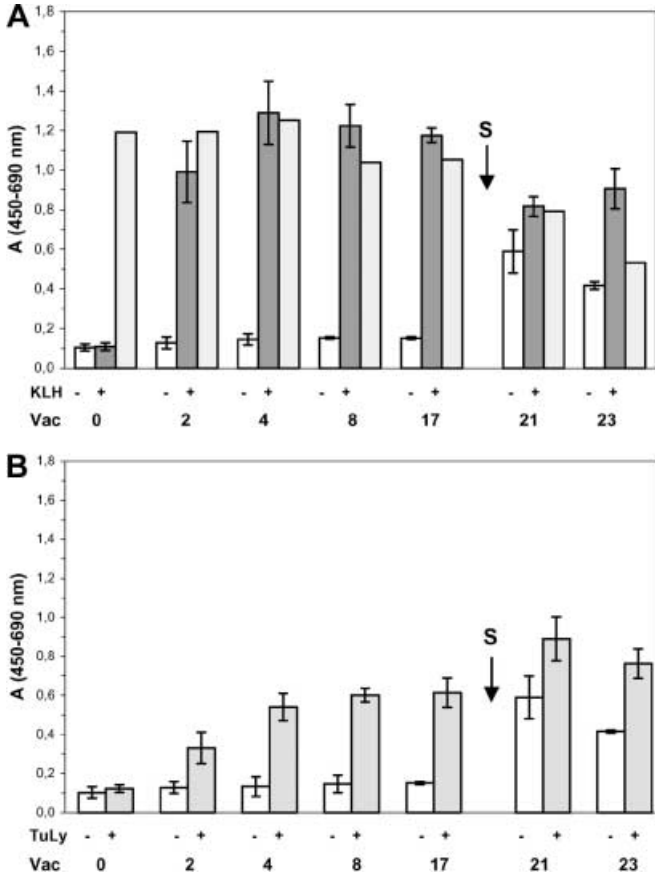


**Fig. 1** Time course of specific proliferative response to KLH (**A**) and autologous tumour cell lysate (**B**). PBMC of patient no. 3 obtained prior to and after subsequent vaccinations with KLH- and tumour lysate-pulsed DC were cultured for 5 days in the presence (+) or absence (–) of antigen (KLH: 25 μg/ml, tumour lysate: 50 μg/ml). BrdU incorporation is reported as the mean values of six replicate measurements above the media background, with error bars representing the standard deviations. PHA (1 μg/ml) was used as a positive control (*dotted column*)

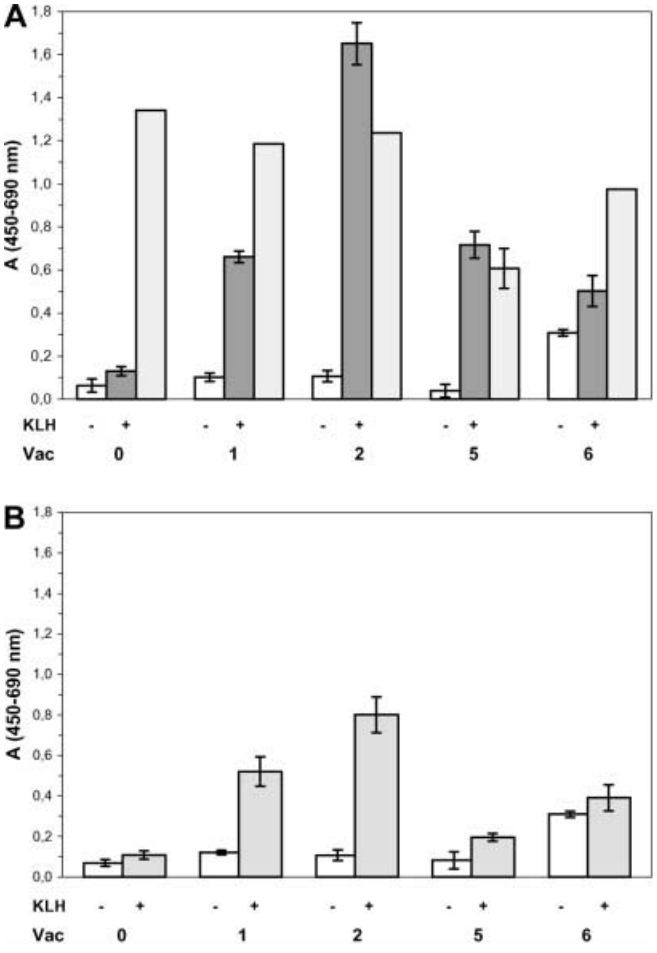


**Fig. 2** Kinetics of KLH- (**A**) and tumour cell lysate-induced PBMC proliferation (**B**). PBMC of patient 4 obtained prior to and after subsequent vaccinations with KLH- and tumour lysate-pulsed DC were cultured for 5 days in the presence (+) or absence (–) of antigen (KLH: 25 μg/ml, tumour lysate: 50 μg/ml, PHA: 1 μg/ml). BrdU incorporation is reported as the mean values of six replicate measurements above the media background with error bars representing the standard deviations

derived from tumour antigens (Figs. 1, 2). Of note, a consistent long-lasting proliferative response to KLH and tumour cell lysate was maintained in patient No. 3 during the vaccination period covering 23 vaccinations (Fig. 1), suggesting an enduring antitumour T cell memory which was significantly paralleled by two prolonged periods of progression-free disease (45 and 23 weeks, respectively; Table 1). Importantly, in patient No. 4 an abrupt decline of KLH- and tumour lysate-stimulated proliferation was observed after the fifth vaccination (Fig. 2), which seemed to be associated with the concurrent emergence of tumour progression, as well as significantly decreased lymphocyte counts (data not shown). Patients Nos. 2, 5, 7 and 8, who developed a strong immune response to KLH, did not show during the vaccination period any detectable tumour lysate-induced proliferation (Fig. 3). The amount of DC administered did not seem to correlate with the magnitude of T cell proliferation to KLH or tumour lysate. Patients Nos. 1 and 6, who displayed partial T cell anergy (a DTH score of 5.5 and 6.6, respectively; Table 2) did not at any time raise a significant lymphoproliferative response either to KLH or to tumour antigens. Significantly decreased proliferative reactivity to the mitogen phytohaemagglutinin was detected in the PBMC from partial responders to recall antigens (Nos. 1, 6, 7 and 8; data not shown) in comparison to that of full responders (Nos. 2, 3, 4 and 5), suggesting an impaired ability of T cells to respond to strong immune stimuli.

The type of vaccination-induced effector response, as well as the clonal size of antigen-specific memory T cells were established by analysing IFN-$\gamma$ production at single-cell level in ELISPOT. Significant expansion of KLH-specific IFN-$\gamma$ secreting T cells was detected after two vaccinations in two ovarian carcinoma patients (Nos. 3 and 4) by in vitro antigen challenge. In addition, a measurable enhancement in the frequency of tumour

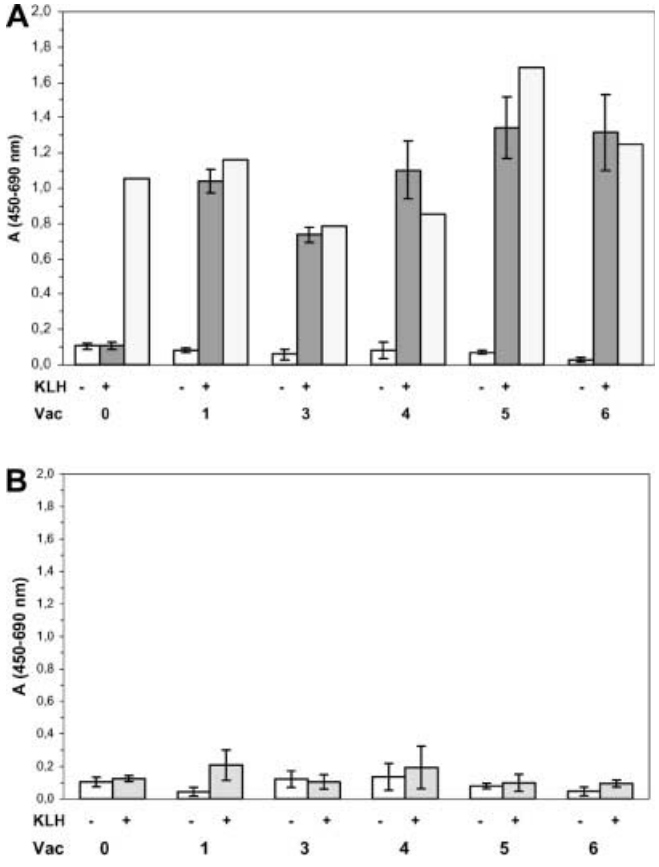


**Fig. 3** Kinetics of KLH- (**A**) and tumour cell lysate-induced PBMC proliferation (**B**). PBMC of patient 5 obtained prior to and after subsequent vaccinations with KLH- and tumour lysate-pulsed DC were cultured for 5 days in the presence (+) or absence (–) of antigen (KLH: 25 μg/ml, tumour lysate: 50 μg/ml, PHA: 1 μg/ml). BrdU incorporation is reported as the mean values of six replicate measurements above the media background with error bars representing the standard deviations

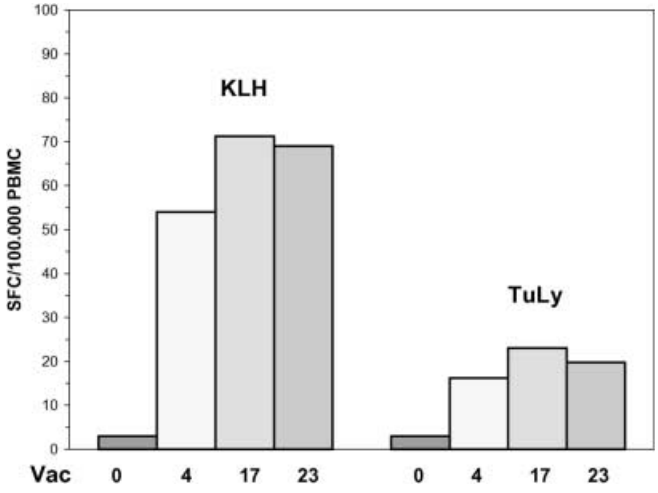


**Fig. 4** Frequency measurements by IFN-γ ELISPOT assays of Ag-stimulated PBMC from patient 3. Antigen-specific T cells were quantified at baseline prior to therapy, as well as after 4 and 17 vaccinations with KLH- and tumour cell lysate-pulsed DC. PBMC were activated with KLH (25 μg/ml) or tumour cell lysate (50 μg/ml) at $2\times10^5$ or $2.5\times10^5$ cells/well, respectively. Results are shown as the number of SFC per $1\times10^5$ PBMC. The data are representative of two experiments. The variation between the six replicate wells was < 25%

antigen-reactive IFN-γ releasing cells was detected in patient No. 3. An 18- to 23-fold increase in the frequency of KLH-reactive T cells (corresponding to 54 and 71 SFC per $1\times10^5$ PBMC), as well as a 5- and 8-fold expansion of tumour cell lysate-specific T cells (corresponding to 16.5 and 23 SFC per $1\times10^5$ PBMC) were observed after the fourth and seventeenth vaccination, respectively (Fig. 4). Furthermore, the number of spots, i.e. the frequency of memory cells producing IFN-γ, remained steady and within a moderate range from vaccination four to 23, demonstrating the stable induction of $T_H1$ type immunity in this advanced-stage, heavily pre-treated FIGO III patient. This finding is of considerable significance in view of the fact that this patient tested positive for KLH- and tumour cell lysate-induced delayed-type hypersensitivity, her PBMC showed consistent and substantial proliferation both to KLH and tumour antigens, and that she concurrently underwent two periods of disease stabilisation lasting 45 and 23 weeks.

## Discussion

The cloning and characterisation of TAA, as well as the development of methods for effective antigen delivery, have opened up new possibilities for immunotherapeutic approaches to cancer [1, 7, 33]. Ovarian carcinoma cells overexpress TAA CA-125 [13], HER2/neu [21] and MUC1 [27]. However, the molecular nature of CA-125 is poorly understood [13] and only a few HER-2/neu- and MUC1-derived CTL-epitopes have so far been identified [2]. In addition, HLA polymorphism precludes their being used in a vaccine with wide population coverage. Pulsing of DC with tumour antigens therefore represents a suitable, widely applicable strategy for eliciting specific multi-epitope immune responses circumventing the need for TAA identification, as well as HLA-restriction constraints. In view of the potential presentation of the entire repertoire of TAA and $T_H$- and CTL-epitopes, this approach reduces, the risk of targeting antigen-loss tumour variants evading immune recognition. Furthermore, priming immunity to epitopes derived from mutational events associated with an individual tumorigenic process might be an important component of an effective antitumour response.

The results of this phase I trial show that vaccination of patients with advanced gynaecological malignancies using DC pulsed with unfractionated tumour antigens and KLH as a helper foreign protein is a safe, feasible and clinically well tolerated procedure. In addition, our data suggest (but do not prove) a possible correlation between the immune response and disease stabilisation. A significant tumour antigen-specific lymphoprolifera-

tive response was detected in two patients (Nos. 3 and 4) after two vaccinations, indicating effective induction in vivo of antitumour T cell immunity in advanced-stage, intensively pre-treated ovarian cancer patients. Most importantly, tumour lysate stimulation in vitro of PBMC of patient No. 3 elicited in a 2-day ELISPOT assay a long-term stable increase of IFN-$\gamma$-producing cells, reflecting substantial enhancement of in vivo primed, tumour-specific $T_H1$ type cytokine reactivity, which is thought to mediate the control of tumour development [22]. Since naive T cells do not produce $T_H1$ or $T_H2$ cytokines and the assay is too short for antigen-induced cell proliferation or differentiation into memory T cells, it appears to be only in vivo differentiated memory T cells that produce IFN-$\gamma$. In support of this, data on natural $T_H$ immunity to HPV16 have shown that IFN-$\gamma$ assessment after brief in vitro antigenic challenge discriminates between primary and secondary T cell responses by detecting only T cells that have met antigen in vivo [34].

Clinical activity has been suggested by progression-free intervals ranging from 25 to 45 weeks in three patients with ovarian carcinoma (Nos. 3, 4 and 8). Patient No. 3, diagnosed with a well-differentiated serous papillary ovarian carcinoma FIGO IIIc, under standard therapy showed progression-free periods of 13 and 15 months respectively. Upon the second and third tumour recurrence, she was vaccinated with Her2/neu-derived peptide- (at another institution) and tumour lysate-pulsed DC, with progression-free intervals of 40 and 45 plus 23 weeks being documented, respectively. Since a significant tumour-reactive response was sustained after the seventeenth vaccination using DC pulsed with cell lysate of the recently excised tumour, it remains unclear whether the T cells induced recognised epitopes that were identical or shared between both tumours or whether alternatively, new clonal populations were generated by differentially expressed epitopes. Patient No. 4, diagnosed as having, a moderately differentiated to dedifferentiated ovarian carcinoma revealed a familial predisposition to ovarian carcinoma. Despite the unfavourable tumour characteristics, under vaccination the arrest of tumour growth lasted 25 weeks. Significantly, the decrease in the proliferative response to KLH and tumour antigens detected after the fifth vaccination coincided with the onset of tumour progression and might have been due to antigen-reactive T cells being sequestered at malignant tissues, tolerance induction or apoptotic destruction of activated cells at the tumour site.

In support of these data, ovarian carcinoma lysate-pulsed DC have been shown to be immunogenic in vitro [24, 37]. Murine data have further demonstrated effective antitumour T cell immunity using tumour lysate-loaded DC [5, 17]. Most importantly, DC pulsed with lysates from melanoma, renal cell carcinoma and paediatric tumours have been recently reported to elicit specific T cell immunity, as well as objective responses [4, 8, 10, 18]. Some of these trials have additionally used KLH as a foreign helper protein in eliciting a potent memory $T_H$ response [8, 10, 11, 18]. Significantly, loading of DC with KLH and tumour lysate has been shown to elicit pronounced enhancement of immune priming, therapeutic efficacy and IFN-$\gamma$ production by tumour-reactive T cells in a murine model [25].

Deficient T cell responses which may promote tumour development have been consistently reported in advanced stages of ovarian cancer [26]. The lack of adverse effects in this trial renders DC vaccination advisable at earlier disease stages when the tumour burden is lower and patients have received less potentially immunosuppressive treatment. The choice of appropriate tumour antigen(s) to arm DC, the improvement of clinically applicable techniques for in vitro generation of DC, as well as the relationships between dosing schedule, possibly combined with other therapeutic agents such as DC activators in vitro and in vivo, the extent of the immune response and clinical outcome warrant further investigations.

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