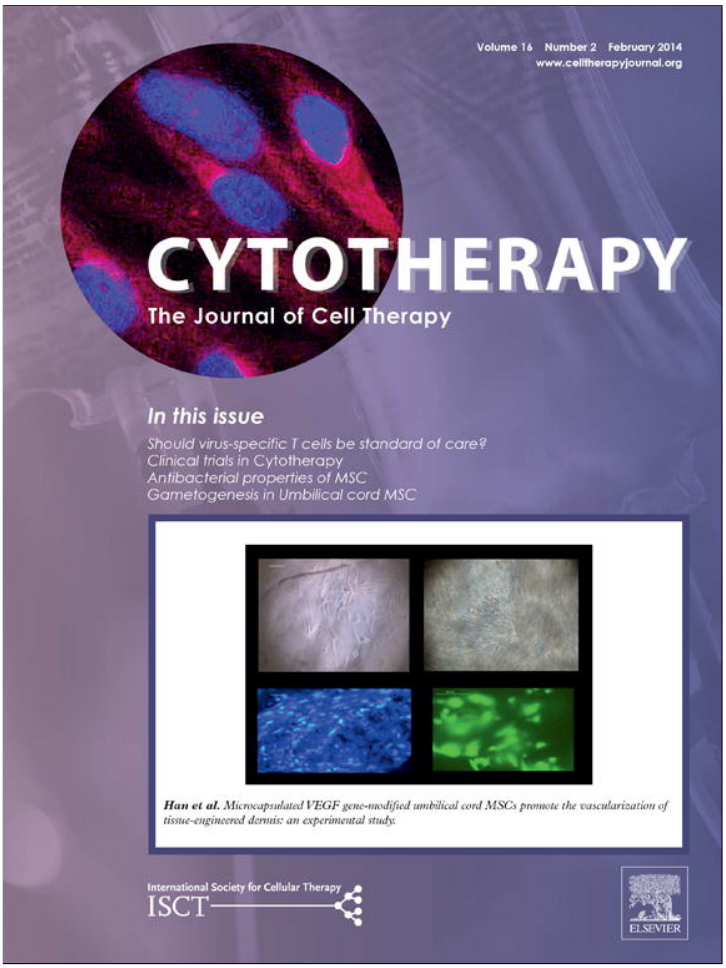

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Open-label, multi-center, non-randomized, single-arm study to evaluate the safety and efficacy of dendritic cell immunotherapy in patients with refractory solid malignancies, on supportive care

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Abstract

Background aims. A phase II clinical trial of an autologous dendritic cell (DC) formulation for the management of refractory solid malignant tumors was conducted across six sites in India with an objective to study safety and efficacy. *Methods.* A total of 51 patients with refractory cancer (either sex) with life expectancy ≥3 months, Eastern Cooperative Oncology Group score ≤2, available tumor tissue and adequate organ and bone marrow function were recruited. Monocytes obtained by leukapheresis, differentiated into DCs by cytokines and primed with autologous tumor lysate (fresh tissue biopsy or paraffin block). On the 8th day, mature DCs were analyzed for expression of CD40, CD80, CD83, CD86, DC205 and DC209. The treatment regime consisted of six doses (intravenous) over 14 weeks with 2 post-treatment follow-up visits, 6 weeks apart. Safety was assessed at all visits and responses were evaluated on days 58, 100 and 184 or at end of the study. *Results.* A total of 38 patients were evaluated for safety and efficacy. One adverse event classified as possibly related was an episode of rigors or chills with mild pyrexia during one infusion. Objective response rate by Response Evaluation Criteria In Solid Tumors was 28.9% (11/38) and immune-related response criteria was 42.1% (16/38); 90% confidence interval for objective response rate was (17.2, 43.3) and (28.5, 56.7) by Response Evaluation Criteria In Solid Tumors and immune-related response criteria, respectively. The median time to treatment progression was >9 weeks. Median overall survival was 397 days. An increase in the expression of interferon-γ was not significant. *Conclusions.* Therapy was safe. The responses, time to treatment progression and survival are encouraging for patients with aggressive refractory disease.

Key Words: *cancer immunotherapy, dendritic cells, monocytes*

Introduction

Metastases are the primary cause of death in patients with solid cancers (1,2). Cancer accounts for approximately 7% of deaths in India and 23% in the United States, with a prevalence of 2.5 million and approximately 0.8 million new cases each year in India (3). Chemotherapy has been in the mainstay of cancer treatment and has been found to be effective in various types of cancer, but metastatic malignancies often develop resistance to standard chemotherapies, which are also responsible for considerable morbidity and death. This has shifted the focus to more specific targeted therapies and immunotherapy. A viable approach in cancer immunotherapy is the use of dendritic cells (DCs) in orchestrating a repertoire of both innate (natural killer cells) and adaptive (T cells) immune responses against cancer. DC-based immunotherapy has emerged as a rational new concept in the treatment of malignant tumors, and there is increasing evidence from animal studies and clinical trials showing that DC-based immunotherapy strategy may be a viable option in cancer treatment.

Discovered by Steinman *et al.* (4) in 1973, for which he received the 2011 *Noble Prize in Medicine*, DCs have evolved from subset curiosity to the most

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sought-after option in immunotherapy. DCs are the most potent antigen-presenting cells that play a key role in programming and regulating tumor-specific immune responses by processing and presenting tumor antigens to naive or effector T lymphocytes (5). Numerous studies show that DCs loaded with tumor-associated antigens, exhibit protective anti-tumor responses that cause therapeutically regression of preexisting tumors or increase time to progression (TTP) without any significant toxicity (6–9).

Various studies have shown that anomaly in DC number and function is linked to malignancies such as breast cancer and multiple myeloma (10,11); further reduced DC counts in the peripheral blood of patients with cancer have also been associated with an accumulation of immunosuppressive immature myeloid cells (12,13), and these form the basis of the concept of DC-based immunotherapy. The other rationale for the success of DC-based immunotherapy is that tumors can evade immune surveillance through tumor suppressor cells, release of inhibitory cytokines (eg, interleukin-10, transforming growth factor -β) (14,15), loss of major histocompatibility complex class I cell surface molecule and structural abnormality of T-cell receptor -CD3 complex and so forth (16); most of these defects may be corrected by *in vitro* maturation of DCs, which can be used as immunotherapy.

Currently clinical trials on DC immunotherapy mainly involve either the use of whole tumor lysate, recombinant protein or RNA transfection strategy to be used as an antigen. This study involves the use of whole-tumor lysate attributable to several advantages in DC-based immunotherapy preparation. First, all patients are eligible for DC-whole tumor lysate therapy because patients are not selected on the basis of their human leukocyte antigen–A2 status. Second, whole-tumor lysate provides a rich array of tumor-associated antigens for both helper and cytotoxic T lymphocytes. This is important because the parallel presentation of antigens to both T-lymphocyte subsets helps in evoking stronger immune responses and could prevent the emergence of tumor escape. The presence of CD4+ T cells also promotes long-term CD8+ T-cell memory (17–19). In addition, DCs pulsed with whole-tumor lysate have shown enhanced efficacy in patients with cancer over DCs loaded with defined tumor-associated peptides or proteins, on the basis of meta-analytical data (20).

Provenge (Sipuleucel-T) was approved by the US Food and Drug Administration on April 29, 2010, for asymptomatic or minimally symptomatic metastatic castrate resistant (hormone-refractory) prostate cancer (21). Various clinical trials on use of DCs in cancer are currently ongoing in Europe, the United States and Asian countries. This multicentric phase II clinical trial in India was undertaken to study the safety, efficacy and tolerability of APCEDEN in refractory solid malignancies. The study was undertaken across six sites in India from September 2011 to December 2012.

Methods

This phase II study was an open-label, multi-centric, non-randomized, single-arm study in patients with refractory solid malignancies who were only receiving symptomatic care. Written informed consent of patients was obtained according to the *Helsinki Declaration*; the study was approved by the respective institutional ethics committees, and the trial was registered with Clinical Trial Registry India (Registration No. CTRI/2011/07/001917). ICH GCP (E6), Indian good clinical practices guidelines and ICMR ethical guidelines for biomedical research on human subjects were followed.

Generation of antigen-loaded mature DCs

APCEDEN is an autologous DC formulation in which DCs are derived from CD14+ blood monocytes as previously described by Romani *et al.* (22) and loaded with whole-tumor lysate. In brief, the process begins with separation of peripheral blood mononuclear cells by apheresis and further isolation of monocytes from apheresis harvest by plastic adherence; culturing in Roswell Park Memorial Institute 1640 media (Lonza, Allendale, NJ, USA) supplemented with cytokines interleukin-4 and granulocyte macrophage colony-stimulating factor (R&D Systems, Minneapolis, MN, USA) and autologous plasma *in vitro* and exposure of the patient's own tumor tissue lysate on the sixth day. For loading of DCs, fresh Trucut biopsy is preferred, but, in the case that an invasive procedure is not possible, paraffin block is used as the source of antigen (23). Tumor lysate was prepared by the freeze-thaw procedure as described by Nestle *et al.* (7), and protein concentration was determined according to Bradford's protein assay (24). On the sixth day, 5 μg/mL of polyinosinic:polycytidylic acid (Poly IC) (InvivoGen, San Diego, CA, USA) was used as maturation stimuli; after 3 h of adding poly IC, 1–20 μg/mL protein was loaded on DCs.

Characterization of DCs

Mature DCs harvested on day 8 were analyzed by means of flow cytometry with the use of fluorochrome-labeled antibody against CD80, CD83, CD86 (BD Biosciences, San Jose, CA, USA), DC205, DC209 and CD40 (Biolegend, San Diego, CA, USA). Viability of cells was assessed by 7AAD staining. Analysis

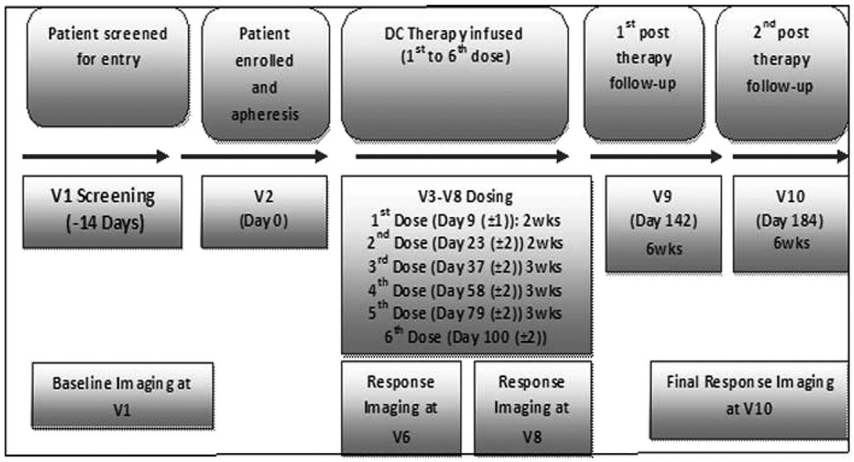

Figure 1. Study schema shows study design.

was performed by means of flow cytometry (FACS-Calibur; Becton Dickinson) with the use of CellQuest software. Adequate cell counts (>1 million DCs) per dose were used. All six doses were prepared at the same time (>1 million DCs/dose) and cryopreserved (10% dimethyl sulfoxide and complete media). Sterility testing was performed according to the procedure described in *US Pharmacopoeia*. Mycoplasma contamination was checked with the use of a MycoAlert-Mycoplasma detection kit (Lonza). Endotoxin was assessed by means of the kinetic chromogenic limulus amoebocyte lysate test (Lonza).

Study objectives

The primary objectives were to determine the safety, tolerability and efficacy of the therapy. Response was evaluated by (i) immune-related criteria (irRC) (25–27) and (ii) Response Evaluation Criteria In Solid Tumors (RECIST) criteria (28).

The secondary objectives were to measure quality of life (QOL) by use of FACT-G (Functional Assessment of Cancer Therapy-General) (29); change in immune response by measurement of pre- and post-therapy immune parameters such as CD4+ and CD8+ count and interferon (IFN)-γ in peripheral blood by flow cytometry; and TTP defined as the time period from the date of enrolment to the date when progression of disease was first documented.

Eligibility criteria

Both male and female adult patients with recurrent solid malignancies, on symptomatic care with at least 3 months of life expectancy, available tumor tissue, ECOG score of ≤2, having adequate organ and bone marrow function were enrolled. Pregnant and lactating women were excluded from participation.

Dosing schedule

Eligible patients underwent leukapheresis for collection of peripheral blood mononuclear cells. These cells were cultured and processed to differentiate into mature DCs. Mature DCs were harvested on day 8 and divided into six aliquots of 2 mL each. A total of six doses of DC formulation were administered over a 14-week period: day 9, day 23, day 37, day 58, day 79 and day 100. There were two post-treatment follow-up visits, 6 weeks apart. Safety assessments were performed at all visits, and response assessment was performed at day 58, day 100 and day 184 or end of study visit.

Study design

The study was designed as an open-label, multicenter, non-randomized, single-arm study in patients with refractory malignancies who were on supportive care. Because the population included patients with different types of solid malignancies who were resistant to different standard chemotherapeutic agents, a

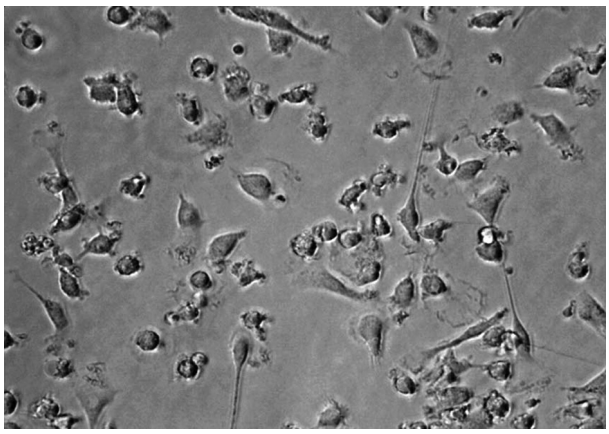

Figure 2. Light micrograph shows DCs on the 8th day of culture (magnification ×20).

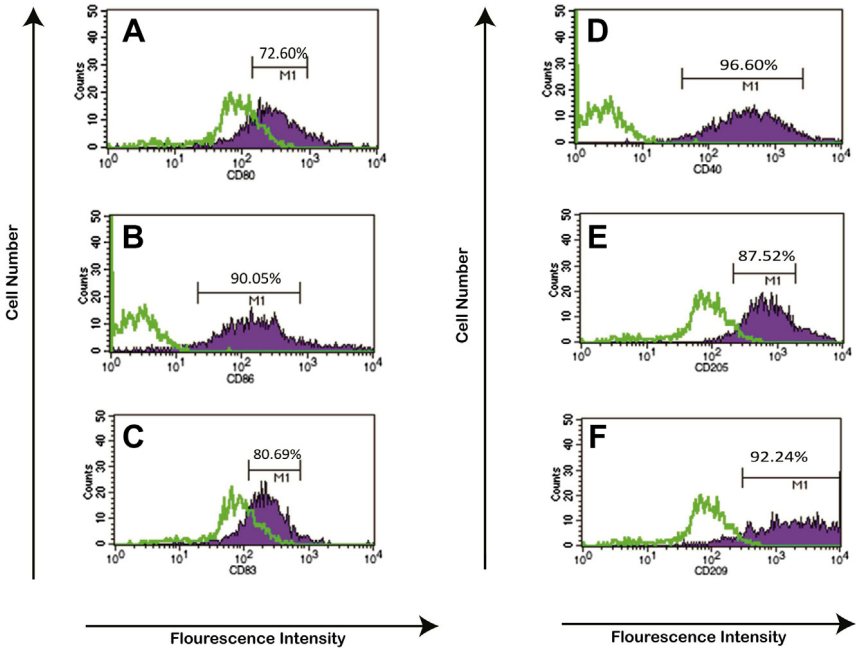

Figure 3. Expression of different surface markers on a mature DCs.

control arm could not be taken. The detailed study design of the trial is represented in Figure 1 (study schema).

Route of administration

Each dose of APCEDEN (>1 × 10^6 cells along with 100 mL of normal saline) was administered through the intravenous route as a slow infusion. Although various studies have preferred intradermal, subcutaneous or intranodal routes of administration, in the present study, DCs were administered through the intravenous route because it has been associated with a significantly higher frequency and titer of Ag-specific antibodies, which is desirable in some clinical situations in addition to the cellular immunity (30).

Safety and efficacy assessment

Primary end points were safety and tolerability as measured by the incidence and severity of treatment-emergent adverse events (TRAE) and incidence of serious adverse events (SAE). Efficacy of therapy was measured by means of tumor response and objective response assessment according to RECIST (version 1.1) and irRC. The objective response rate (ORR) for this study included the percentage of patients who showed complete remission (CR), partial remission (PR) and stable disease.

Secondary end points were to assess QOL as measured by the FACT-G (31) and change in the immune response by measuring immune parameters before and after therapy by means of flow cytometry and TTP.

Safety events were graded by use of the revised National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.0 (published May 28, 2009) (25,26,32). All Safety events were coded by use of MedDRA (version 13) (33).

Assessment of immune response by intracytoplasmic IFN-γ release assay

Intracellular staining for IFN-γ production of lymphocytes was performed as described by Kern *et al.*

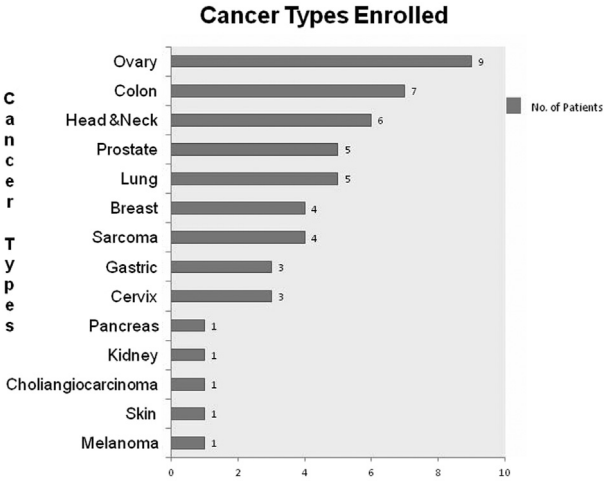

Figure 4. Graph represents different types of cancers enrolled in the study.

Table I. Summary of clinical profiles of patients receiving at least one dose (38 patients inclusive of cohort 1 and cohort 2).

S. No.	Patient ID	Sex	Age, y	Source of antigen	Diagnosis	Period of disease in months	Mets site	No. of chemo-failures	Date of enrollment in trial	Best response (visit/criteria/ response)	No. of DC infusions received	Status	TTP in days	Survival in days
Cohort 1	01HCH	M	59	FT	Head and neck	20	1	3	11/10/2011	V6/irPD/PD	5	Dead	60	95
	02RCH	M	64	FFPE	Head and neck	15	2	2	12/10/2011	V6/irPD/PD	5	Dead	60	157
	03GKK	M	63	FFPE	Colon	67	2	2	10/11/2011	EOT/ irPD/PD	3	Dead	45	72
	04KSK	M	38	FT	Sarcoma	60	4	2	04/11/2011	V6/irPD/PD V8/irPD/PD	6	Alive	60	397
	05RYP	F	31	FFPE	Ovary	63	3	3	05/12/2011	V6/irPD/PD	3	Dead	60	81
	06HMM	F	56	FFPE	Head and neck	45	2	4	01/12/2011	V6/irPD/PD	4	Dead	60	117
	07PDC	F	50	FFPE	Cervix	53	2	2	15/12/2011	V6/irPD/PD	4	Dead	60	74
	08SRG	M	69	FT	Head and neck	7	3	2	29/12/2011	V6/irPD/PD V8/irPD/PD V10/irPD/PD	6	Alive	60	342
	09GNK	M	62	FT	Lung	17	1	2	06/01/2012	V6/irPD/PD	4	Dead	60	184
	10NDT	M	70	FT	Colon	26	1	3	28/12/2011	V6/irPD/PD	4	Dead	60	116
	11N-B	F	51	FT	Breast	30	3	6	09/01/2012	V6/irPD/PD V8/irPD/PD	6	Dead	60	136
	12JMK	M	59	FT	Prostate	70	1	2	06/03/2012	V6/irPD/PD	3	Dead	60	149
	13VSS	M	70	FFPE	Cholangiocarcinoma	6	1	1	17/03/2012	V6/irPD/PD	4	Dead	60	123
Cohort 2	01JHN	M	71	FT	Colon	72	2	3	14/09/2011	V6/irSD/PD V8/irPD/PD	6	Dead	60	171
	02ARI	F	66	FFPE	Ovary	24	1	3	30/09/2011	V6/irSD/SD V8/irPD/PD	6	Dead	105	173
	03MKN	M	55	FT	Prostate	60	3	3	12/10/2011	V6/irSD/PD V8/irPD/PD	6	Dead	105	315
	04THI	F	57	FFPE	Sarcoma	48	1	2	18/10/2011	V6/irSD/PD V8/irPD/PD	6	Dead	105	152
	05SPA	F	54	FFPE	Ovary	30	2	5	28/10/2011	V6/irSD/PD V8/irPD/PD	6	Dead	105	198
	06BSR	F	51	FFPE	Ovary	35	2	2	15/11/2011	V6/irSD/PD	5	Dead	176	176
	07SEN	M	67	FT	Prostate	55	2	5	13/02/2012	V6/irSD/SD V8/irSD/SD	6	Dead	296	356
	08TAM	M	62	FFPE	Melanoma	26	1	1	09/12/2011	V6/irSD/PD V8/irSD/PD	6	Dead	207	207
	09M-B	F	70	FT	Sarcoma	39	1	2	10/01/2012	V6/irSD/PD V8/irPD/PD	6	Alive	450	450
	10VSM	F	40	FFPE	Lung	25	1	4	24/10/2011	V6/irSD/SD V8/irSD/SD V10/irSD/SD	6	Alive	528	528
	11GIB	F	42	FT	Ovary	56	1	2	06/01/2012	V6/irSD/PD V8/irSD/SD V10/irSD/SD	6	Alive	454	454

(*continued*)

Table I. Continued

S. No.	Patient ID	Sex	Age, y	Source of antigen	Diagnosis	Period of disease in months	Mets site	No. of chemo-failures	Date of enrollment in trial	Best response (visit/criteria/ response)	No. of DC infusions received	Status	TTP in days	Survival in days
	12RVT	M	73	FT	Prostate	72	1	3	02/01/2012	V6/irSD/SD V8/irSD/SD V10/irSD/SD	6	Alive	458	458
	13GBD	F	36	FFPE	Colon	60	1	1	29/11/2011	V6/irSD/SD	4	Dead	87	87
	14RVB	F	60	FFPE	Ovary	48	3	3	23/11/2011	V6/irSD/SD	5	Alive	498	498
	15SSK	F	60	FT	Ovary	40	2	2	29/12/2011	V6/irSD/PR V8/irSD/PR V10/irSD/PR	6	Alive	462	462
	16CSN	M	68	FT	Prostate	101	1	3	28/12/2011	V6/irSD/SD V8/irSD/SD V10/irSD/SD	6	Alive	463	463
	17A-A	F	42	FFPE	Cervix	24	2	3	12/11/2011	V6/irSD/PD	5	Dead	246	246
	18C-N	M	56	FFPE	Head and neck	12	1	1	14/11/2011	V6/irSD/PD V8/irPD/PD	6	Dead	193	193
	19K-B	F	61	FFPE	Head and neck	132	2	0	28/11/2011	V6/irSD/PD V8/irSD/PD V10/irSD/PD	6	Alive	493	493
	20G-H	F	33	FFPE	Head and neck	17	1	0	06/12/2011	V6/irSD/PD V8/irPD/PD V10/irPD/PD	6	Alive	485	485
	21I-V	F	38	FFPE	Breast	10	2	4	09/01/2012	V6/irSD/PD V8/irSD/PD V10 irPD/PD	6	Alive	451	451
	22JKR	M	57	FT	Lung	8	1	2	13/01/2012	V6/irSD/SD V8/irSD/SD V10/irPD/PD	6	Dead	447	417
	23BPS	F	54	FT	Cervix	17	2	2	17/03/2012	V6/irSD/SD V8/irSD/SD	6	Alive	383	383
	24V-S	F	54	FFPE	RCC	14	2	1	22/02/2012	EOT/irSD/PD	2	Alive	397	397
	25SKT	F	54	FFPE	Colon	44	2	4	22/11/2011	EOT/irSD/SD	3	Dead	379	439

FFPE, formalin; FT, fresh tissue.

in 1998 (34). In brief, 5 × 10^6 CD14-depleted peripheral mononuclear cells, obtained before vaccination (T0) and after the fourth vaccination (T6), were cocultured with 1 × 10^6 mature tumor lysate–pulsed DCs for 18 h. Monensin (10 μmol/L; Sigma, Vienna, Austria) was added during the last 3 h to block protein secretion. T0 and T6 cells, with and without exposure to tumor lysate, were used as controls. In a parallel set of experiments, 500 ng/mL of ionomycin (Sigma) and 50 ng/mL of phorbol myristate acetate (Sigma) were added to the cell suspensions. Cells were harvested, washed and permeabilized with a permeabilization agent (Immunotech S.A.S., Marseille, France), according to the manufacturer's protocol. Cells were double-stained with phycoerythrin-labeled anti-CD69 or anti–IFN-γ and fluorescein isothiocyanate (FITC)-labeled anti–CD3-specific antibody (Immunotech). Appropriate immunoglobulin G1 antibodies were used as isotype controls. Samples were analyzed with the use of a flow cytometer (FACSCalibur, Becton Dickinson, USA). The tests were performed before therapy was started and after the 6th vaccination was completed.

Ratio of CD4+ and CD8+ cells

Analysis of CD4 and CD8 lymphocyte count was performed according to the procedure earlier discribed by Borowitz *et al.* in 1992 (35). Briefly 2–3 mL of peripheral blood was incubated with the following anti-human monoclonal antibodies: anti–CD3-PC5, anti–CD4-FITC, anti–CD8-PE and anti–CD16-FITC (Becton Dickinson). After immunofluorescent staining, the cells were fixed with 1% paraformaldehyde and were then analyzed by means of a FACSCalibur flow cytometer with the use of CellQuest-PRO software (Becton Dickinson). The acquisition and analysis gates were restricted to the lymphocyte gate as determined by their characteristic forward and side-scatter properties. Cell expressing CD markers were acquired and analyzed in the FL1 or FL2 logarithmic scale through the use of the set gates.

Statistical analysis

Statistical analysis was performed with the use of the SAS package (SAS Institute Inc, Cary, NC, USA, and Version 9.1.3). A detailed statistical analysis plan (25,26,36) was prepared before locking the database and performing the final analysis for the study.

Results

DC characterization

To determine quality control of the DC formulation, criteria proposed by Figdor *et al.* (37) were followed concerning phenotype and purity. Light microscopy of 8th-day harvested cells showed predominantly mature DCs (Figure 2).

Cells were positive for CD80, CD83, CD86, DC 205, DC 209 and CD40+ (Figure 3). As a quality control criterion, a cell viability of 70% was considered as release criteria. Cells were free of microbial contamination and endotoxins.

Sample size

A total of 51 patients were enrolled for study and qualified as an intention-to-treat population. Thirteen patients died before they could receive the first dose, hence, 38 patients who received at least one dose were evaluated for safety and response. A total of 12 patients completed the study, which included six doses of DC immunotherapy and two post-treatment follow-ups in the course of 12 weeks.

Each sex had equal representation. Median age was 53 years. Most cases had multiple chemotherapy failure and metastatic disease. Most patients enrolled had cancer of the ovary, followed by colon and head and neck cancer. On-going co-morbid conditions such as hypertension, diabetes, chronic obstructive pulmonary disease and coronary artery disease were controlled with medication. A total of 14 different types of solid cancer were studied (Figure 4).

To analyze comparative data, the patients were divided into three cohorts. Cohort I included patients with progressive disease (PD), cohort II included patients with objective response (OR) and cohort III included non-evaluable patients; these were early dropouts and did not undergo response evaluation. Progression-free survival and overall survival were calculated from the date of enrollment and date of first infusion, respectively.

Baseline characteristics, source of antigen and instrument response of patients with OR are summarized in Table I. The cutoff date for analysis of data was December 5, 2012.

Safety and tolerability

Of 51 patients enrolled, 38 cases were evaluated for safety and efficacy, because 13 subjects died before the first dose could be given. Patients receiving at least one dose were evaluated for safety and response evaluation. In all, 225 infusions were administered and all were well tolerated, barring only one incident of chills and rigors associated with mild pyrexia reported during a single infusion. The patient continued on the study; symptoms were resolved and did not reappear during subsequent infusions. According to investigators, this could have been "possibly related" to study therapy.

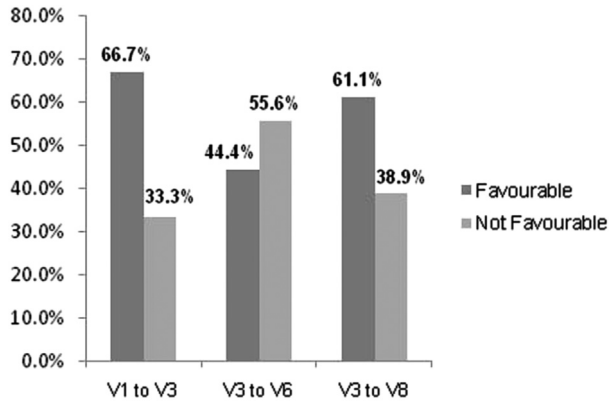

Figure 5. FACT-G scores change status (%) at different follow-up periods.

Adverse events were reported in 29 patients (56.9%) irrespective of causal relationship to study therapy. Twelve patients (23.5%) had TRAEs reported as unlikely to be caused by therapy. Only one TRAE, chills and rigors, was adjudged as "possibly related" to study therapy by the investigator. SAEs were reported in 21.6% of the patients but not related to the study therapy. All SAEs were attributed to the primary cancer condition and associated metastases and not adjudged related to study therapy. A total of 13 (25.4%) patients died while on study. All the deaths were caused by disease progression and were unlikely to be related to study therapy.

Efficacy

Response rates were summarized as follows: ORR by RECIST was 28.9% (11/38) and irRC was 42.1% (16/38); 90% confidence interval for ORR was (17.2, 43.3) and (28.5, 56.7) by RECIST and irRC, respectively. Intervals above 0 indicated that ORR was estimated with sufficient precision. Nine of 12 (75%) patients who completed the study continued to show OR by RECIST (version 1.1) as well as irRC. One patient from this group showed PR throughout the study. Best overall response was recorded for 43 cases. Eleven of 43 (25.6%) cases showed objective response.

QOL (FACT-G) deteriorated in 33.3% of the patients from visit 1 to visit 3 when no intervention was performed, which shows the baseline disease status of the patients. From visit 3 to visit 6, there was further deterioration in 55.6% after three cycles, which may be indicative of tumor flare associated with immunotherapy. Subsequent improvement in 61.1% patients from visit 3 to visit 8 after five cycles could be indicative of disease stabilization and therapeutic effect (Figure 5).

Immune response was evaluated as follows: Levels of IFN-γ from CD3+ cells could be performed in only 25 patients and is represented in Figure 6. The Mann-Whitney rank sum test showed that the increase in IFN-γ was not statistically significant ($P = 0.064$).

There was also an increase in the mean value of CD4:CD8 values (Figure 7); the Mann-Whitney rank sum test revealed that this increase was not statistically significant ($P = 0.151$).

Evaluable patients with progressive disease (PD) had a median TTP of 67.5 and 75 days by RECIST and irRC, respectively. Of 38 patients who took atleast one doses, 11 did not show progression by RECIST and 16 patients did not progress by irRC. Among completers (12 patients completed both follow-ups), TTP was computed to be 71 and 110 days by RECIST and irRC, respectively. Median TTP by both methods was well above 9 weeks for all evaluable cases.

TTP analysis was done across cohorts, which showed that cohort II showed significant delay in onset of disease progression (Table II).

Survival analysis was not part of initial trial protocol, but it was performed to get a better representation of the contribution of DC immunotherapy to the overall survival. On the cutoff date of December 5, 2013, more than 50% patients were surviving in cohort 2; therefore, median survival could not be calculated. April 26, 2013, was considered the cutoff date

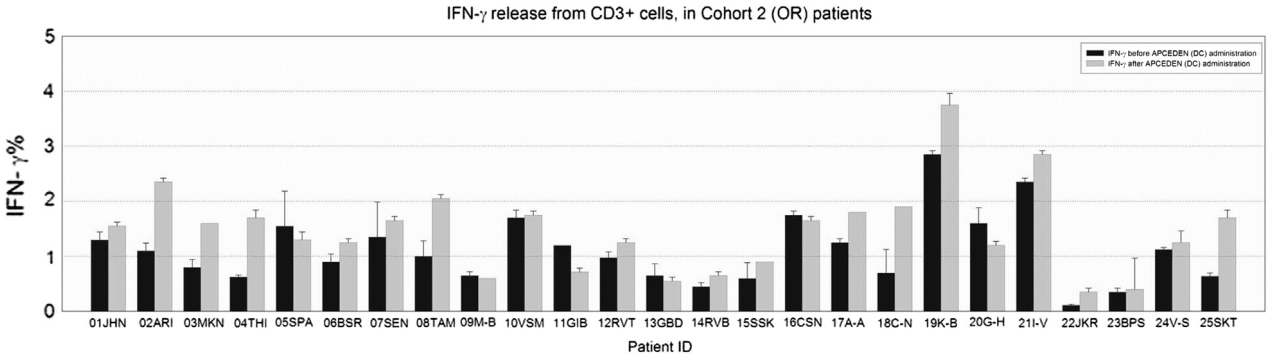

Figure 6. Graph shows levels of IFN-γ by CD3+ cells for patients with objective response (cohort 2).

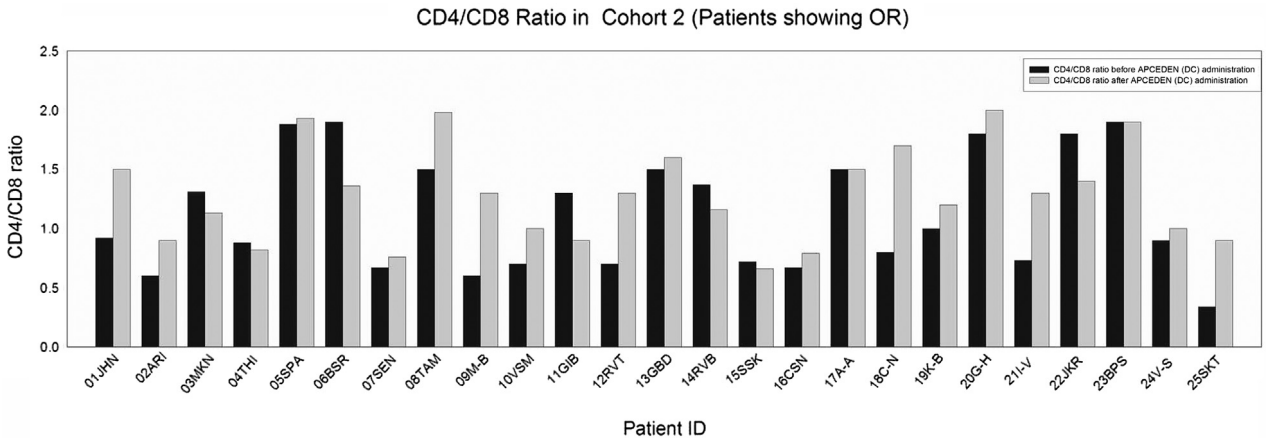

Figure 7. Graph shows ratio of CD4 and CD8 T lymphocytes in patients with objective response (cohort 3).

for calculating median survival. In patients showing objective response (n = 25), median survival was calculated to be 397 days and 123 days (Figure 8) for those with PD (n = 13), days for non-evaluable cases (n = 13).

Discussion

The primary aim of this study was to assess the feasibility, safety, tolerability and efficacy of tumor lysate–pulsed mature DCs in malignant solid tumors. The final formulation of whole-tumor lysate–loaded mature DC was found to meet the quality control specifications according to Figdor *et al.* (37). This clinical trial has demonstrated that the administration of APCEDEN leads to no major toxicity and is safe. Furthermore, the therapy was well tolerated and feasible to be performed on an outpatient basis.

In patients with objective response, we observed significant improvement in QOL, and overall median survival of 397 days was recorded in patients with objective response (cohort 2). This is particularly remarkable, given the far advanced clinical stage of the disease, multiple line of chemotherapy failures and the current lack of any conventional treatment options for these patients.

Moreover, median TTP of >9 weeks is also comparable to the Provenge study (38), in which most patients progressed at 8 weeks. ORR of the current study is better than in most of the DC-based trials. Draube *et al.* (39) performed a meta-analysis of 29 clinical trials in prostate cancer and renal cell carcinoma (RCC) and observed objective response rates of 7.7% in prostate cancer and 12.7% in RCC. The ORR of 28.9% and 42.1% by RECIST and irRC, respectively, is also good, considering (40) that only a single report of infusion reaction is also encouraging, which can establish it as a viable choice for cancer therapy. One patient showed PR, and none of the patients was found to meet the formal criteria for complete responses. In the current study,

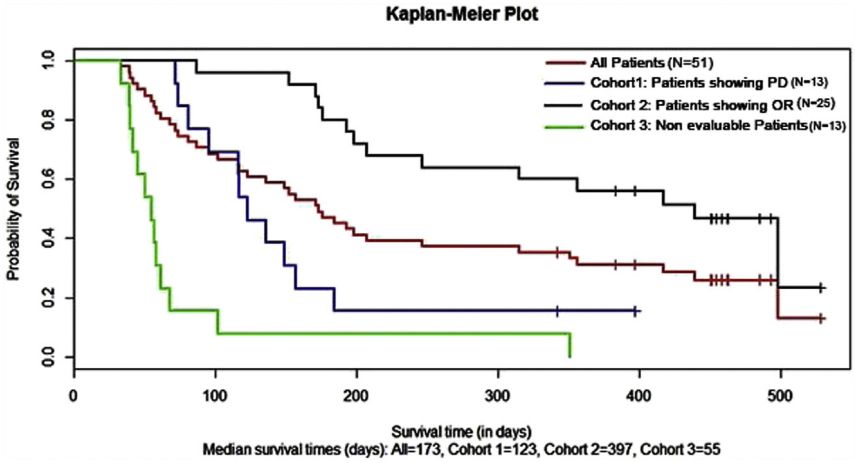

Figure 8. Kaplan-Meier survival plot.

Table II. Time to treatment progression, evaluable patients (n = 38).

	Cohort 1 (PD) n = 13	Cohort 2 (OR) n = 25	Overall n = 3 8
Mean (months)	1.96	7.52	5.62

no CR was observed; however, in other DC-based trials even CR has been achieved. In a study by Holtl *et al.* in 2002 (40), autologous tumor lysate–pulsed DCs were given through intravenous route for the treatment of RCC; two patients (of 27) showed CR and one patient showed PR.

The clinical data set the stage for further research in an appropriate clinical setting, right patient population and appropriate surrogate marker analysis for response and survival. Minimal residual disease and in combination with other modalities may be an area to explore. Other aspects of vaccine optimization, immune marker analysis and recombinant proteins for antigen preparation, could be considered for future research.

The current study shows that DCs can be used for adoptive immunotherapy.

In this clinical trial, only patients with advanced tumor stage were treated. However, minimal residual disease may be the optimal clinical setting to apply such a noninvasive and nontoxic therapeutic approach. Several aspects of vaccine optimization, antigen preparation and method of application are the foci of ongoing and forthcoming studies.

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