Research Article

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Immunotherapy

A retrospective analysis comparing APCEDEN® dendritic cell immunotherapy with best supportive care in refractory cancer

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Aim: A retrospective survival benefit analysis of APCEDEN®, APAC BIOTECH Pvt Ltd 69, Jacranda Marg, DLF PHASE II, Gurugram, Haryana, India, an autologous dendritic cell-based product for management of refractory solid malignancies, was performed in comparison with a control group. **Methods:** Subjects (retrospective data) whose survival data, geographical region, age, gender, ECOG performance status and stage of disease that could be matched with the treatment group were considered for analysis. **Results:** The analysis suggests a significant survival benefit of 199 days for the APCEDEN therapy treatment group when compared with the control group (356 vs 157 days). The event-free survival time of APCEDEN therapy was 439 days in patients who demonstrated an objective response at first evaluation as per immune-related response criteria. **Conclusion:** APCEDEN demonstrated highly convincing survival benefits when compared with the control group.

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Keywords: APCEDEN® • cancer • dendritic cells • immunotherapy • Kaplan–Meier • monocytes • survival

The immune system is capable of recognizing both danger signals (i.e., stress ligands) and high affinity T-cell epitopes from nascently transformed cells, destroying the developing tumor and protecting the host from neoplastic disease [1]. Cancer immunosurveillance is broadly comprised of three different phases referred to as elimination, equilibrium and escape [2]. Tumor cells can escape immune surveillance by exploiting the immune balance between antitumor immune cells like effector T cells or NK cells and protumor immune cells like regulatory T cells, myeloid-derived suppressor cells, M2 macrophages and other IL-10 secreting regulatory cells that include innate B cells and mast cells [2–8]. One paradigm of immunotherapy for cancer thus dictates a need for interference with immune suppression of tumor cells and a shift in balance back toward protective antitumor responses. Antitumor immunity develops from a multifaceted interaction between innate and adaptive immune responses. Frontline innate immune responses dictated by pattern recognition and stress ligands are followed by specific adaptive immune responses characterized by immunocytes expressing clonal antigen receptors. In recent years, there has been an explosion in the use of immunomodulatory techniques in cancer treatment. This revolution has been accompanied by novel approaches such as checkpoint inhibition that seek to overcome immune resistance as well as an increased use of cancer vaccines utilizing tumor antigens to augment specific adaptive responses [9–14].

Therapeutic vaccination of cancer aims at blocking the spread of the disease by skewing the T-cell response in favor of tumor elimination and induction of memory T cells. This strategy requires components of the immune system that present antigens to induce $CD8^+$ responses and also induce an appropriate population of T helper cells supporting an antitumor microenvironment. Dendritic cells (DCs), also known as nature's adjuvant, capture

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and process antigens effectively in their immature state, whereas upon maturation develop an enhanced capacity for antigen presentation, costimulation and T-cell activation. Stimulation of DC by tumor antigens has been achieved by both *in vivo* targeting and *ex vivo* loading. The choice of antigen preparation can determine overall success of the therapy with the most impressive objective clinical response observed following the use of whole proteins, tumor lysates, or killed tumor cells [15–18]. These results might be attributed to the broad availability of total tumor-associated and specific antigens in the above mentioned sources [16]. Additionally, exogenous antigens may be cross-presented to MHC-I to generate $CD8^+$ responses along with classical activation of $CD4^+$ cells [19]. *Ex vivo* generated DC vaccines like Sipuleucel-T (Dendreon Pharmaceuticals, WA, USA) involve the loading of DC-like antigen presenting cells with tumor antigens in culture followed by reinfusion of the activated autologous antigen presenting cell product into the patient [20] and is associated with a 4-month prolonged median survival in patients with asymptomatic, metastatic prostate cancer [21]. Other cell-based vaccination strategies have also yielded intriguing results. Holtl *et al.* reported two subjects with objective complete remission (CR) and one with partial remission (PR), out of 27 evaluable subjects with metastatic renal cell carcinoma [22]. Recently, Bapsy *et al.* demonstrated safety and efficacy of APCEDEN®, an autologous DC immunotherapy in patients with refractory solid malignancies. The protocol for APCEDEN vaccination involves collection of peripheral blood mononuclear cells, *in vitro* culturing and differentiation, and maturation of the DC product by loading with tumor lysate from the patient in the presence of the TLR-3 agonist polyinosinic:polycytidylic acid (poly I:C). The complete treatment regimen consists of six doses administered over a period of 14 weeks [23].

Comparing different immunotherapeutic vaccines is challenging because of a lack of standardized criteria for correlation between clinical and immune parameters as well as variability in tumor regression or disease stability among various tumor types. As Response Evaluation Criteria in Solid Tumors (RECIST) for determining the efficacy in immunotherapy remains controversial, immune responses and overall survival are other acceptable parameters to determine the efficacy of a vaccine. In the present study, the retrospective data of patients with no active systemic treatment were compared with an APCEDEN therapy treatment group to understand the survival patterns of subjects receiving the DC therapy versus comparable controls. In addition, the efficacy and safety data obtained in the *Bapsy et al.,* study were compared with that of the published literature.

Materials & methods

Vaccine preparation

APCEDEN is an autologous DC formulation in which DCs are derived from $CD14^+$ blood monocytes as previously described by Romani *et al.* [24] 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, NJ, USA) supplemented with cytokines, IL-4 and GM-CSF (R&D Systems, 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 case an invasive procedure is not possible, paraffin block is used as the source of antigen [25]. Tumor lysate was prepared by the freeze–thaw procedure as described by Nestle *et al.* [26], and protein concentration was determined according to Bradford's protein assay [27]. On the sixth day, 5 μg/ml of poly I:C (InvivoGen, CA, USA) was used as maturation stimuli; after 3 h of adding poly I:C, 1–20 μg/ml protein was loaded on DCs.

Treatment 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. The detailed study design of the trial is represented in Figure 1 (study schema).

Retrospective selection of control populations

Retrospective control subjects were chosen by considering the geographical region, age, gender, ECOG performance status, stage of disease and availability of survival data. The subjects whose demographic profile matched the subjects used for APCEDEN therapy and were not undergoing current active systemic treatment were considered for survival analysis. A list of the subjects and their respective last received supportive therapy is provided in Supplementary

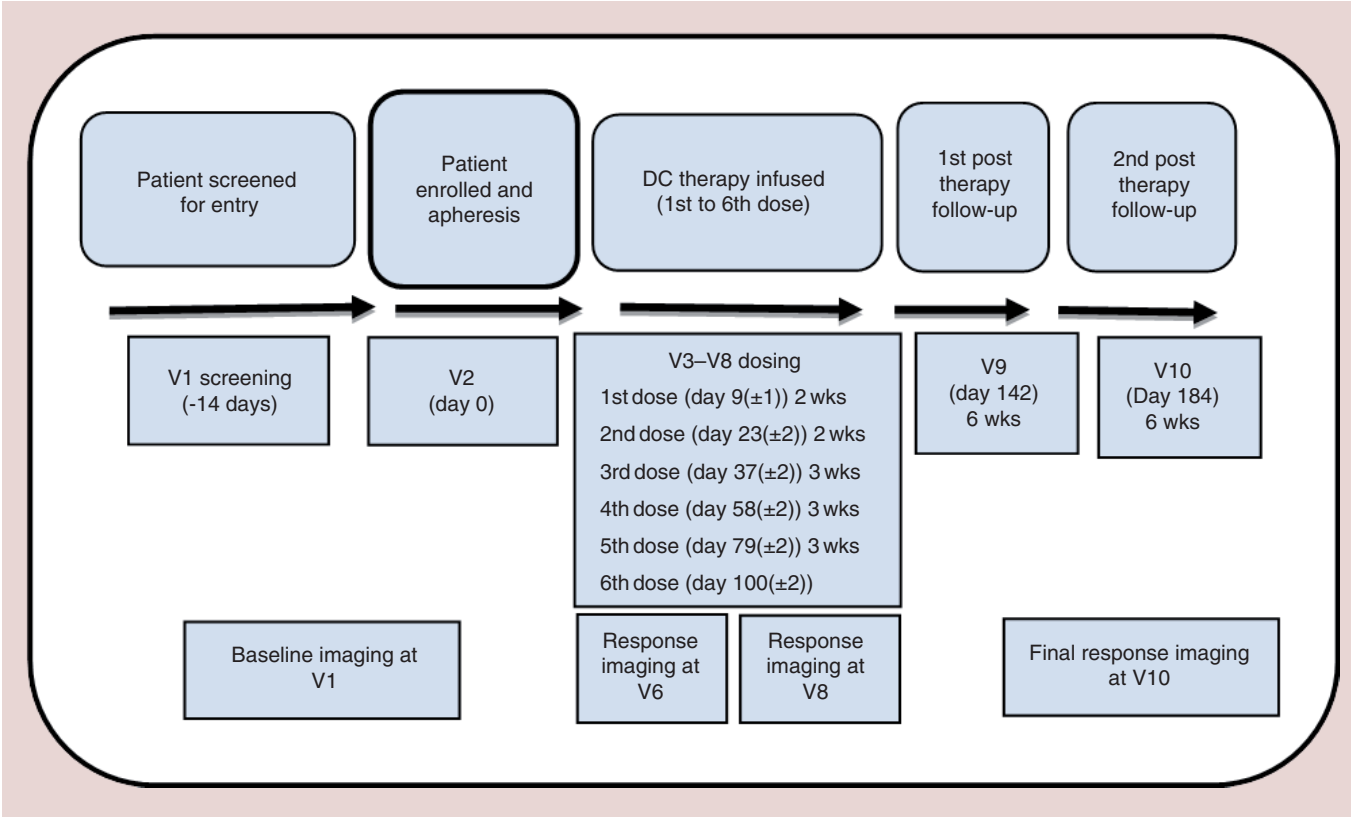

Figure 1. Study schema shows the study design.

Table 1A. The retrospective data from the subject's medical records with prior independent ethics committee's approval were collected using a data capture form predesigned for subjects who received APCEDEN therapy [23]. The data were collected from three different centers across different geographical regions in India (BiBi General Hospital, Hyderabad; Curie Manavata Cancer Center, Nasik; Sri Venkateshwara hospital, Bangalore; Ruby hall clinic, Pune). The ethical committee approval was taken from these hospitals to conduct the study. The retrospective data collected from individuals receiving no active systemic treatment are referred as the control group. The study details of APCEDEN therapy were described previously by Bapsy *et al.,* [23]. Survival data derived from the original APCEDEN study are presented in Supplementary Table 1B.

Statistical methodology

Kaplan–Meier (KM) survival analysis was used to plot survival curves and estimate median survival time to end points that are based on time to event analysis. The cut-off date for survival analysis was 26 April 2013 for the APCEDEN treatment group [23]. Subjects alive at that time point are included in censored values along with subjects who lived less than 100 days to compare the APCEDEN therapy to the control group. The complete treatment regimen consists of six doses administered over a period of at least 14 weeks or 98 days. Patients who survived for less than 100 days are not expected to comply with full treatment of APCEDEN therapy. Hazard ratio estimation of treatment effect between APCEDEN therapy and control group was calculated using the Wald's test and Cox proportional hazard model using the APCEDEN data as the numerator. Two-sided 95% confidence intervals were calculated as described [28]. SAS® 9.3 software was used for survival analysis, Graphpad Prism 5.0 was used to generate plots and fisher exact test to enumerate the overall response rates of APCEDEN therapy compared with the published literature.

Results

Survival analyses of subjects given APCEDEN treatment

Figure 2 details cancer diagnoses of the APCEDEN treatment group and the corresponding control group. Subjects

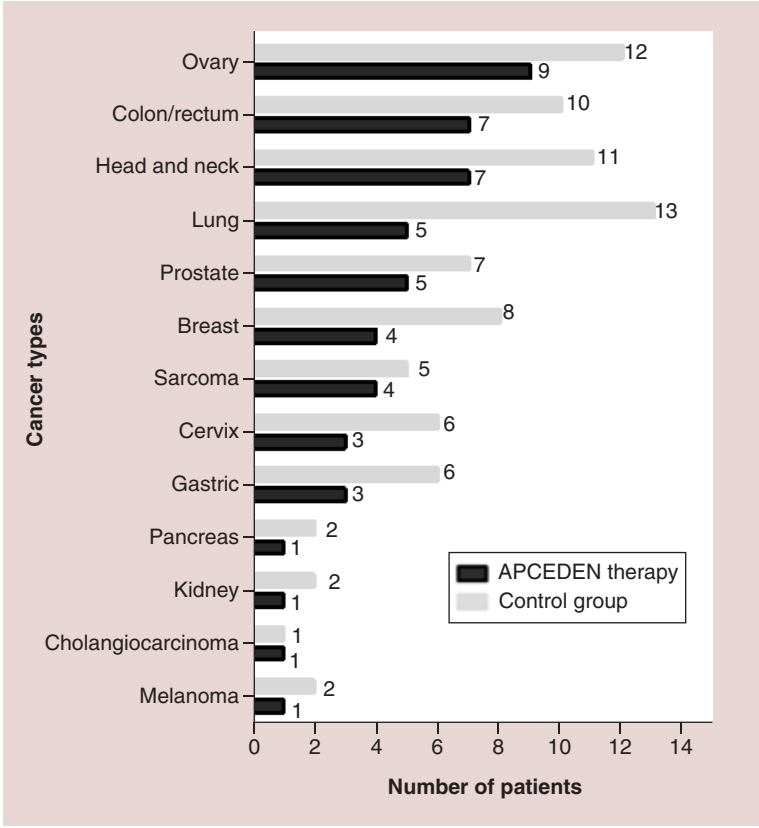

Figure 2. Bar graph indicated different types of cancer diagnoses of subjects enrolled in both the APCEDEN® treatment and control groups.

Table 1. Summary of survival rates for APCEDEN® therapy (by cohort).

Overall survival	APCEDEN	
	Cohort 1†	*Cohort 2*‡
	n = 13	*n = 25*
Median, days (95% CI)	149 (116–NC)	439(207–NC)
Mean days(SE)	150 (10)	335(26)

†Included patients who showed progressive disease at first evaluation.
‡Included patients who showed objective response at first evaluation.
NC: Not calculated as the horizontal line at 50% survival does not intersect the confidence interval; SE: Standard error.

that received APCEDEN treatment (n = 51) excluding the nonevaluable subjects (n = 13) were categorized based on their response at first evaluation based on immune-related response criteria (irRC). Subjects with progressive disease at first evaluation were analyzed in cohort 1 (n = 13) and the subjects with an objective response were assigned to cohort 2 (n = 25). KM survival analysis for cohorts 1 and 2 indicates the higher probability of prolonged survival in cohort 2 subjects (Figure 3). The analysis also illustrates median survival days as 149 for cohort 1 which is significantly less in comparison to 439 days for cohort 2 (Table 1).

Comparative survival analyses between subjects given APCEDEN treatment & control subjects

Survival data comparing the treatment group (APCEDEN) and the control group (supportive care only) is shown in Table 2. The data were analyzed in two clusters, the first including all subjects and the second excluding those designated as non-evaluable. In survival analysis I, the total number of subjects n = 51 who received the complete APCEDEN treatment regimen were considered for analysis. In survival analysis II, 13 subjects who were early dropouts and could not be assessed for a response even once and did not receive the complete treatment regimen were excluded. In survival analysis I, the percent of censored values in the APCEDEN treatment was 60.8 versus 64.7% for the control group. In survival analysis II, 50% of APCEDEN treated subjects and 64.7% of controls were censored. KM analyses of survival for APCEDEN versus the control group indicated a significantly higher

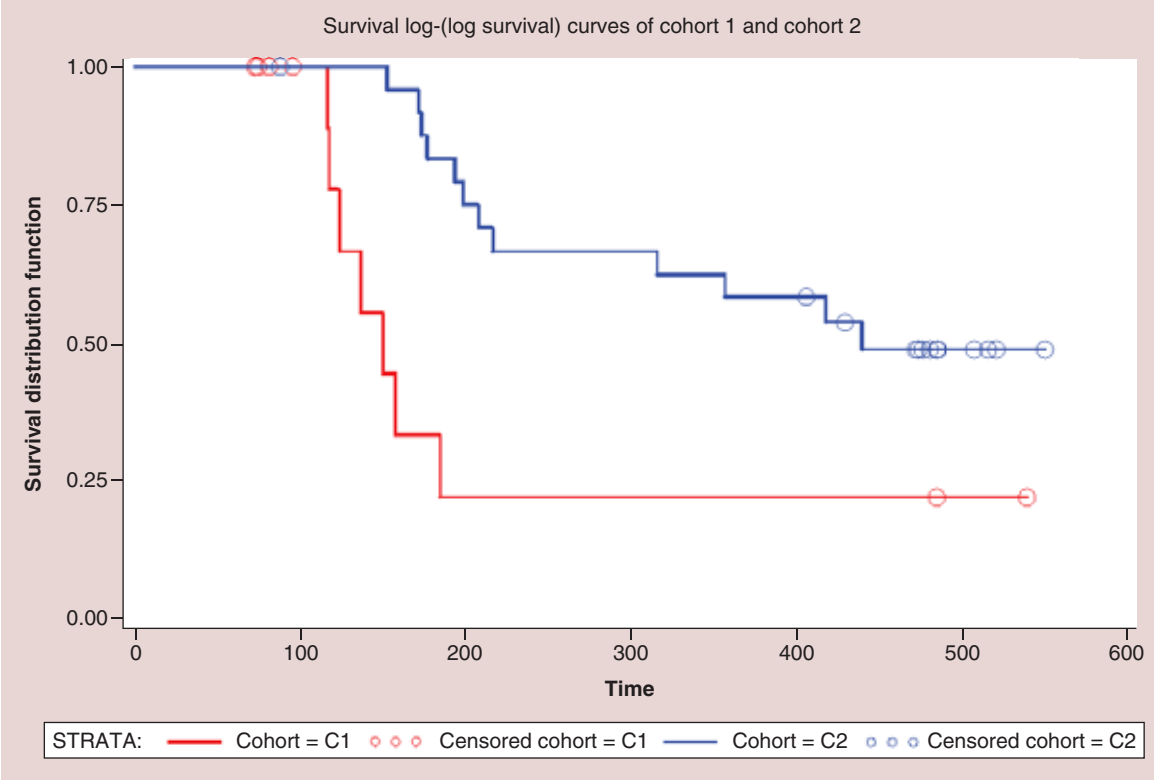

Figure 3. Kaplan–Meier event-free survival analysis of patients treated with APCEDEN® therapy stratified by response at first evaluation.
Blue line (top): Objective response; Open circles: Censored data; Red line (bottom): Progressive disease.

Table 2. Summary of survival rates in APCEDEN® therapy versus control group.

Overall survival	Survival analysis I†		Survival analysis II‡	
	APCEDEN	*Control group*¶	*APCEDEN*	*Control group*¶
	n = 51	*n = 85*	*n = 38*	*n = 85*
Median, days (95% CI)	356 (184–NC)	147 (140–182)	356 (193–NC)	147 (140–182)
Mean days (SE)	305 (24)	215 (28)	307 (24)	215 (28)
Hazard ratio§ (95% CI)	0.364 (0.203–0.656)		0.356 (0.196–0.646)	

†Survival analysis was performed including all the 51 subjects who received at least one dose of APCEDEN and continued in the study for sufficient time. Patients who survived less than 100 days are censored.
‡Survival analysis was performed excluding 13 subjects out of 51 subjects who were early dropouts and could not be assessed for a response even once. Patients who survived less than 100 days are censored.
§APCEDEN therapy with event-free survival data is used as numerator to calculate the hazard ratio.
¶Retrospective data collected from advanced solid malignancies patients receiving no active systemic treatment.
NC: Not calculated; SE: Standard error.

probability of prolonged survival in subjects receiving APCEDEN treatment (Figure 4). Median survival for subjects that received APCEDEN therapy was significantly higher than the control group in both survival analyses (356 vs 147 days). The reduced risk of death is further highlighted by the hazard ratios of 0.364 and 0.356 for survival analysis I and II, indicating a survival advantage of roughly 64% following treatment with APCEDEN therapy.

Discussion

Though immunotherapy is becoming increasingly prevalent in cancer patients, standard methodologies to characterize efficacy are still being accurately developed. Frequent contradiction between evaluations of disease progression by RECIST and irRC, identify cases that can be categorized as pseudoprogression [29]. While such cases can often look similar to progressive disease by standard imaging technologies, apparent increase in tumor burden is in reality

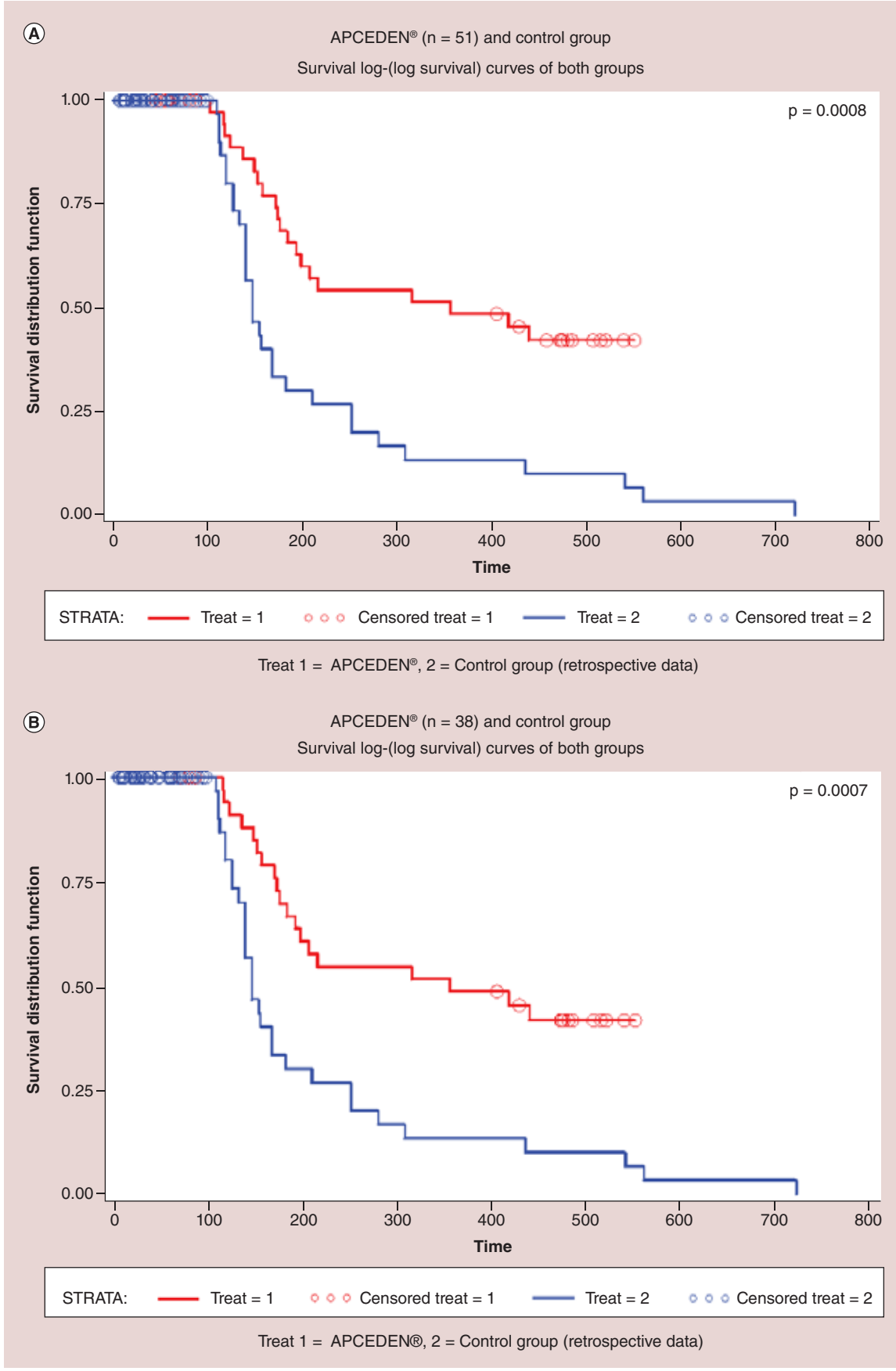

Figure 4. Kaplan–Meier overall survival analysis. (A) APCEDEN® (n = 51) and control group**. (B)** APCEDEN (n = 38) and control group.
Blue lines (bottom): Controls; Open circles: Censored data; Red lines (top): APCEDEN.

due to an influx of infiltrating immune cells. Cases of pseudoprogression have been reported by many different groups. For example, Wolchok *et al.* reported that 10% patients who demonstrated an objective clinical response per irRC would have been wrongly categorized as progressive disease (PD) by WHO criteria [30]. Unlike the conventional cytotoxic chemo- and radiotherapies, immunotherapeutic interventions require an increased amount of optimum to reach a quantifiable clinical end point. This observation explains the often delayed separation of KM plots in immunotherapy versus chemotherapeutic treatment [31]. Overall survival analysis and estimation of hazard ratio over a pre-established time frame might be considered a more appropriate method to analyze the efficacy of immunotherapeutic agents.

The retrospective control group was selected from different geographical locations across India and was performed primarily on refractory patients. A match of the tumor type of the control group with the APCEDEN treatment group was not achieved due to the lack of statistically significant number of patients to carry out the analysis. Hence, it was opted to consider 13 different tumor entities with different treatments to enrol a significant number of patients into this retrospective study. The data of the previous treatments were also unavailable as the patients had consulted multiple hospitals for their preceding treatments.

The median event-free survival time following APCEDEN therapy was approximately 11 months and compared extremely favorably to the median survival time of approximately 5 months in control group patients (i.e., Table 2A). The survival curve for APCEDEN therapy is well above the survival curve of control group, thus exhibiting better survival benefit with the DC-based APCEDEN therapy (HR = 0.35). The median survival time for subjects in the APCEDEN treatment group who showed an objective response per irRC at their first evaluation was 439 days (14.6 months), significantly better than the patients who exhibited progressive disease at first evaluation (149 days – approximately 4 months). Survival data for cohort 1 subjects were similar to that of the control group.

Following APCEDEN treatment, the objective response rate by RECIST was 28.9% (11/38; 90% CI: 17.2–43.3); however, irRC was 42.1% (16/38; 90% CI: 28.5–56.7). Median time to progression was >9 weeks [23]. These results indicate that the response rates for APCEDEN therapy were at least as good as previously published studies. In one published study, the serious adverse events (SAEs) reported after Provenge® (Dendreon Pharmaceuticals, WA, USA) treatment was 24%; however, this was statistically indistinguishable from the 25.1% SAE rate of that study's control group [32]. The reported SAE rate for APCEDEN was 21.6% [23]. The total number of adverse events observed following APCEDEN treatment was 45 AEs in total of 51 patients, whereas 591 AEs in 146 patients were observed following Provenge treatment.

In conclusion, APCEDEN personalized DC-based therapy has been shown to work effectively in various cancer types with an adverse event profile and quality of life better than that exhibited by the retrospective control subjects identified in this study [23]. These results have important clinical relevance for patients with advanced solid malignancies as the long-term survival benefit observed among at least 30% subjects receiving APCEDEN opens a promising area for further development.

This retrospective survival comparison analysis was performed on the recommendation of Indian Council of Medical Research, apex body in India for formulation, coordination and promotion of biomedical research. The study was further reviewed and scrutinized by Central Drugs Standard Control Organization, the national regulatory body for pharmaceuticals and medical devices and ICMR. We present here the same data approved by both the central bodies.

Financial & competing interests disclosure

S Kohli, S Chiliveru and B Sharan are employees of APAC Biotech, however, given that the data were collected, analyzed and handled at all times by a third party CRO (ClinSync Clinical Research Pvt. Ltd. Hyderabad, India), the authors are highly confident that the presented data are free of bias or inappropriate manipulation. None of the other study authors have conflicts of interest. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors have obtained appropriate institutional ethical review board approval or have followed the principles outlined in the Declaration of Helsinki for all human experimental investigations. In addition, for all the investigations done in this study, informed consent has been obtained from the participants involved.

Summary points

- APCEDEN®, is an autologous dendritic cell-based immunotherapy product for management of refractory solid malignancies.
- The present retrospective survival benefit analysis was performed in comparison with control group on the recommendation of Indian Council of Medical Research and Central Drugs Standard Control Organization.
- The median event-free survival time following APCEDEN therapy was approximately 11 months in comparison to the 5 months of control group.
- The objective response immune-related response criteria for APCEDEN treatment group at first evaluation was 439 days significantly better than 149 days for patients showing progressive disease.
- The long-term survival benefit observed among at least 30% subjects receiving APCEDEN opens a promising area for further development.

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