Article

Potential immunotherapy of dendritic cell-cytokine-induced killer cells for refractory metastatic colorectal cancer: a preliminary experience of single tertiary hospital

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Abstract: Background: Successful treatment for patients with metastatic colorectal cancer who failed to response to first-line treatment has been a challenge due to their low response rate for later-line treatment and poor progression free survival and overall survival. The application of dendritic cell-cytokine-induced killer cells (DC-CIK) immunotherapy combined with conventional treatment, either surgical resection or chemotherapy, showed improvement in survivals in first-line treatment for metastatic colorectal cancer. In this retrospective study, we aimed to evaluate the benefit of dendritic cell-cytokine-induced killer cells (DC-CIK) immunotherapy for patient with refractory metastatic colorectal cancer. **Methods:** A total of 20 patients with refractory metastatic colorectal cancer receiving cell-cytokine-induced killer cells (DC-CIK) immunotherapy were enrolled to this study. Among these patients, 11 patients responded to the treatment and the remaining 9 patients did not. All patients were followed for at least one years and the determination of treatment response was mainly based on image study at 6 months after the completion of treatment. Data were analyzed with Kaplan-Meier method and log-rank test. **Results:** The treatment response rate of the study group is 55% (11/20). The median progression free survival (PFS) and median overall survival (OS) of responsive patients was 7 months and 12 months, respectively. The median overall survival of irresponsive patients was 9.5 months. Four responsive patients received subsequent metastectomy or cytoreduction plus hyperthermic intraperitoneal chemotherapy surgery (CRS + HIPEC). **Conclusion:** DC-CIK cell-based immunotherapy may provide benefits for patients with refractory mCRC with improved response rate and progression free survival.

Keywords: DC-CIK; immunotherapy; colorectal cancer

1. Introduction

Colorectal cancer (CRC) is one of the most common solid malignant tumors worldwide. It is also top three malignancies of cancer-related cause of death. [1-3] Over 20% of patients diagnosed with CRC have distant metastasis. Which has poor prognosis of 5-year survival about 10-20%. [1, 4-6] First

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line treatment of metastatic CRC (mCRC) is chemotherapy and/or radiotherapy combine with surgical resection. [5, 7] Various options of chemotherapy regimens are available with biosynthetic agents while therapy also may be determined according to genetic profile of the tumor. However, treatment for mCRC is still difficult especially in patients who failed in first-line treatment and had higher disease severity such as tumor burden and site of metastasis. Recently, cell-based immunotherapy has emerged as a promising treatment option for metastatic colorectal cancer. Dendritic cell (DC)-cytokine-induced killer (CIK) cell therapy has gained enormous attention due to its potential to enhance anti-tumor immunity and improve patient outcomes.

Dendritic cell-cytokine-induced killer cells (DC-CIK) therapy involves the use of two types of immune cells: dendritic cells and cytokine-induced killer cells. Dendritic cells are antigen-presenting cells that play a critical role in initiating immune responses against foreign substances, including cancer cells. Cytokine-induced killer cells are T cells that have been activated by cytokines to specifically target cancer cells. In DC-CIK therapy, dendritic cells are initially isolated from the patient's blood and then exposed to cancer antigens to activate. These activated dendritic cells are then combined with cytokine-induced killer cells and infused back into the patient's bloodstream. [22, 23]

There are few studies revealed an efficacy of dendritic cell-cytokine-induced killer cells (DC-CIK) immunotherapy especially in progression-free survival (PFS), disease-free survival (DFS) and overall survival (OS). The preliminary results of a combination of DC-CIK immunotherapy with first line treatment seemed promising. [8-10] However, the benefit and clinical outcomes of DC-CIK immunotherapy combine with second- or later- line chemotherapy are still not definitely revealed or discussed.

In this retrospective study, CRC patients who received DC-CIK cell-based immunotherapy as adjuvant therapy of standard second- or later- line chemotherapy during July 2020 to September 2021 were recruited and their characteristics and clinical outcomes were analyzed. Figure 1 shows the timeline of the DC-CIK cell-based immunotherapy.

2. Materials and Methods

2.1. Patients

Patients with pathologically confirmed diagnoses of colorectal cancer who received DC-CIK cell-based immunotherapy as adjuvant therapy of standard chemotherapy were retrospectively enrolled from China Medical University Hospital during July 2020 to September 2021. All patient. The tumor node metastases (TNM) staging system of the American Joint Committee on Cancer/Union for International Cancer Control was used to confirm CRC stage or differentiation. The inclusion criteria were: (1) a pathological diagnosis with colorectal cancer; (2) receiving 2 full cycles of DC-CIK therapy; (3) receiving DC-CIK cell-based immunotherapy combined with second- or later-line chemotherapy. Exclusion criteria were: (1) having a follow-up period less than 12 months; (2) being lost during followed up or having incomplete data; (3) receiving DC-CIK cell-based immunotherapy combined with first-line chemotherapy. Finally, a total of 20 patients was included in this study. Figure 2 is the flow chart of this study.

Medical records of the patients were reviewed for treatment response, and we focused on both followed up image finding (CT, MRI and PET) and tumor marker (CEA and CA19-9) at 6 months after the completion of DC-CIK cell-based immunotherapy. Recruited patients were then divided into two group, responder and non-responder, based on the treatment response. Definition of treatment response is a more than 20% decrease in tumor volume as shown by follow-up image, either CT, MRI or PET. Progression free survival (PFS) was defined as the time from the date of completing DC-CIK cell-based immunotherapy to the date of first progression or last contact. Overall survival (OS) was defined as the time from the date of completing DC-CIK cell-based immunotherapy to either the date of death or the date of last follow-up.

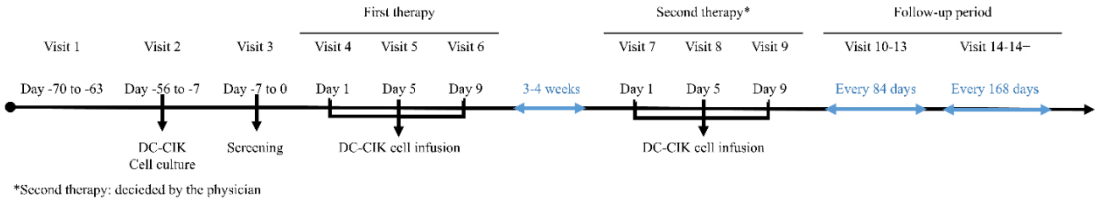

Figure 1. Timeline of DC-CIK cell-based immunotherapy.

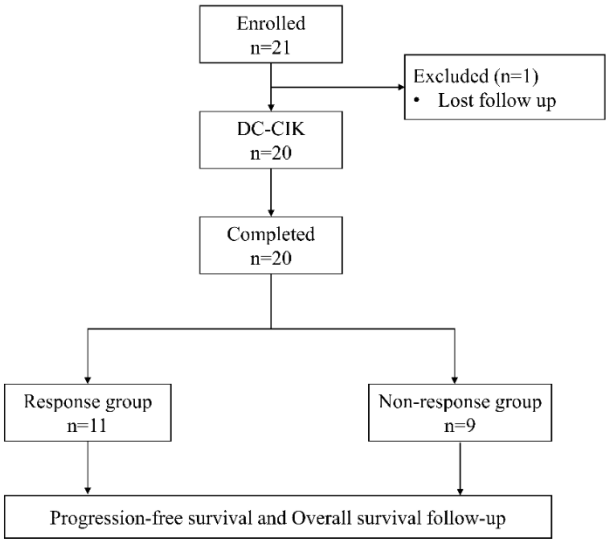

Figure 2. Flow chart.

2.2. Generation of DC and CIK cells

Peripheral blood mononuclear cells (PBMCs) were harvested from peripheral blood by apheresis (COBE BCT) and separated by Ficoll-pague (GE) gradient centrifugation. For the induction of DC-CIKs, PBMCs were firstly incubated for 7 days in the presence of IL-4 (500 U/mL; R&D Systems, Inc.), TNF-a (10 ng/mL; R&D Systems, Inc.) and GM-CSF (800 U/mL; R&D Systems, Inc.) in vitro to generate autologous DCs. Then, PBMCs were activated in vitro with recombinant cytokines IL-2 at 1,000 U/mL (R&D Systems, Inc.), IFN-γ at 1,000 U/mL (R&D Systems, Inc.) and CD3 antibody at 50 ng/mL (Takara Bio USA, Inc.) for 7 to 10 days. The phenotypes of DCs (CD3, CD86 and HLA-DR) and CIKs (CD3, CD56, CD86 and NKG2D) were characterized by flow cytometry. The proportion of CD3 cells and CD86 cells reached less than 2% and greater than 80%, respectively, among the cultured cells in the autologous DC-specific cultures. The cultured autologous DCs were then mixed with cultured CIKs at a proportion of 1:100, and then DC–CIK were harvested for intravenous administration to patients.

2.3. DC-CIK treatment and follow up

Each patient received 2 cycles of DC–CIK cell infusions. One cycle of DC-CIK immunotherapy included three cellular infusions to give a total of 3×10^9 cells, spanning from 3 to 4 weeks. Patients received an infusion at Days 1, 5, and 9 during the first cycle and repeatedly the second cycle was given after the standard treatment such as chemotherapy administrated.

All patients were routinely followed at our clinic every 2 or 3 months, including blood examination, carcinoembryonic antigen (CEA) and CA19-9 levels. computed tomography (CT) scan, magnetic resonance imaging (MRI) and positron emission tomography (PET) scan to evaluate treatment response and status of the disease.

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2.4. Statistical analysis

The baseline characteristics were summarized by number with percentage for categorical variables and median with range for continuous variables. The Fisher's exact test and Wilcoxon signed-rank test were applied to examine the difference of categorical and continuous, respectively, between two groups. The survival curves were plotted by the Kaplan-Meier method and compared by the log-rank test. The significant level was set as 0.05. All analyses were performed by software R version 4.2.1.

3. Results

3.1. Baseline characteristics of patients

Twenty patients pathologically diagnosed with colorectal cancer, all at stage IV, who received DC-CIK cell-based immunotherapy combined with 2nd- or later-line chemotherapy were retrospectively enrolled in this study. Among them, 11 were responder and 9 were non-responder.. The clinical characteristics of two groups were shown in Table 1 to show that there was no significant difference in clinical characteristics or demographic between these two groups. Treatment strategy and medical management were also similar between these two groups.

Table 1. Patient Characteristics.

	Response group (N=11)		Non-response group (N=9)		
Characteristic	**n**	**%**	**n**	**%**	**p-value**
Gender					>0.999
Male	6	55%	4	44%	
Female	5	45%	5	56%	
Age (years)					0.3816
Median	60		53		
Range	45-72		46-70		
BMI					0.710
Median	25		25		
Range	18.5-29.6		16.0-29.3		
Tumor location					0.794
Right side colon	2	18%	1	11%	
Left side colon	7	64%	5	56%	
Rectum	2	18%	3	33%	
Gene profile					
MSS/MSI-L	8	89%	5	100%	
MSI-H	1	25%	0	0%	
All RAS Mutation	3	30%	4	44%	0.377
BRAF Mutation	1	10%	0	0%	
Organs of metastasis					
Liver	9	82%	7	78%	>0.999
Lung	3	27%	4	44%	0.642
Peritoneum	4	36%	6	67%	0.370
Pelvic lymph node	3	27%	0	0%	0.218
Bone	1	9%	4	44%	0.127
Ovary	1	9%	2	22%	0.566
Number of metastasis site					0.059
1	6	55%	1	11%	
2	1	9%	5	56%	
≥3	4	36%	3	33%	
Radiotherapy	3	27%	4	44%	0.642
Treatment cycle (weeks)					0.675
Median	42		62		
Range	28-132		30-109		
Baseline CEA					0.095
Median	8.87		103.29		
Range	0.82-11374		2.30-4645.3		
Baseline CA19-9					0.224

Median	20.8	331.25
Range	7.8-3093.1	11.5-4694

Categorical variables were tested by the Fisher's exact test; Continuous variables were tested by the Wilcoxon signed-rank test; BMI: body mass index; HTN: hypertension; DM: diabetes mellitus; HLP: hyperlipidemia; HBV: hepatitis B virus; HCV: hepatitis C virus; BPH: Benign prostatic hyperplasia; SCC: Squamous-cell carcinoma; CEA: carcinoembryonic antigen; CA: Cancer Antigen; MSS/MSI-L: microsatellite stable/ microsatellite instability-low; MSI-H: microsatellite instability- high.

3.2. Clinical outcomes

During the follow-up period, median PFS in the responder group was 7 months (with a range of 3-14 months) and median OS was 12 months (with a range of 8-19 months). Median OS in the non-responder group was 9.5 months (with a range of 2-16 months). No significant difference in OS between 2 groups (P= 0.147). Four (36.6%) patients in the responder group received surgery with curative intended 6 months after DC-CIK cell-based immunotherapy. Among these 4 patients, 2 received metastectomy (hepatectomy and splenectomy) and the other 2 underwent CRS and HIPEC surgery. The results were shown in Table 2 and Figure 3.

Table 2. The median of PFS and OS time.

	Response group	**Non-response group**	**Wilcoxon signed-rank test**
	(N=11)	(N=9)	p-value
PFS time (months)			0.638
Median	7	4	
Range	3-14	3-16	
OS time (months)			0.147
Median	12	9.5	
Range	8-19	2-16	

PFS: progression free survival; OS: overall survival;.

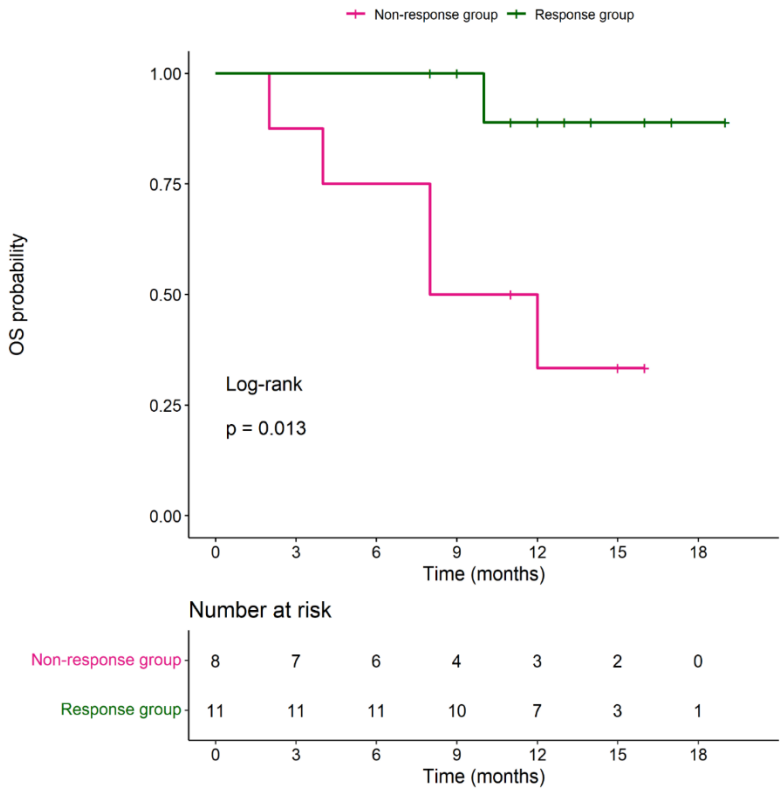

Figure 3. The overall survival (OS) probability.

3.3. Side effects of DC-CIK cell infusion

There were 3 (15%) patients had mild fever, chillness and fatigue during DC-CIK infusion. One (5%) patient developed chest tightness and tachycardia. No major event such as anaphylaxis, leukopenia, abnormal liver function tests (LFTs) or impair kidney function was noted.

4. Discussion

DC-CIK therapy has shown promising results in the treatment of advanced gastrointestinal cancer. In a recent clinical trial, patients with advanced gastrointestinal cancer who received DC-CIK therapy had a significantly longer progression-free survival and improved quality of life compared to those who received standard chemotherapy alone. The therapy was well-tolerated, with few adverse effects reported. [24]

In Taiwan, cell-based immunotherapy is under a strict regulation by the Ministry of Health and Welfare. As for solid malignant tumors, DC-CIK cell-based immunotherapy is only indicated for patients with metastatic disease. In the study of Xie, Y., et al, 142 patients with stage III/IV CRC patient were recruited to show a significant difference in 5-year overall survival rate and 5-year progression-free survival rate between patients with/without DC-CIK cell-based immunotherapy. [10] Studies of Lin, T., et al. and Niu, J., et al. also reported similar results. [8, 9] However, these studies all aimed at patients with stage III/IV CRC receiving first-line treatment combined with DC-CIK cell-based immunotherapy. Publications or reports focusing on the use of DC-CIK cell-based immunotherapy with second- or later-line treatment for refractory mCRC patients are limited.

In our study, 20 patients with refractory mCRC who were treated at least with first-line chemotherapy were recruited. All patients showed progression or relapse afterward. However, after combining DC-CIK cell-based immunotherapy, 11 out of 20 patients (55%) responded to the treatment. Among these 11 responsive patients, 4 patients (36%) converted to surgical treatment (metastectomy or hyperthermic intraperitoneal chemotherapy) with a median PFS of 7 months. The preliminary results, although not statistically significant and without a control group, was encouraging. Afterwards, second- or later-line treatment for mCRC patients were searched with a focus on treatment response rate and PFS below.

The efficacy of current treatment options for patients with late-stage metastatic colorectal cancer (mCRC) have been limited, as shown by the median progression-free survival (PFS) reported in the literature. Epidermal growth factor receptor (EGFR) inhibitors, such as Panitumumab and Cetuximab, combined with chemotherapy have been widely used in second- or later line settings, with a median PFS of 3 to 7 months and a response rate of 18%. [11-15] Bevacizumab, a vascular endothelial growth factor (VEGF) inhibitor, was also used to yield a median PFS of 7 months. [12, 16] In third- or later-line settings, Regorafenib or Trifluridine-Tipiracil (TAS-102) was used as single agent or combined with other medications, but the median PFS was only 1.7 to 4.6 months. [17-20] A review by Bekaii-Saab et al. focused on the clinical outcomes of mCRC patients, especially in third- and later-line treatments. [21] The study found that the median PFS was only 1.9 to 4.5 months, indicating the limited efficacy of current treatment options for this patient population. Conversion to surgery after second- or third-line treatment in mCRC is also limited due to the nature of the disease and the decreased treatment response in these patients.

However, our study indicated that combining DC-CIK cell-based immunotherapy with conventional chemotherapy may be a potential treatment combination for mCRC. The results showed a greater response rate and median PFS than the references, with four of the patients even receiving subsequent curative surgery. DC-CIK therapy has the potential to enhance anti-tumor immunity by stimulating a broad immune response against cancer cells, leading to a more durable response and potentially fewer side effects. Overall, the combination of DC-CIK immunotherapy with conventional chemotherapy may provide a promising treatment option for patients with mCRC who have failed previous lines of therapy. Further research is needed to optimize the therapy and identify which patients will benefit the most.

The limitations of the current treatment options for patients with late-stage metastatic colorectal cancer (mCRC) highlight the need for alternative therapies that can improve patient outcomes. DC-

CIK cell-based immunotherapy, a form of adoptive cell transfer (ACT) therapy that involves infusing activated immune cells into the patient's bloodstream, [25] has emerged as a potential treatment option for mCRC. DC-CIK cells are a type of immune cell that is derived from the patient's own blood and are stimulated in the lab to enhance their anti-tumor activity. These cells are then infused back into the patient to stimulate a broad immune response against cancer cells. Preclinical studies [27, 28] have shown that DC-CIK cells have potent anti-tumor activity against various types of cancers, including colorectal cancer. In clinical trials, the combination of DC-CIK therapy with conventional chemotherapy has demonstrated promising results in patients with advanced-stage colorectal cancer. The therapy has shown good tolerability, with few adverse effects, and has produced encouraging response rates and improved survival outcomes.

One of the key advantages of DC-CIK immunotherapy is its ability to enhance the patient's immune response against cancer cells. [29, 30] Unlike traditional chemotherapy, which targets both healthy and cancerous cells, DC-CIK therapy is more targeted and specifically activates the patient's own immune system to recognize and attack cancer cells. This approach has the potential to produce more durable responses and fewer side effects compared to conventional chemotherapy. While DC-CIK immunotherapy shows promise as a treatment option for mCRC, further research is needed to optimize the therapy and identify which patients will benefit the most. This includes exploring different treatment combinations, dosing schedules, and patient selection criteria. Nonetheless, DC-CIK immunotherapy offers an exciting new avenue for the treatment of patients with advanced-stage colorectal cancer and may provide a more effective and targeted treatment option than current therapies.

However, DC-CIK therapy has its own challenges and limitations. One limitation is the difficulty in isolating and activating dendritic cells. Additionally, the therapy may not be effective in all patients, and more research is needed to determine which patients may benefit the most. [31]

There are also several limitations in our study. First of all, this was a retrospective study without randomized comparison. Secondly, the case number of our study was relatively small. Thirdly, the follow up period was short. Despite of these limitation, conventional chemotherapy combined with DC-CIK cell-based immunotherapy seemed to have potential treatment efficacy in refractory mCRC. Studies of larger scale with randomized control and longer follow up are needed to further support our preliminary outcome.

5. Conclusions

DC-CIK cell-based immunotherapy may have some therapeutical benefit for refractory mCRC patients. Based on our data, the combination of DC-CIK cell-based immunotherapy with second- or later- line chemotherapy has a response rate over 50% with PFS of 7 months while 36% of responsive patients converted to subsequent surgical treatment, which is rare in refractory mCRC. Although without a statistical significance in this study, a larger prospective randomized control study is needed to provide more and solid support the abovementioned finding.

Author Contributions: T.-W.K., C.-W.H., W.T.-L.C., W.-C.S. and L.-B.J. conceived the study concept and design. T.-W.K., C.-W.H. and S.-C.C. performed the acquisition of data. T.-W.K., C.-W.H., W.T.-L.C., W.-C.S., and L.-B.J. contributed to the first drafting of the manuscript. C.-T.H., C.-K.T., C.-L.C., C.-L.L., and H.-T.Y. carried out the interpretation of data and statistical analysis.W.-L.H., W.-C.S., C.-H.T., D.-Y.C., and L.-B.J. supervised the study. T.-W.K., C.-W.H., W.T.-L.C., W.-C.S. and L.-B.J. contributed to the data access and responsibility and had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This retrospective study was reviewed and approved by the Research Ethics Committee of China Medical University Hospital (CMUH111-REC3-054).

Informed Consent Statement: Not applicable for the retrospective study.

Data Availability Statement: The data presented in this study are available in the article.

Conflicts of Interest: C.-H.T. was the founder of Ever Supreme Bio Technology. W.-C.S., D.-Y.C., and L.-B.J. were stockholders of the Ever Supreme Bio Technology. W.-L.H. and W.-C.S. were employed by Ever Supreme Bio Technology and China Medical University Hospital. C.-T.H., C.-K.T., and C.-L.C. were employed by Ever Supreme Bio Technology. **T**he remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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