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CASE REPORT

Sequential Autologous CIK/NK Cells Combined with Chemotherapy to Induce Long-Term Tumor Control in Advanced Rectal Cancer: A Case Report

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Objective: Colorectal carcinoma (CRC) is the third most common malignancy. In addition to comprehensive cancer treatments, such as surgery, chemotherapy, and radiotherapy, the adoptive immune cell therapy (ACT) has played an increasingly important role in recent years, and the adaptive transfusion of autologous NK cells and CIK cells is a brand-new approach to cellular therapy for solid tumors.

Case Presentation: A 57-year-old man underwent a radical resection of microsatellite stable (MSS) rectal cancer with synchronous liver metastases. After surgery of the primary lesion surgery, he was treated with autologous CIK/NK cells combined with XELOX translational therapy. Each cycle can obtain over 10×10^9 CIK cells or over 6×10^9 NK cells combined chemotherapy of XELOX every 3 weeks. After 2 cycles of therapy, he achieved partial response (PR). He immediately underwent a hepatic metastasis resection. After surgery, the patient continued to receive autologous CIK/NK cells in combined with 4 cycles of XELOX. To date, he has achieved and maintained no evidence of disease (NED) for over 40 months.

Conclusion: This is a case of successful treatment of rectal cancer with liver metastasis using ACT in conjunction with first-line chemotherapy. The advantage of this treatment plan is that it has few side effects and achieves long-term control of tumor recurrence by improving the patient's immune function. However, its responsiveness and benefit rate still need further investigation.

Keywords: autologous CIK cells, autologous NK cells, chemotherapy, advanced rectal cancer

Introduction

Colorectal carcinoma (CRC) is the third most common malignancy and the second most common cause of cancer-related death. Its incidence rate has fallen from 60.5 per 100,000 people in 1976 to 46.4 in 2005, and more dropped to 38.7 in 2016. However, the proportion of rectal cancer within CRC has increased from 27% in 1995 to 31% in 2019.[1] First-line treatment for metastatic rectal cancer is 5-FU-based cytotoxic chemotherapy, such as folinic acid/5-FU/oxali platin, folinic acid/5-FU/irinotecan, capecitabine/oxaliplatin, or folinic acid/5-FU/oxaliplatin/irinotecan. The addition of anti-VEGF therapy to these regimens has also been shown to improve survival and has the addition of anti-EGFR therapy in patients with wild-type RAS disease.[2]

The liver is the most common site of colorectal cancer metastasis, and the occurrence of liver metastases has a significant impact to patient prognosis. Therefore, effective treatment of colorectal liver metastases (CRLM) is key to improving patient survival rates and quality of life. Radical surgery is recognized as the best treatment for CRLM. However, the conversion rate for CRLM is not high, only 20–40%, and the postoperative recurrence rate is over 70%. Most recurrences of CRLM occur within 2 years post-surgery.[3] Current clinical guidelines, both domestically and

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internationally, recommend that resectability of CRLM based on the premise of radical surgery R0 resection. However, scattered tumor cells, also known as minimal residual disease (MRD), can remain after primary tumor resection, and can be the main factor in tumor recurrence.[3] Total neoadjuvant therapy (TNT), an emerging treatment modality that involves completion of all treatment modalities, including both chemoradiotherapy and chemotherapy prior to transabdominal resection, is now recognized as the preferred standard for the treatment of locally advanced rectal cancer. Its advantages include higher rates of pathologic complete response (pCR) rates and longer disease-free survival (DFS), minimizing the length of time patients need an ileostomy, facilitating resection, and improving the tolerance and completion rates of chemotherapy.[4] Pelvic cavity magnetic resonance imaging (MRI) is the recommended diagnostic procedure for rectal cancer staging. MRI-diffusion weighted imaging (DWI) can help to distinguish post-treatment changes from residual active tumor using apparent diffusion coefficient (ADC) values. It is recognized as a non-invasive method for monitoring the response to preoperative radiochemotherapy.[5]

Limitations in the treatment of advanced rectal cancer primarily stem from the insufficient individualized treatment for different patients, resulting in poor efficacy and limited responses in some patients to existing targeted therapies and immunotherapies, which restrict treatment options. ACT has become the fourth treatment following surgery, chemotherapy, and radiotherapy. ACT typically includes cytokine-induced killer (CIK) cells,[6,7] dendritic cells (DC)-CIK,[8,9] cytotoxic T lymphocytes (CTL),[10] tumor-infiltrating lymphocytes (TIL),[11] and natural killer (NK) cells.[12,13] These cell therapies are designed to use the body's natural defense mechanisms to target and eliminate cancer cells and usually have fewer side effects or risks. On the other hand, cell therapies based on chimeric antigen receptor (CAR) technology have emerged, such as CAR-T cell, CAR-NK, or CAR macrophages (CAR-M), with typically utilize either autologous stem cells, allogeneic or xenogeneic cells, or genetically modified cells that require higher levels of manipulation and are considered high risk.

CIK cells possess a high proliferation rate and potent antitumor effects with MHC-unrestricted cytotoxicity. CIK cells are heterogeneous immune effector cells with a mixture of T cell and NK cell-like phenotypes, making them a type of T cell at the interface between the innate and adaptive immune systems.[14] These are not restricted by MHC and have broad-spectrum anti-tumor and viral effects. The clinical trials of CIK cells (or CIK cells combined with DCs) have exceeded 80 globally for a variety of solid tumors, such as lung cancer, breast cancer, brain cancer, and colon cancer. Alongside other immunotherapies, conventional chemotherapies, or radiot herapies, CIK therapy suggested a synergistic anti-tumor effect.[15] NK cells are the primary effector cells of the innate immune system. They can non-specifically kill tumor cells and virus-infected cells without prior sensitization.[16] The abundance and function of NK cells are associated with clinical outcomes in various tumors, as NK cells not only participate in cancer immune surveillance but also support treatment responses induced by various therapies, including chemotherapy, radiotherapy (RT), targeted anticancer drugs, and peptide-mediated oncolysis. Oncological treatments, particularly chemotherapy, attenuate both the abundance and the function of patient's endogenous NK cells. Those factors support the therapeutic principle of adoptive NK cell transfer. Currently, over 40 clinical trials are ongoing to evaluate the safety and efficacy of adoptively transferred NK cells (often combined with other therapeutic modalities) in patients with solid tumors, but signals of efficacy remain sporadic.[17] Most of these studies are on allogeneic NK cell therapy, because unlike cytotoxic T cells, their MHC independence does not trigger Graft vs Host Disease (GvHD). However, autologous cells have become a promising strategy when allogeneic NK cells are limited or unavailable.

The inevitable resistance to chemotherapy, narrow mechanisms of action and toxicity, limit its efficacy. Multiple studies have shown that chemotherapy combined with immune cell therapy prolongs the overall survival (OS) and disease-free survival (DFS), improves immune function and quality of life, and reduces chemotherapy side effects.[18,19] In this report, the combination of alternating autologous CIK cells and NK cells with standard first-line chemotherapy in TNT treatment mode has successfully treated advanced rectal cancer patients. The infusion of immune cells improves the immune function status of patients, achieving long-term control of tumors.

Case Presentation

The patient, a 57-year-old man was diagnosed as rectal cancer with synchronous liver metastasis stage IV (T4aN0M1) in September 2020 and underwent a radical resection of rectal cancer. The primary tumor measured 6.0 × 5.0cm.

Histological examination revealed moderately differentiated adenocarcinoma with serosal invasion (Figure 1A and B). Vascular invasion was observed, and nerve infiltration was not. Immunostaining showed positive for MLHI, MSH2, MSH6, PMS-2, negative for cerbB2, and PD-L1 Tumor Proportion Score (TPS) <1%. Next-generation sequencing (NGS) results show HLA-C, TP53, APC, SMAD2, SOX9, ARID1A, PTCH1 gene mutations, KRAS, NRAS, BRAF, NTRK1, NTRK2, NTRK3 gene wild type, tumor mutation burden (TMB) 6.09, MSS. The images of contrast-enhanced CT showed masses in the 2nd segment of the left hepatic lobe and the 6th segment of the right hepatic lobe, with sizes of 2.3 × 2.0cm and 0.6 × 0.5cm, respectively (Figure 2A). The multidisciplinary team (MDT) meeting evaluated potential resectable liver metastases. Liver metastasis surgery can be performed after two cycles of translational therapy.

The patient received 1 course of autologous CIK cells or 1 course of autologous NK cells intravenous infusion combined chemotherapy of XELOX [Oxaliplatin (130 mg/m^2 intravenously on Day 1) and Capecitabine (1500 mg orally twice daily on Days 1–14)] every 3 weeks (Figure 3A). A total of 40 mL of peripheral blood was collected from patients 1–3 days prior to chemotherapy. PBMCs were isolated and enriched by Ficoll density gradient centrifugation. After 14

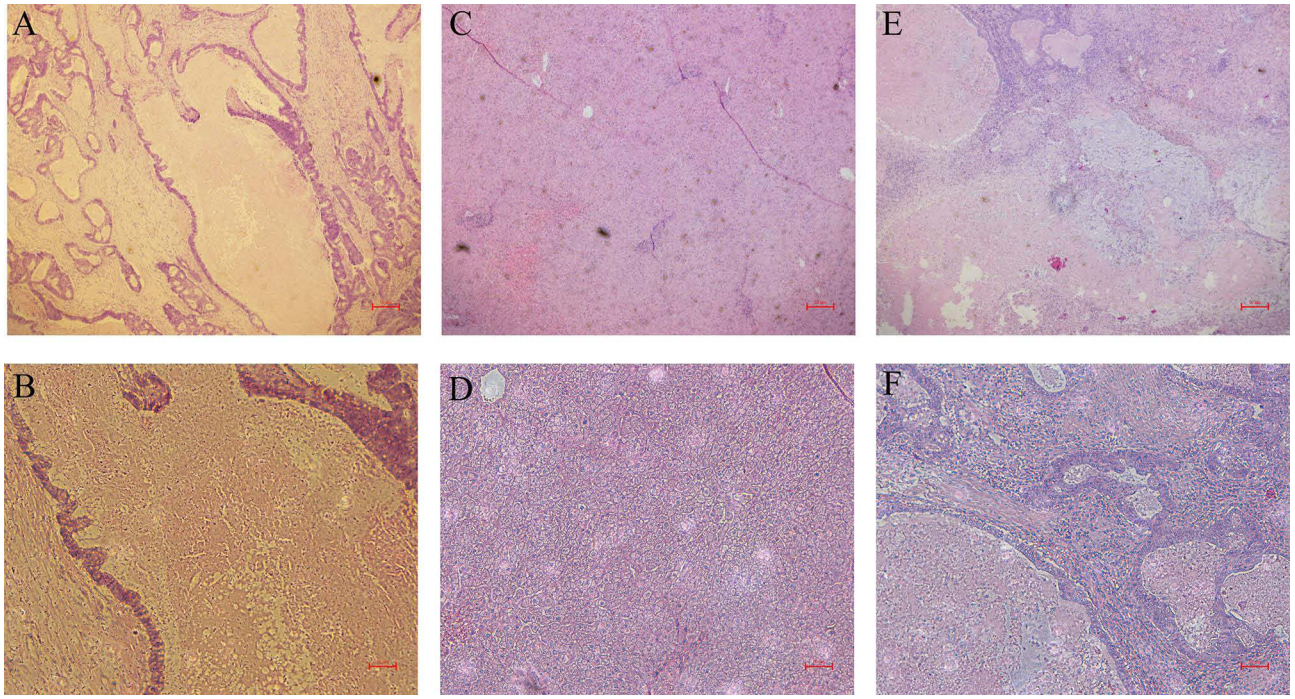

Figure 1 Histological and immunohistochemical staining results. Hematoxylin and eosin (HE) staining of the rectal lesions (**A** and **B**). HE staining of the 2nd segment of the left hepatic lobe lesions (**C and D**). HE staining of the 6th segment of the right hepatic lobe lesions (**E and F**). (**A, C** and **E**) magnification, ×40; (**B, D** and **F**) magnification, ×100.

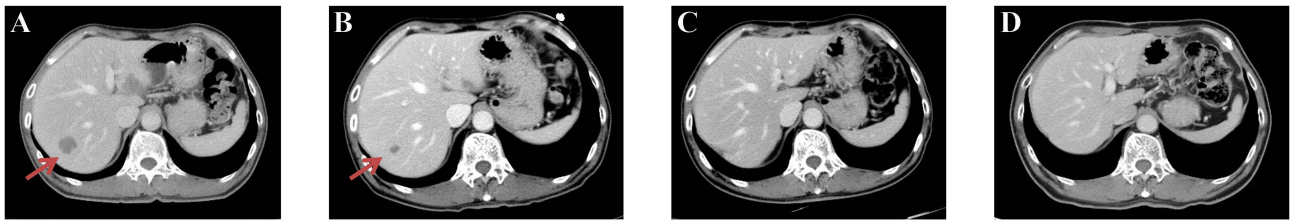

Figure 2 Upper abdominal imaging examination. Liver metastases before combination therapy (**A**). After 2 cycles of treatment, the liver metastases significantly reduced in size (**B**). Imaging after 6 cycles of combined treatment and resection of liver metastases (**C**). Follow up imaging after 2 years of treatment (**D**). Red arrows indicate liver metastases.

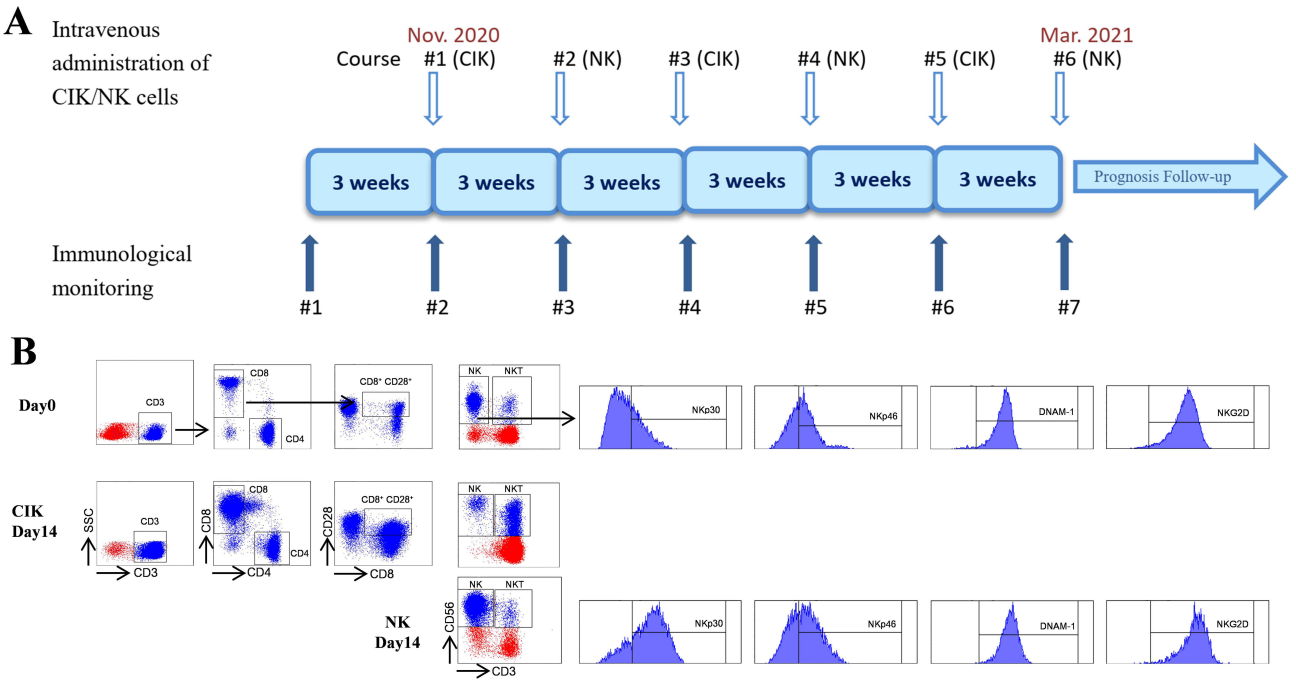

Figure 3 Schematic diagram on schedules of CIK/NK cell treatments (**A**) and immunophenotypes monitoring (**B**).

days of culture, the cells were harvested and resuspended in Ringer's lactate solution (100 mL) supplemented with 0.1% human serum albumin (HSA). Their phenotype was analyzed by flow cytometry method (Figure 3B). Each cycle can obtain over 10×10^9 CIK cells or over 6×10^9 NK cells. Routine laboratory parameters (differential blood counts, RBC parameters, white blood cell counts), blood chemistry (creatinine, AST/SGOT, ALT/SGPT, γ-GT, and LDH), and serum tumor markers of the digestive system were determined after each treatment and during the follow-up period. The CT imaging showed that the liver lesions were reduced by 47%, achieved PR according to Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 (Figure 2B). The patient underwent a hepatic metastasis resection on December 30, 2020. The histological examination showed that the larger nodule was hepatocellular nodular hyperplasia with atypical hyperplasia (Figure 1C and D), and the smaller one was metastatic adenocarcinoma originating from the gastrointestinal tract (Figure 1E and F). The results indicate that the two cycles of translational therapy were successful.

After surgery, the patient continued to receive 4 cycles of autologous CIK/NK cells combined with 6 cycles of XELOX [Oxaliplatin ($100 mg/m^2$ intravenously on Day 1) and Capecitabine (1500 mg orally twice daily on Days 1–14)]. He received a total of 6 cycles of treatment for 4.5 months starting on November 12, 2020 (Figure 3A). Follow up and contrast-enhanced CT examination showed no tumor metastasis or recurrence (Figure 2C and D).

A total of 6 cycles of ACT and 8 cycles of chemotherapy were completed. Tumor markers were within the normal limits for carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 199, CA242, CA50, and CA274 (Figure 4). During treatment, the patient experienced one episode of mild nausea and one episode of grade 2 granulocytopenia. According to the EORTC Core Quality of Life Questionnaire (QLQ-C30), the patient's quality of life improved after treatment (Table 1). No complications related to CIK/NK cells infusion were observed.

Plasma was obtained after centrifugation of peripheral blood (10 min at 3000rpm). Aliquots (300 μL) were prepared and directly stored at −80°C. Plasma cytokines and lymphocyte subpopulations were determined by flow cytometry on a FACSCanto II instrument (BD Biosciences, San Jose, CA, USA). The number of lymphocytes declined rapidly in the Cycle 1 and Cycle 2 treatment, including total lymphocytes (Figure 5A), CD3+ T cells (Figure 5B), CD4+ T cells (Figure 5C), $CD8^+$ T cells Figure 5D), NK cells (Figure 5E) and NKT cells (Figure 5F). After 2 to 3 cycles of chemotherapy combined with ACT, there was a significant recovery. Throughout the treatment, the expression of regulatory T cells (Tregs) (Figure 5G) and immune checkpoint PD-1 molecule gradually (Figure 5H) decreased, while

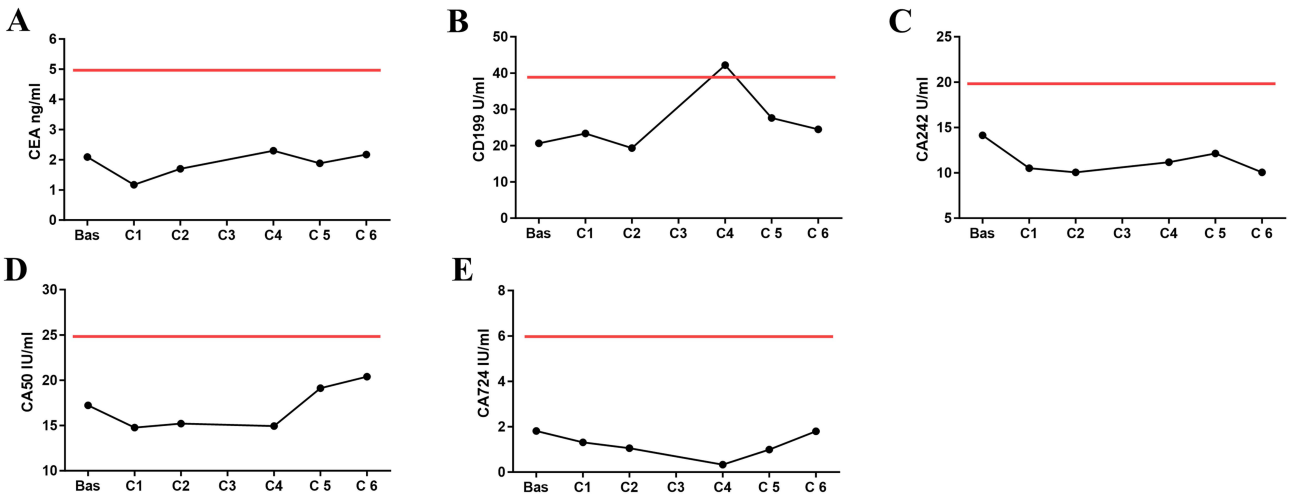

Figure 4 Blood tumor markers examination. All markers were maintained at a low lever before and after treatments, showing no difference in the level of CEA (**A**), CA199 (**B**), CA242 (**C**), CA50 (**D**) and CA724 (**E**). In these graphs, checking point "Bas" means baseline, while checking points "C1" - "C6" indicate after the first time to the sixth ACT.

the proportion of cytotoxic $CD8^+CD28^+$ T cells (Figure 5I) significantly increased. IFN-γ and IL-2 increased during treatment and returned to baseline levels after treatment (Figure 5J), whereas TNF-α and IL-10 showed a downward trend (Figure 5K). IL-6 was much higher than the other cytokines and continued to increase (Figure 5K). Overall, Th2-type cytokines showed predominant expression. The patient received regular follow-up examinations. As of now, the patient

Table 1 Evaluation of the Patient's Quality of Life Before and After Treatment

	Before Treatment	After 2 Cycles of Treatment	After 6 Cycles of Treatment
Function subscales			
Physical function	73.33	73.33	80
Role function	33.33	83.33	100
Emotional function	66.67	66.67	66.67
Cognitive function	83.33	83.33	83.33
Social function	33.33	66.67	66.67
Symptom subscales/ items			
Fatigue	33.33	33.33	33.33
Nausea/vomiting	16.67	16.67	16.67
Pain	33.33	33.33	33.33
Dyspnea	33.33	33.33	33.33
Insomnia	33.33	33.33	33.33
Appetite loss	33.33	33.33	33.33
Constipation	33.33	33.33	33.33
Diarrhea	66.67	33.33	33.33
Financial difficulties	33.33	33.33	33.33
Global health / Quality of Life	50	66.67	66.67

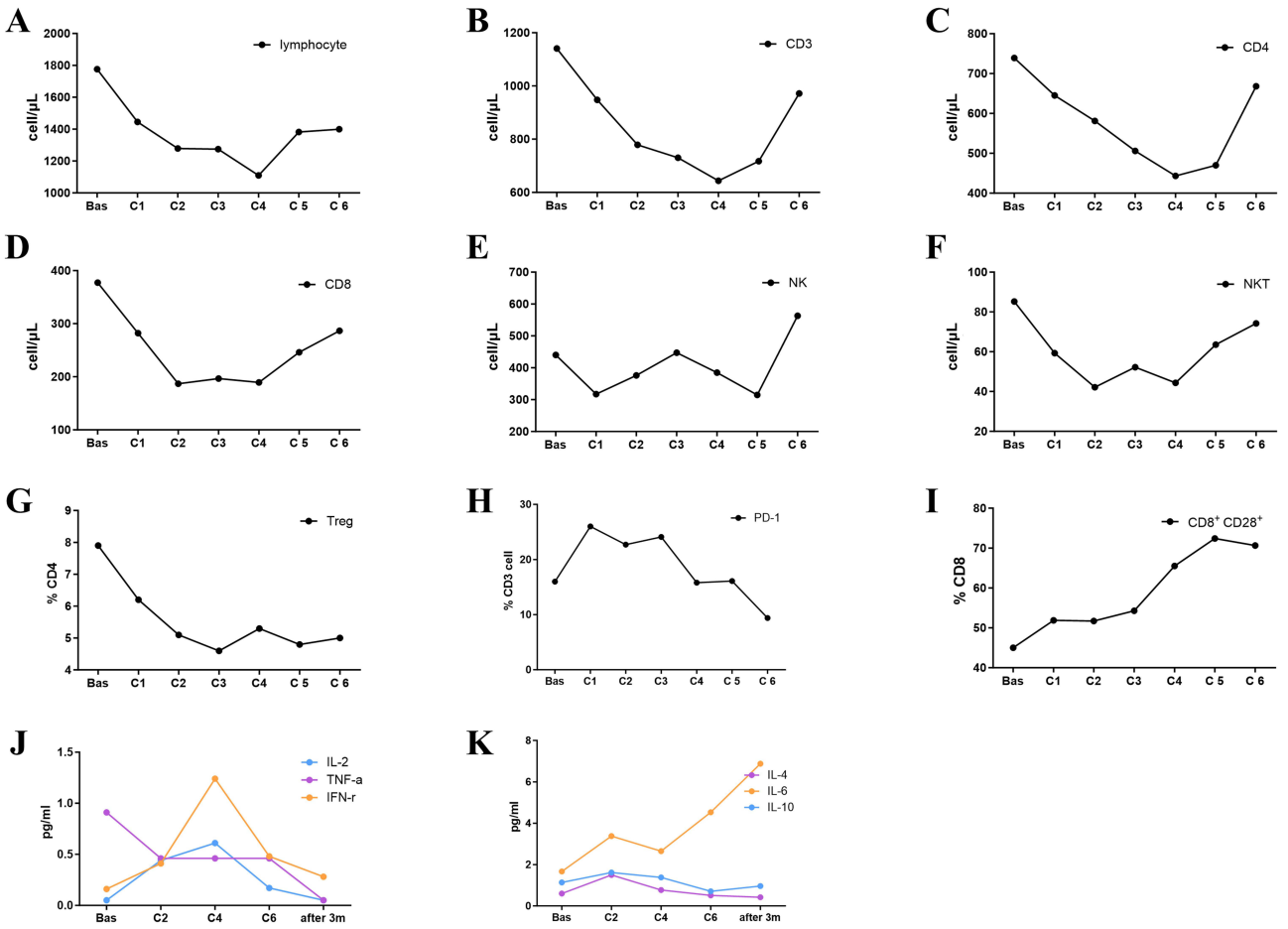

Figure 5 Changes in immunophenotyping and cytokines during treatment. There are total lymphocytes (**A**), $CD3^+$ T cells (**B**), $CD4^+$ T cells (**C**), $CD8^+$ T cells (**D**), NK cells (**E**), NKT cell (**F**), Tregs (**G**), $CD3^+PD\text{-}1^+$ cells (**H**), $CD8^+CD28^+$ (**I**), Th1 cytokine (**J**), and Th2 cytokine (**K**). In these graphs, checking point "Bas" means Baseline, while checking points "C1" - "C6" indicate after the first time to the sixth ACT.

remains in NED. These results suggest that autologous CIK/NK cells combined with chemotherapy were safe and effective for this patient with advanced rectal cancer.

Discussion

This is a successful case of cellular immunotherapy combined with chemotherapy for patients with MSS liver metastatic rectal cancer. Approximately 70% of patients with stage IV colorectal cancer had synchronous CRLM.[20] Research statistics among 977 patients with localized liver diseases, underwent CRLM resection rate is 35%. In this case, the patient's liver metastases decreased by 47% and was given the opportunity for surgery after 2 cycles of treatment. The pathological results of liver metastasis surgery showed that there were no tumor cells in the larger mass. The tumor regression grade (TRG) was 0 which meant the larger lesion of liver metastasis had reached pathological complete response (pCR). The evaluation results of CT image and pathology strongly suggested that the appropriate combination of autologous CIK/NK cells and chemotherapy could have significant effects.

The vast majority of patients with metastatic colorectal cancer are MSS, with microsatellite instability (MSI)-H patients accounting for only 5%. It is usually considered that MSI-H type cancer is immune-inflamed type, whereas MSS type is immune-exclude or immune desert type. MSS CRC patients have a poor prognosis and are generally ineffective with single immunotherapy.[21,22] Those patients typically have fewer tumor-infiltrating lymphocytes than those of MSI-H CRC patients, such as cytotoxic cells, $CD8^+$, Th1, Th2, follicular T helper cells, and T cells. Intratumoral T cells can be replenished with fresh, non-exhausted replacement cells from sites outside the tumor.[23] The increase in peripheral

lymphocytes may contribute to the infiltration of lymphocytes within the tumor. ACT may alleviate lymphopenia caused by chemotherapy. In this case report, the patient's lymphocyte depletion caused by chemotherapy was significantly restored after 2 to 3 cycles of ACT. Regulatory T cells (Tregs) are usually recognized as a specialized subset of $CD4^+$ T cells function in the establishment and maintenance of immune tolerance.[24] They constitutively express T lymphocyte antigen 4 (CTLA-4) and compete with CD28 to bind to CD80/CD86 (CTLA-4 has a higher affinity for CD80/CD86 than CD28),[25] thereby activating the secondary signal of T cells and reducing the anti-tumor activity of effector T cells. During the treatment, the patient showed a decrease in the proportion of $CD4^+CD25^+Foxp3^+$ Tregs and an increase in the proportion of cytotoxic $CD8^+CD28^+$ cells. Meanwhile, the expression of immune checkpoint PD-1 molecules on the surface of T cells was reduced.

Th1 cytokines play a vital role in the anti-tumor immune response, while Th2 cytokines play an anti-Th1 response role in the process.[26] The patient exhibited lower levels of Th1 cytokines than those of Th2 cytokines, and immune balance shifts to Th2. This situation is generally seen in cancer patients. Studies have found that the secretion of IL-2 and INF-γ in peripheral blood of the patients with advanced tumors decreased, and the secretion of IL-10 increased. Th2 cell drift is positively correlated with the malignancy of tumors, such as breast cancer,[27] cervical cancer,[28] melanoma,[29] etc. The patient's IL-2 and INF-γ levels increased, while IL-4 and IL-10 levels decreased slightly during treatment. However, the patient's IL-6 level continues to increase. It suggests that the cell therapy has played a certain role in regulating Th1/Th2 balance, but this effect is limited. The above changes in the patient's immune parameters suggest that CIK/NK cell immunotherapy may improve the patient's immune function and alleviate immune suppression.

The immune function of the body is closely linked to the occurrence, development, and recurrence of tumors. ACT can not only exert anti-tumor effects but also improve or rebuild immune function to restore immune surveillance function, thereby preventing tumor recurrence. Research has shown that the recurrence rate within 12 months of the CRLM surgery was as high as 44.7%.[30] The existing research is focused on the combination of CIK cells alone with or NK cells alone with radiotherapy and chemotherapy. In order to improve the responsiveness of the ACT, we adopted a treatment plan of alternating infusion of CIK cells and NK cells, and achieved good outcome. The patient has been in the NED state for 40 months and will continue to maintain this state. In addition, ACT could alleviate pain and improve quality of life.[31] His quality of life has significantly improved.

In addition to effectiveness, this regimen has an excellent safety profile. During treatment, one episode of mild nausea and one episode of degree 2 granulocytopenia were reported, with no adverse reactions related to the ACT. Although CIK and NK are not able to recognize tumor-specific antigen-like CAR technology and TIL cells, they are safer, more practical and more cost-effectiveness. However, this treatment plan has limitations, including variable efficacy depending on individual patient differences, and the potential for immune-related side effects. Its main side effects are mainly low-grade fever, as well as chills, rashes, fatigue, headaches, etc. Therefore, in clinical applications, it is necessary to comprehensively consider the overall situation of the patient and develop personalized treatment plans, such as monitoring the patient's immune status during the treatment process.

Conclusion

In summary, the success of the case provides a new first-line treatment for advanced MSS type rectal cancer, especially APC gene mutations and KRAS, NRAS, BRAF gene wild type. We have also achieved good clinical results with cell therapy alone and chemotherapy without the use of targeted drugs. The advantages of this treatment method include minimal side effects and a high level of general applicability, particularly in its ability to achieve long-term tumor control by improving the patient's immune status. However, further research and clinical trials are needed to verify its efficacy and populations that benefit from it. At present, early activation of the immune system may be a factor in screening the types of beneficiaries.

Ethics Approval and Informed Consent

Studies involving human participants were reviewed and approved by the Ethics Committee of the Northern Theater Command General Hospital. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or

data included in this article. As reported in the case, the patient showed improvement in multiple immune indexes after 2 to 3 cycles of treatment.

Consent for Publication

The authors have obtained informed consent from the patients for publication of the case details and any accompanying images, and the ethics committee of The General Hospital of Northern Theater Command approved this consent process and the publication of case details.

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Disclosure

The author(s) report no conflicts of interest in this work.

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