Adoptive Chemoimmunotherapy Using Activated αβ T Cells for Stage IV Colorectal Cancer

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Abstract. *Background/Aim: Adoptive immunotherapy of cancer is evolving with the development of novel technologies that generate proliferation of large numbers of αβ and γδ T cells. We evaluated the safety and efficacy of the combination of adoptive immunotherapy using αβ T cells with chemotherapy for stage IV colorectal cancer (CRC). Patients and Methods: Fifteen patients with advanced or recurrent CRC received XELOX + bevacizumab + ex vivo expanded αβ T lymphocytes as a first-line chemoimmunotherapy. Results: Median age of the 15 patients (4 men, 11 women) was 65 years (range=49-80). Median progression-free survival was 21.3 months. Response rate was 80% (complete response (CR)=26.7%, partial response (PR)=53.3%, stable disease (SD)=20% and progressive disease (PD)=0%). Most adverse events were mild to moderate regarding their intensity and immunotherapy-associated toxicity was minimal. Conclusion: Combination of adoptive αβ T cell immunotherapy with chemotherapy for stage IV CRC is feasible and safe.*

T lymphocytes can be divided into two subsets, αβ and γδ T cells, based on their expression of T cell antigen receptors (TCRs). The majority of αβ T cells recognize antigenic peptides in the context of major histocompatibility complex (MHC) class I or class II and produce effector molecules that mediate the regulation and differentiation of other cells in the immune system. Adoptive immunotherapy of cancer is evolving with the development of novel technologies for generating a large number of activated killer cells, such as αβ T cells, γδ T cells and natural killer cells. With advancing technology, these killer cells are exploitable for adoptive immunotherapy as *ex vivo*-expanded killer cells. *Ex vivo*-expanded αβ T cells have been studied (1-3) and used to treat cancers, such as hepatocellular carcinoma (4) and lung cancer (5). Tumor-infiltrating T cells may be a valuable prognostic tool in the treatment of colorectal cancer (CRC) (6).

Programmed cell death 1 (PD-1) is a key immune-checkpoint receptor expressed by activated T cells; T cell activation is inhibited by ligation of PD-1 with its ligand PD-L1 expressed on the tumor cells, thereby protecting cancer from immune attack. Inhibitors of PD-1 (and its ligand programmed death-ligand 1 (PD-L1)) are becoming a promising immunotherapy approach (7, 8).

Historically, the clinical development of cancer immunotherapies avoided the combination with chemotherapy assuming that cytotoxic agents might abrogate or suppress immunological antitumor activities (9). However, it seems that chemotherapeutics can induce several beneficial effects on the immune system (10). For example, 5-fluorouracil (5-FU) appears to up-regulate tumor antigen expression on colorectal and breast cancer cells (11). Furthermore, regulatory T cells, which suppress immune reaction, are depleted by several chemotherapeutics resulting in enhanced T-cell reactivity (12, 13). Oxaliplatin induces immunogenic death of CRC cells by stimulating pre-apoptotic calreticulin and triggering high-mobility group box 1 protein, with this effect determining its therapeutic efficacy in CRC patients (14). Anti-vascular endothelial growth factor (VEGF) antibody has been reported to enhance the antitumor activity of adoptively transferred antitumor T cells by augmentation of lymphocyte infiltration into tumor (15).

To our knowledge, there have been no reports on prospective trials of combination treatment of adoptive immunotherapy with first-line chemotherapy for stage IV

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CRC. We evaluated the safety and efficacy of combination treatment of αβ T cell therapy with XELOX and bevacizumab for stage IV CRC.

Patients and Methods

Patients. The medical records of patients diagnosed with CRC between June 2012 and June 2015 were retrospectively reviewed. Those with histologically proven metastatic and unresectable colorectal adenocarcinomas, with no prior chemotherapy or who completed adjuvant chemotherapy during the last 6 months were enrolled in the study. All patients provided their written informed consent prior to chemotherapy.

Eligibility criteria. Eligible patients were ≥20 years of age, with histologically confirmed CRC without prior chemotherapy for metastatic disease. They also met the following criteria: Eastern Cooperative Oncology Group (ECOG) performance status 0-2; life expectancy ≥12 weeks; white blood cell count ≥3,000/mm^3; neutrophil count ≥1,500/mm^3; platelet count ≥75,000/mm^3; hemoglobin ≥8.5 g/dl; total bilirubin ≤2.0-times the upper limit of normal; aspartate aminotransferase and alanine aminotransferase ≤3.0 times the upper limit of normal; serum creatinine ≤2.0 mg/dl. Patients with any of the following conditions were excluded: interstitial lung disease; autoimmune disease; clinically significant cardiovascular disease; active infection; a history of serious hypersensitivity to drugs; systemic steroid administration; pregnancy; multiple primary cancers within the past 5 years; microbiologically positive for human immunodeficiency virus or human T-cell lymphotropic virus type I; and any other condition making a patient unsuitable for this study.

Treatment. Patients received XELOX plus bevacizumab therapy (7.5 mg/kg of bevacizumab and 130 mg/m^2 of oxaliplatin on day 1 plus 1,000 mg/m^2 of capecitabine twice daily on days 1-14, every 3 weeks) for advanced or recurrent CRC (Figure 1) (16-18). Dose reductions were required for all grade 3 or 4 toxicities attributed to the study medications. The dose of bevacizumab was not reduced. Treatment was continued until disease progression, unacceptable toxicities or withdrawal of consent. Study treatment was delayed if any of the following criteria were applicable on the day of scheduled administration or the previous day: a neutrophil count <1,000/mm^3, a platelet count <75,000/mm^3, active infection with fever ≥38.0˚C, grade 2 or worse peripheral sensory neuropathy (PSN) and other grade 2 or worse non-hematological toxicities. The oxaliplatin dose was reduced to 100 mg/m^2 if grade 3-4 neutropenia or thrombocytopenia, persistent grade 2 or reversible grade 3 PSN or any grade 3-4 non-hematological toxicities occurred. The study was terminated if grade 3 toxicity persisted after a 21-day washout period or if grade 4 PSN or a grade 2-4 allergic reaction occurred. The study was also terminated if the patient required longer than 4 weeks recovery from an adverse event.

Peripheral blood mononuclear cells were harvested by centrifugation and over 1×10^6 harvested cells were cultured with an immobilized anti-CD3 antibody and interleukin (IL)-2 for 14 days. Over 5×10^9 lymphocytes were obtained on average. The cultured lymphocytes comprised 61±15% CD8$^+$, 30±15% CD4$^+$ (CD4$^+$:CD8$^+$ ratio, 0.8 on average) and a small percentage of natural killer cells and natural killer T cells. This indicated that CD8+ T lymphocytes proliferated more intensively than CD4$^+$ T lymphocytes during the 2-week culture period (19). Over 5×10^9 αβT lymphocytes cultured *ex vivo* were injected intravenously into patients on day 17 or 18, once every 3 weeks for 4.5 months or longer.

Evaluation of toxicities and effect of chemotherapy. All patients underwent physical examination, chest radiography and computed tomography (CT) scans of the abdomen, pelvis and chest before treatment. All patients were included in the safety and efficacy analyses. The severity of adverse effects was evaluated according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC), version 4.0. Tumors were measured at 6- to 8-week intervals by CT image and response was evaluated according to the response evaluation criteria for solid tumors (RECIST), version 1.1. The evaluation of response was based on radiologist-reported measurements. Complete response (CR) and partial response (PR) required subsequent confirmation of response after an interval of at least 4 weeks.

Results

Baseline patients' characteristics. Out of 22 patients who received chemoimmunotherapy for metastatic CRC, seven were excluded (five patients with other regimens were excluded and two patients were excluded because of inadequate hematological, renal and liver functions). Patients' characteristics are presented in Table I. The median age of the 15 included patients (4 men, 11 women) was 65 years (range=49-80). ECOG performance status scores were 0 in all patients.

Treatment. XELOX + bevacizumab: The median number of treatment cycles was 15 (range=4-27). Thirteen patients (86.7%) continued treatment through eight cycles, while the reasons for discontinuing treatment were adverse events in one patient and personal reasons in another. Treatment was delayed in 7 patients (46.7%) due to neutropenia in one patient, thrombocytopenia in four, fatigue in one and diarrhea in one. Three patients (20%) required dose reduction at least once within eight cycles, with the reason being neutropenia in one, fatigue in one and diarrhea in one. αβ T lymphocytes: The median number of treatment cycles was 12 (range=4-21). The average cell number was 5.9×10^9 (range=4.5×10^9-7.9×10^9) for each infusion. Eleven patients (73.3%) continued treatment through eight cycles, while the reasons for discontinuing treatment were refusal or personal reasons in four patients. Treatment with chemotherapy was delayed in 7 patients due to adverse events (46.7%).

Efficacy. The confirmed response rate was 80% (CR=26.7% (n=4); PR=53.3% (n=8); stable disease (SD)=20% (n=3); progressive disease (PD)=0%) (Table II); disease control rate was achieved in 100%. Median progression-free survival was 21.4 months (Figure 2).

Safety. Adverse events for 15 patients are summarized in Table III. Grade 3 or higher hemotoxicity and grade 3 or higher non-hematological toxicity was noted in 6.7% and

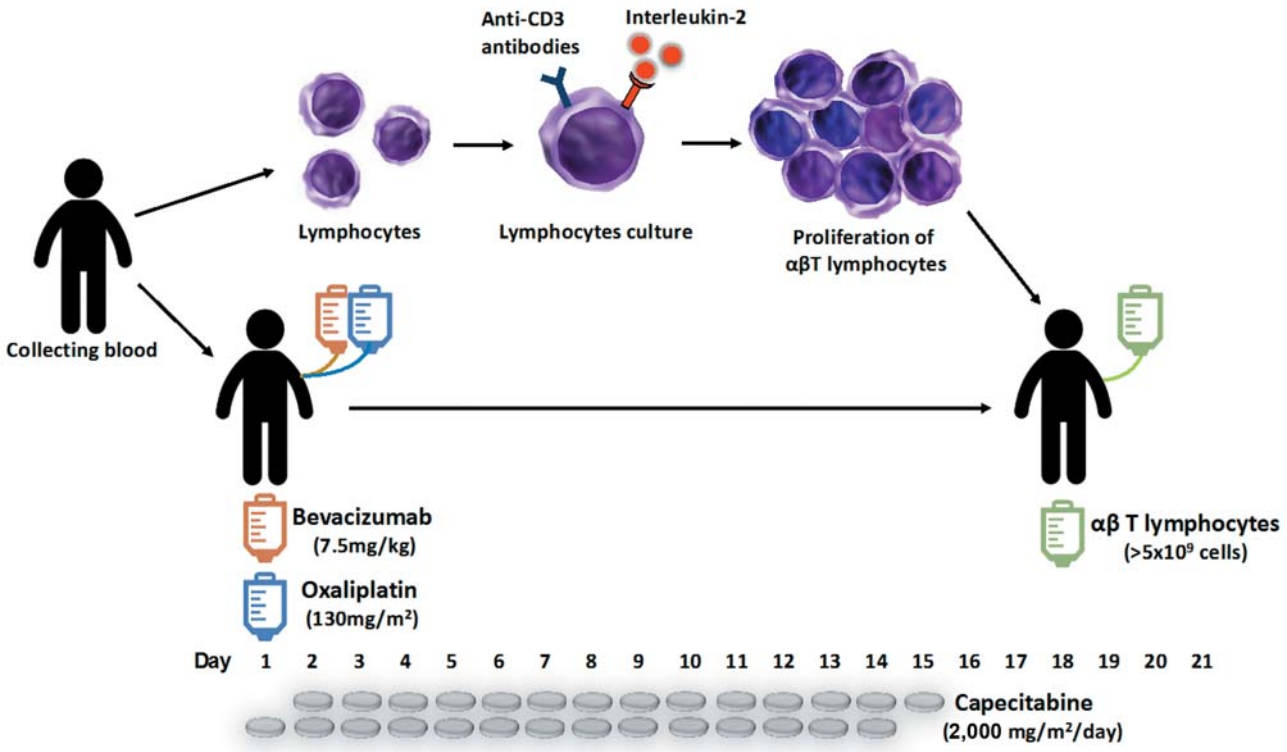

Figure 1. *Chemoimmunotherapy approach. Patients received XELOX + bevacizumab + αβ T lymphocyte therapy.*

20.0% of patients, respectively. Five patients developed Grade 2 thrombocytopenia. There were no other severe treatment-related adverse events and no deaths during treatment.

Discussion

In this study, XELOX + bevacizumab + *ex vivo* expanded αβ T lymphocyte therapy in 15 patients with CRC led to 100% disease control rate (80% of response rate), with an acceptable toxicity profile.

Maeda *et al*. reported that FOLFOX regimens induce not only direct cytotoxicity but also enhancement of antitumor immunity *via* Treg depletion (20). A previous clinical report has indicated that combination chemoimmunotherapy, followed by subcutaneous administrations of granulocyte macrophage colony-stimulating factor and IL-2, induces strong immunologic and antitumor activity in metastatic colon cancer patients (21). A progressive increase in lymphocyte and eosinophil counts, amplification in central memory, a marked depletion of immunosuppressive Tregs and activation of colon cancer-specific cytotoxic T-cells were observed. Thus, FOLFOX chemotherapy may be suitably combined with immunotherapy. However, FOLFOX was

Table I. *Patients' baseline characteristics.*

Age	65(49-80)
Gender	Male 4:female 11
Primary	Cecum (3):Ascending (2):Transverse (1): Descending (2):Sigmoid (2):Rectum (5)
Chemotherapy (cycles)	15 (4-27)
Immunotherapy (cycles)	12 (4-21)
Metastatic site	Liver (9):Lung (3):Peritoneum (3) Bone (1):LN (5)

Table II. *Efficacy of chemoimmunotherapy.*

RECIST	N	%
CR	4	26.7
PR	8	53.3
SD	3	20.0
PD	0	0

RECIST, Response Evaluation Criteria in Solid Tumors; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table III. *Adverse events during chemoimmunotherapy.*

	Hematological	Non-hematological
Grade≥3	Neutropenia:1	Diarrhea:1 Fatigue:1 Hypertension:1
Grade 2	Thrombocytopenia:5 Neutropenia:1	Hand foot syndrome:3 Neuropathy:2 Hypersensitivity:1

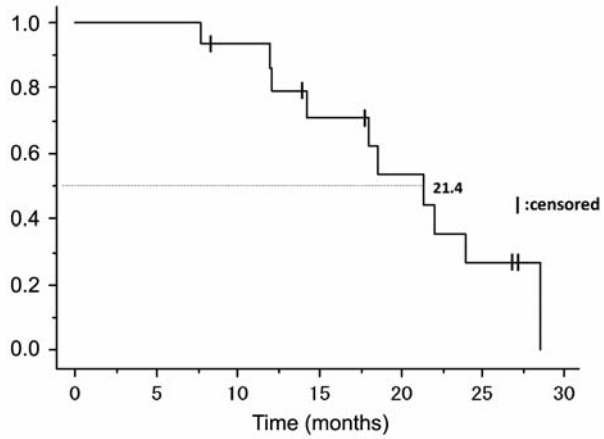

Figure 2. *Kaplan-Meier survival curve. Progression-free survival over time.*

associated with more grade 3/4 neutropenia and febrile neutropenia than XELOX (22, 23). Therefore, we have treated with XELOX, that may be as effective as FOLFOX.

Even if CRC appears to have been eradicated by chemotherapy and radiation, it is known that a small number of cancer stem cell (CSC) fraction can self-propagate and frequently sustain tumor growth, leading to relapse and therapeutic failure. Although CSCs are often resistant to a variety of treatments, including chemotherapy and radiotherapy, immunotherapy may still be effective (24-26). A combined approach that uses chemotherapy to kill the bulk of cancer cells and immunotherapy to keep residual CSCs and differentiated cancer cells in check may abrogate the recurrence of CRC cells (27). Furthermore, treatment with chemotherapy, such as cyclophosphamide or gemcitabine, can augment the antitumor effects of immunological activities by depleting Treg cells, potentially enhancing antitumor immune responses (28). Therefore, chemotherapy may simultaneously kill cancer cells and boost antitumor immune responses (29, 30).

The limitations of surgery and chemotherapy in treating CRC patients necessitate the development of novel approaches. Immunotherapy alone may be insufficient for treating metastatic CRC patients. The findings of this study indicate a potential application for other immunotherapies that enhance T cell immune responses, including anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA4) mAb, ipilimumab (31), anti-PD-1 antibody (32) and anti-PD-L1 antibodies (33). Further basic studies and clinical trials will give us additional clues on how to develop and establish successful immunotherapy approaches using αβ T cells.

In this original work, the low number of patients considered cannot lead to well-defined conclusions. There is an imbalance between males and females regarding the number of patients, that may have, in some way, influenced the results. The present work is, therefore, only a first step in examining the association between chemotherapy and immunotherapy.

This study confirms that chemoimmunotherapy is a safe and feasible treatment option for cancer patients. The results strongly support further testing of the combined use of adoptive immunotherapy with chemotherapy.

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Conflicts of Interest

The Authors declare that they have no conflicts of interest.

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