IL-15-Induced Lymphocytes As Adjuvant Cellular Immunotherapy For Gastric Cancer

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Abstract

To test the antitumor potential of adoptive cell therapy (ACT)-transferred lymphocytes in a mouse model of human gastric cancer, and evaluate the clinical efficacy and safety of combining lymphocytes as adjuvant therapy with first-line chemotherapy in patients with gastric cancer. A human gastric cancer xenograft model was constructed in sublethally irradiated 6–8-week old NCG male mice. MKN-45 cells (1×10^6 cells/mouse) were subcutaneously injected into the flanks. After tumors had become palpable, the mice were randomized into control, $ACT^{IL\text{-}2}$, and $ACT^{IL\text{-}15}$ groups. Human lymphocytes were injected into the mouse tail veins. In addition, 63 patients who had histological or cytologically confirmed stage III–IV gastric cancer randomly received S-1 plus oxaliplatin plus $ACT^{IL\text{-}15}$ (a combination therapy group) or S-1 plus oxaliplatin alone (chemotherapy group). In the mice, treatment with $ACT^{IL\text{-}15}$ cells inhibited tumor growth upon adoptive transfer, and mice that received $ACT^{IL\text{-}15}$ cells had a significantly longer survival ($P < 0.05$, $ACT^{IL\text{-}15}$ vs. $ACT^{IL\text{-}2}$). In the human study, the median survival of patients in the combination therapy group was 472 days (95% confidence interval (CI), 276–668 days), whereas the median survival of patients in the chemotherapy group was 266 days (95% CI, 200–332 days; $P < 0.05$). Eleven percent (7/63) of patients had adverse reactions, but the reactions did not interfere with treatment. Adoptive transfer of $ACT^{IL\text{-}15}$ cells in a mouse model of gastric cancer and patients with advanced gastric cancer treated with S1 and oxaliplatin improved survival with an acceptable safety profile.

Introduction

Gastric cancer is challenging to treat, with few patients eligible for extensive surgery and a median survival of 3–5 months for those with an unresectable disease[1]. Clinical trials with various treatment regimens have produced improved results, but the median survival is still less than one year[2-5]. No standard third-line therapy is available for patients with advanced gastric cancer who have not responded to two or more lines of chemotherapy. Thus, a strategy for improving the efficacy of the present chemotherapy without increasing the toxicity is urgently needed.

The oral anticancer drug fluoropyrimidine, S-1, combined with 1 M tegafur, 0.4 M 5-chloro-2, 4-dihydroxypyrimidine, and 1 M potassium oxonate, is effective against gastrointestinal cancer cells in vivo[6, 7]. The standard of care for the first-line treatment of unresectable advanced or metastatic gastric/gastroesophageal junction cancer is oral fluoropyrimidine (e.g., capecitabine or S-1) or capecitabine plus cisplatin or oxaliplatin[8-10]. Apart from the effects of chemotherapy on tumor-cell replication, it has been proposed that chemotherapy produces an antitumor effect through modulation of the immune system[11, 12]. For example, oxaliplatin can induce immunological death of tumor cells and enhance the efficacy of immunologic agents[13].

Novel agents that can improve survival in patients with gastric cancer are needed. Immunotherapy agents targeting the adaptive immune response have shown promising results in several cancers[14].

Adoptive cell therapy (ACT), i.e., the administration of ex vivo-expanded autologous or allogeneic T lymphocytes, can help control tumor growth. However, the therapeutic efficacy of ACT in vivo is affected by the number of cells with antitumor properties[15]. The optimal lymphocytes for ACT are central memory (T_{CM})-like populations that have higher expression of lymphoid-homing molecules than effector memory (T_{EM})-like populations [16]. The lack of IL-15 has been associated with poor proliferation of T_{CM}-like adoptively transferred cells, a result suggesting that IL-15 is critical in this process [17]. In addition, the antitumor effects of IL-15 have been documented in human gastric cancer [18], gastric cancer liver metastases [19], and xenografted human gastric cancer [20]. Motivated by these studies, we have tested the ability of IL-15 to promote the growth of large numbers of lymphocytes with T_{CM}-like phenotype. Thus, we generated lymphocytes cultured in the presence of either IL-15 or the conventional IL-2.

We evaluated the antitumor potential of ACT lymphocytes in tumor-bearing NCG mice that had received grafts of human gastric carcinoma cells. We then assessed the clinical efficacy and safety of combining lymphocytes as adjuvant therapy with first-line chemotherapy in patients with gastric cancer.

Materials And Methods

Chemicals, cell lines, and animals

The human cell lines AGS and NCI-N87 (subsequently referred to as N87) are Lauren intestinal-type gastric adenocarcinoma cell lines, and MKN-45 is a Lauren diffuse-type gastric adenocarcinoma cell line. AGS, N87, and MKN-45 cells were obtained from the America Type Culture Collection and maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and L-glutamine 2 mmol/L (“regular media”). The cancer cell lines were passaged within six months from the time that they were received. The United Kingdom Coordinating Committee on Cancer Research guidelines was followed [21].

Patients

Sixty-three patients who had histological or cytologically confirmed stage III–IV gastric cancer participated in the study. The Ethical Review Board of the Medical Ethics Committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College approved the study protocol. All subjects gave written informed consent in accordance with the Declaration of Helsinki. After enrollment, all patients provided a complete medical history and underwent a physical examination. The demographics and baseline characteristics of the patients are summarized in Table 1. The criteria for patient selection were 1) age 18–80 years; 2) expected survival of ≥ 3 months; 3) Karnofsky performance status score > 40%; 4) peripheral blood white blood cell count ≥ 4×10^9/L; 5) platelet count ≥ 10×10^{10}/L; 6) serum aspartate aminotransferase/alanine aminotransferase values

below the normal upper limit; 7) no cardiac arrhythmias, congestive heart failure, or severe coronary artery diseases; and 8) not pregnant or lactating.

Table 1

Demographics and baseline characteristics of gastric patients with cancer.

Demographic variable	Combination therapy (n = 34)	Chemotherapy (n = 29)	P
Age (years)	55.7 ± 10.89	56.2 ± 9.88	0.36
Sex, n (%)			
Female	9 (26.5%)	7 (24.1%)	0.78
Male	25 (73.5%)	22 (75.9%)	
ECOG Performance status			
0	11	7	0.63
1	23	22	
Histology			
Poorly differentiated	27	17	0.58
Well/Moderately differentiated	7	12	
Number of organs with metastasis			
≤2	24	18	0.42
≤3	10	11	

ECOG, Eastern Cooperative Oncology Group.

ACT cell generation

Peripheral blood mononuclear cells were obtained using Ficoll density centrifugation[22]. The cells were resuspended at a density of 3×10^6 cells/mL in X-VIVO 15 medium (Lonza, Switzerland), supplemented with 10% heat-inactivated autologous plasma and primed by adding 1,000 U/mL IFN-γ on day 0, and 100 ng/mL anti-CD3 antibody (MACS GMP CD3 pure, Miltenyi Biotech, Bergisch Gladbach, Germany) and 500 U/mL IL-2 was provided on day 1. On day four of culture, the cell density was adjusted to 1×10^6 cells/mL. Fresh medium with IL-2 (500 U/mL) or IL-15 (50 ng/mL) was added every three days. On day 14 of culture, ACT cells were harvested and used for analysis. Cells cultured in IL-15 and IL-2 were designated as $ACT^{IL\text{-}15}$ and $ACT^{IL\text{-}2}$ cells, respectively.

In vitro cytolytic assays and cytokine release

Europium release assay was used for in vitro cytotoxicity analysis as described[22]. Target cells (MKN-45, AGS, or N87) were co-cultured with ACT cells in triplicate at an effector-to-target cell ratio of 50:1, 10:1, 5:1, and 1:1 in U-bottom 96-well culture plates (NUNC, Roskilde, Denmark). The supernatant from each well was collected and co-incubated with europium solution (Europium, Perkin Elmer, Turku, Finland) in flat-bottom 96-well plates (NUNC, Roskilde, Denmark). Fluorescence was measured with a time-resolved fluorometer (1420–018 Victor, Perkin Elmer, Waltham, MA, USA). The percentage of specific cytolysis was calculated for each well as described [22], and the mean ± SD was calculated for each duplicate or triplicate.

Flow cytometry assay

Expression of surface markers in the cell was analyzed by flow cytometry. All antibodies (FITC-conjugated anti-CD3, PECy7-conjugated anti-CD56, APC-conjugated anti-CD25, PE-conjugated anti-CD45RO, PerCP-conjugated anti-CD62L, APC-conjugated anti-CCR7) were purchased from BD Biosciences. Samples were analyzed using a FACS Calibur flow cytometer and CellQuest software (Becton Dickinson).

Tumor xenograft model

For establishing human gastric cancer xenograft model in mice, 6–8-week old NCG mice (Institute of Zoology, Chinese Academy of Sciences, Shanghai, China) were housed under specific pathogen-free conditions. The mice were irradiated with 200 cGy for 24 h. MKN-45 cells (1×10^6 cells/mouse) were subcutaneously injected into the flanks. When tumor nodules became palpable (seven days after injection), mice were randomized into three groups: blank control (injected with physiological saline via the tail vein), $ACT^{IL\text{-}2}$ (injected with $ACT^{IL\text{-}2}$ cells via the tail vein), and $ACT^{IL\text{-}15}$ (injected with $ACT^{IL\text{-}15}$ cells with the tail vein). Animals were euthanized on day 32 after transplantation of malignant cells, or they were monitored for survival. Mice that showed signs of physical abnormalities or poor health were sacrificed by carbon dioxide asphyxiation followed by cervical dislocation. Toxicity was defined as 20% or more bodyweight loss or toxic death. Body weight was measured once a week, and survival was calculated from the time of tumor-cell injection until death. Animals were euthanized when they exhibited a lack of a righting reflex or lack of response to stimuli to avoid suffering. Tumor volume was measured every 2–3 days in a blind fashion and calculated according to the following formula: tumor volume = length × $width^2$ /2.

Immunohistochemistry

Tumors were harvested and fixed for 24 h with 10% buffered formalin before being embedded in paraffin. Serial sections 5 μm thick were cut for histologic analysis and were stained with hematoxylin-eosin according to standard procedures. For immunohistochemistry, sections were incubated with anti-CD3 (1:200), then incubated with Dako ChemMateTM EnVision System (Dako, Glostrup, Denmark) for 30 min. Staining was visualized using diaminobenzidine and sections were counterstained with hematoxylin.

Enzyme-linked immunosorbent assay (ELISA)

Single-cell suspensions from tumors were cultured in the medium at 37°C for 6 h. The secretion of IFN-g, TNF-a, IL-4, and IL-10 by ACT cells was measured in the supernatant of the culture media with ELISA kits (R&D Systems) according to the manufacturer instructions.

Statistical analysis of animal studies

The differences in the mean relative tumor volume (RTV) between the treated and control groups on day 25 were analyzed with the two-tailed Student's t-test. All statistical analyses were performed by SPSS 20.0 software. $P < 0.05$ was considered statistically significant.

Study design

Subjects were randomly assigned to receive S-1 plus oxaliplatin plus $ACT^{IL\text{-}15}$ cells (a combination therapy group) or S-1 plus oxaliplatin alone (chemotherapy group). To avoid bias, randomization was performed (Bellsystem24, Tokyo, Japan). Assignment to the treatment groups was balanced according to stratified factors, including Eastern Cooperative Oncology Group performance status and whether the cancer was unresectable or recurrent. A unique random sequence generated by an independent statistician was sequentially applied to each patient allocation by the biased coin method. The two groups were matched for gender, age at onset of disease, pathology, tumor size, metastases, and stage at the first visit.

All patients in the chemotherapy group and the combination therapy group received the same chemotherapy component of the treatment, including doses and cycles. S-1 was orally administered at 80 mg/m^2 divided into two daily doses for 12 days, followed by nine days off. Oxaliplatin was administered intravenously at 130 mg/m^2 over 1–3 h on day 1, then every 21 days until the disease had progressed or unacceptable toxic effects had developed. The dose of S-1 was calculated according to the patient's body surface area. Patients in the combination therapy group received $ACT^{IL\text{-}15}$ cell therapy on day 14 and again after the second chemotherapy treatment. The mean lymphocytes count in the $ACT^{IL\text{-}15}$ cell agent was 5.9×10^9 cells.

Assessments during treatment and follow-up

Toxicity assessments, compliance with S-1, and blood test results were recorded after each cycle of treatment. Tumors were assessed after every other cycle. Computed tomography was performed every eight weeks until cancer progressed. The therapy was discontinued if the disease progressed, the patient refused, unacceptable toxicity occurred, or the patient died. Tumor status was assessed by imaging approximately every two months or until death.

Toxicity was evaluated according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0. Response to treatment was evaluated according to the Response Evaluation Criteria in Solid Tumors (www.cancer.gov/). Radiographic evidence of response to treatment was independently reviewed. An independent data-monitoring committee oversaw the safety and efficacy of the trial and other aspects of the study conduct.

Statistical analysis of human studies

Statistical analyses were conducted with SPSS 20.0 and the GraphPad program (version Prism 5). Differences in demographic and clinical variables between the two groups were evaluated with the Pearson χ^2 test, while Fisher's exact test was used for categorical variables. The Kaplan–Meier method was used to analyze the progression-free survival (PFS) and overall survival (OS). PFS was calculated from the time of the first treatment to the time of the first disease progression or the last follow-up. OS was calculated from the date of the first treatment to the date of death resulting from any cause or the date of the last follow-up. All P values were two-tailed, and significance was set at $P < 0.05$.

Results

Phenotypic polarization and functional polarization of cells cultured in IL-15

We evaluated the quality of the cell phenotype and compared cell composition at the end of cultivation. No significant differences were found in the composition of the cells between culturing with IL-2 or IL-15 (Figure 1A). Expression of the activation antigen CD25 on $CD3^+CD56^-$ or $CD3^+CD56^+$ T lymphocytes cultured with IL-15 was significantly higher than with IL-2 (Figure 1B).

$ACT^{IL\text{-}15}$ cells maintained positive staining for the T_{CM} marker. While the T_{CM} markers CD62L and chemokine receptor (CCR7) were virtually absent on $ACT^{IL\text{-}2}$ cells (0.3%), $ACT^{IL\text{-}15}$ cells (62.1%) retained the expression of CD62L and CCR7 (Figure 2A). Lymphocytes were also counted at indicated time points. Figure 2B illustrates that lymphocytes similarly expanded in media containing IL-2 and IL-15.

We also assessed the functional activity of the generated lymphocytes. ACT^{IL-15} and ACT^{IL-2} cells were added to target cells AGS, N87, and MKN-45 in an effector-to-target ratio of 50:1 and tested in europium release assays. ACT^{IL-15} cells induced significantly more cytotoxicity against AGS and N87 than did ACT^{IL-2} cells, whereas the in vitro cytotoxicity of ACT^{IL-15} cells against MKN-45 cells was not higher than that of ACT^{IL-2} cells (Figure 3A). We also evaluated cytokine secretion and found that ACT^{IL-15} cells significantly increased IFN-g secretion from AGS and N87 cells but not from MKN-45 cells (Figure 3B). In sum, ACT^{IL-15} cells exhibited cytotoxicity against gastric cancer cells and their cytotoxicity against AGS and N87 cells was stronger than that of ACT^{IL-2} cells.

We considered that ACT^{IL-15} cells might have differential effects in vivo and in vitro and, therefore, may be effective against MKN-45 cells in vivo. After culture in medium containing IL-2 or IL-15, lymphocytes proliferated extensively and similarly. In three independent experiments, ACT cells were transferred into MKN-45 gastric carcinoma-bearing mice, and lymphocyte tumor infiltration and persistence were compared. We measured tumor infiltration of adoptively transferred human cells and the secretion of cytokines by ACT cells in tumor sites. ACT^{IL-15} cells promoted tumor infiltration and increased IFN-g secretion potential of adoptively transferred lymphocytes (Figure 4).

We next measured tumor volume and survival in mice receiving ACT^{IL-2} or ACT^{IL-15} cells compared with these measures in untreated controls. Treatment with ACT^{IL-15} inhibited tumor growth upon adoptive transfer (Figure 5A). Moreover, mice that received ACT^{IL-15} cells had a significantly improved survival ($P = 0.049$, ACT^{IL-15} vs. ACT^{IL-2}) (Figure 5B).

These data indicated that the adoptive transfer of ACT^{IL-15} cells is preferable to ACT^{IL-2} cells in tumor immunotherapy.

The role of ACT^{IL-15} in human gastric cancer immunotherapy

The combination with immunotherapy and chemotherapy has been proposed as a therapeutic strategy with the potential for improved survival rate and prognosis for gastric patients with cancer[23]. Thus, we assessed the clinical efficacy and safety of ACT^{IL-15} administered with S-1 plus oxaliplatin for treating patients with advanced gastric cancer.

Clinical evaluation of patients with gastric cancer

Seventy-three patients were enrolled at the National Cancer Center/National Clinical Research Center for Cancer/Cancer Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College from November 2014 to February 2019; three were excluded before randomization (Figure 6). The remaining 70 patients were randomized into the combination therapy (ACT^{IL-15} plus S-1/oxaliplatin) group or the

chemotherapy (S-1/oxaliplatin) group (35 patients per group). One patient from the combination therapy group and six patients from the chemotherapy group were eliminated because of poor adherence, leaving 34 patients in the combination therapy group and 29 patients in the chemotherapy group for final analysis (Figure 6). The characteristics of all patients are detailed in Table 1.

Effector cell treatment and assessment of safety

$ACT^{IL\text{-}15}$-related self-limiting adverse drug reactions, including pyrexia, chill, myalgia, and fatigue, were reported in 11% (7/63) of patients; however, the reactions did not delay or stop the treatment. No patients exhibited pulmonary or renal symptoms, signs of infection, hepatic deterioration, or autoimmune disorders.

The effect of $ACT^{IL\text{-}15}$ on OS and PFS

As of March 1, 2019, the median survival of patients in the combination therapy group was 472 days (95% confidence interval (CI), 276–668 days), whereas that of patients in the chemotherapy group was 266 days (95% CI, 200–332 days; $P < 0.05$, Figure 7A). The 1-year OS rate of the combination therapy group (66.7%) also was significantly higher than that of the chemotherapy group (40.5%; $P < 0.05$).

The median PFS of the combination therapy group was 153 days (95% CI, 109–197 days), whereas the median PFS of the chemotherapy group was 136 days (95% CI, 103–169 days; $P > 0.05$, Figure 7B). The 1-year PFS rate of the combination therapy group (18.2%) was significantly higher than that of the chemotherapy group (14.7%; $P < 0.01$). These results are evidence that the addition of $ACT^{IL\text{-}15}$ to a standard chemotherapy regimen improves patient survival.

Discussion

This study found that IL-15 sustained the growth of memory T cells and the proliferation of adoptively transferred cells. Also, IL-15 was better than IL-2 in rescuing or generating potentially therapeutic cells from peripheral blood. In the model of human gastric cancer, mice that received $ACT^{IL\text{-}15}$ cells had significantly longer survival than did mice that received $ACT^{IL\text{-}2}$ cells. Most importantly, the combination of $ACT^{IL\text{-}15}$ and chemotherapy was superior to chemotherapy alone for treating patients with advanced gastric cancer. Thus, IL-15 appears to be a versatile cytokine that increases the activity of adoptively transferred antitumor lymphocytes in vivo and may have more therapeutic potential than IL-2. The transfer of $ACT^{IL\text{-}15}$ cells was well-tolerated, and treatment was not interrupted by side effects. These findings suggest that $ACT^{IL\text{-}15}$ plus S-1/oxaliplatin has the potential for being a safe and effective treatment for advanced gastric cancer.

In addition to the effects of chemotherapy in inhibiting tumor replication, immunotherapy plus chemotherapy has been proposed as a comprehensive treatment that might improve outcomes for

treating human gastric cancer[15]. Research has suggested that the antitumor effects of chemotherapy occur through regulation of the immune system[12]. There are no reports of well-tolerated chemotherapy regimens in patients with advanced gastric cancer that give a median survival time of one year or longer. We believe that the median survival time of 1.3 years and median PFS of 5.1 months in patients with gastric cancer in our study was the result of synergism of $ACT^{IL\text{-}15}$ and S-1 plus oxaliplatin.

S-1 or S-1 plus oxaliplatin remains the backbone of gastric cancer chemotherapeutics, and it has been widely used for first-line therapy for advanced gastric cancer [24, 25]. Based on this experience, we selected S-1/oxaliplatin as chemotherapy treatment. Cancer immunotherapy has been found potentially useful in the control of tumor growth and patient survival [26], and ACT combined with chemotherapeutic regimens for treating gastric cancer was more productive than treatment with chemotherapy alone [23, 27]. Recent studies have shown that chemoresistant cancer cells are sensitive to the cytotoxic effect of ACT lymphocytes [23, 28, 29], and theoretically, ACT lymphocytes have potential to eradicate residual tumor cells following chemotherapy. Our findings suggest that the combination of ACT with S-1/oxaliplatin favorably modulates the immune milieu of the host, which could be beneficial in instances of chemoresistance. Therefore, we hypothesized that patients would benefit from $ACT^{IL\text{-}15}$ along with S-1/oxaliplatin.

Another study[30] has found that the mere generation of large numbers of highly differentiated lymphocytes is insufficient to achieve tumor regression. The effects of ACT on OS and PFS were most notable for patient with lymphocyte preparations that had high levels of $CD45RO^{+}$ T-cells. CD45, which is also known as the leukocyte common antigen, functions as a tyrosine phosphatase in leukocyte signaling[31]. CD45RO is a marker of effector memory T lymphocytes following adoptive transfer[32] and has been demonstrated to closely represent the activation status of T cells[33]. Memory T cells are known to be generated during cell-mediated immune responses and survive for months, and even years after the antigen is eliminated [34, 35]. In our study, IL-15, unlike IL-2, uncouples differentiation from proliferation to generate a large number of more effective, less-differentiated memory lymphocytes. Therefore, our findings suggest that the combination of $ACT^{IL\text{-}15}$ with S-1/oxaliplatin favorably modulates the immune milieu of the host, which could be beneficial in instances of chemoresistance. Taken together, these results validate the use of $ACT^{IL\text{-}15}$ along with chemotherapy for improving the response in gastric patients with cancer.

Although this trial was a randomized, there were several limitations. First, studies on the treatment of gastric patients with cancer were not conducted in a blind manner. Although the randomization of patients to a combination of $ACT^{IL\text{-}15}$ and chemotherapy vs. chemotherapy alone was appropriate, the lack of blinding could have introduced bias in assessing the survival outcomes and adverse effects. Another limitation is that the number of gastric patients with cancer studied (63 divided into two groups) is modest, and the study is single-center. Thus, the representativeness of the results needs testing in larger samples from multiple centers.

Conclusions

Adoptive transfer of ACT^{IL-15} cells in a mouse model of gastric cancer and patients with advanced gastric cancer (treated with the transferred cells along with S-1 and oxaliplatin) improved survival. These findings support efforts toward the improved treatment of gastric patients with cancer and the evaluation of ACT^{IL-15} cells in earlier treatment of patients with advanced disease.

Declarations

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Competing interests

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Author contributions

Yuefeng Hu and Jingwei Liu: Conceptualization, Design and Writing-original draft preparation; Dong Liu, Peilin Cui and Xu Lu: Data Curation, Writing-Reviewing and Editing; Wen Zhang, Hao Chen and Yongcheng Lu: Resources, Methodology and Validation; Chunmei Piao: Funding acquisition; Xuesong Liu and Yue Wang: Validation.

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Not applicable.

Ethics approval and consent to participate

The study was approved by the Ethical Review Board of the Medical Ethics Committee of the National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences, and Peking Union Medical College. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analyzed during the study are included in the published article.

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Figures

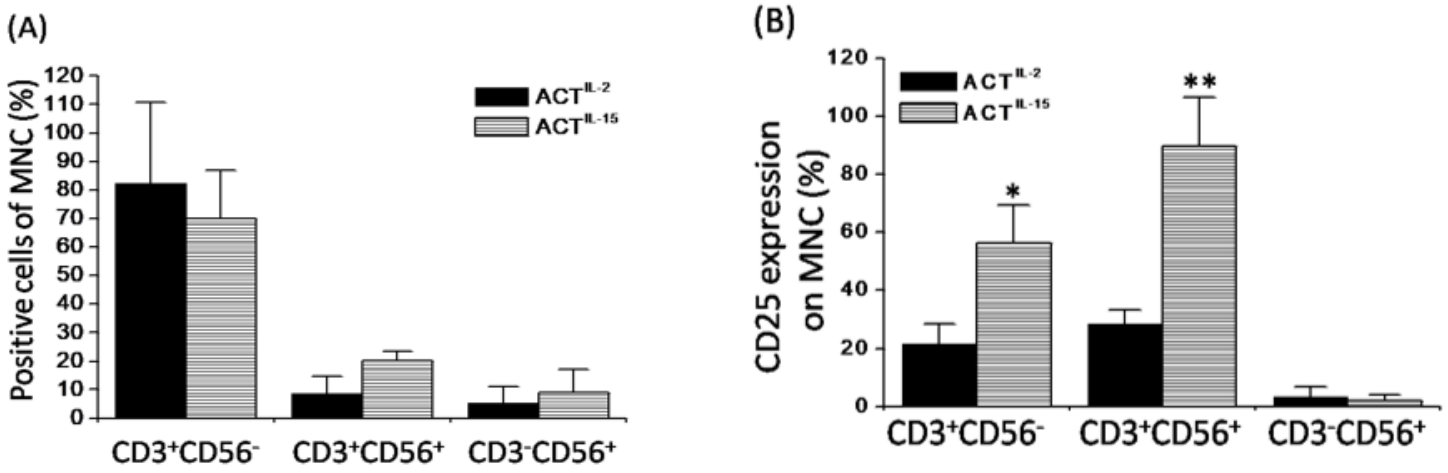

Figure 1

Phenotypic analysis of ACTIL-2 and ACTIL-15 cells during culture. (A) The composition of CD3+CD56− T, CD3+CD56+ T-NK, and CD3−CD56+ NK lymphocytes was not significantly different between ACTIL-2 and ACTIL-15 cells. (B) CD25 expression on CD3+CD56− T cells and CD3+CD56+ T-NK cells was significantly higher in ACTIL-15 cells than in ACTIL-2 cells. *P < 0.05; **P < 0.01. ACT, adoptive cell therapy. Data shown are representative of 3 independent experiments.

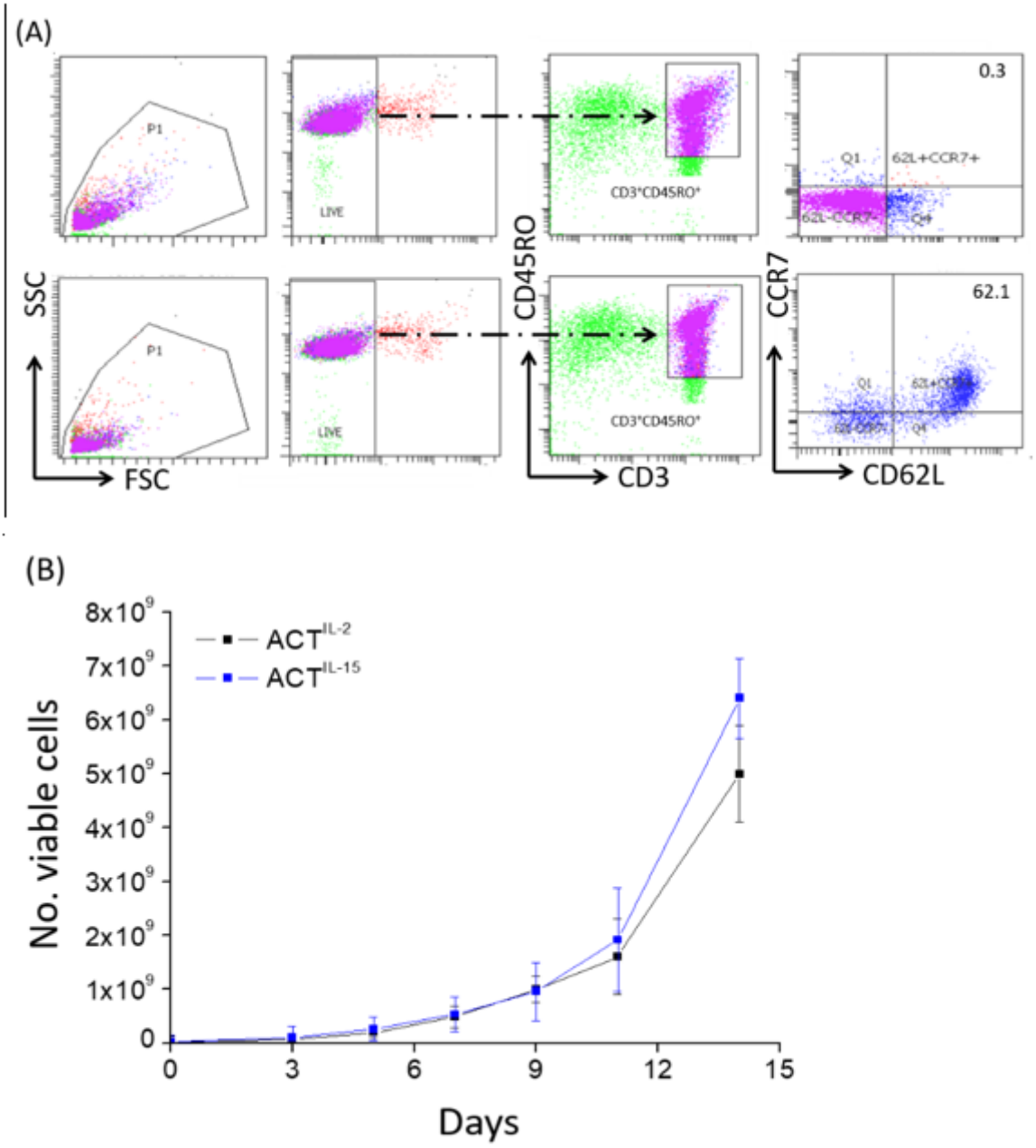

Figure 2

IL-15 uncouples lymphocyte differentiation from proliferation. (A) IL-15 preserves lymphoid-homing molecule expression after multiple in vitro stimulations. Flow cytometric analysis for surface expression of CD62L and CCR7 on ACTIL-2 and ACTIL-15 cells. (B) Lymphocytes similarly expand in media containing IL-2 and IL-15. Lymphocytes were counted at indicated time points following trypan blue exclusion. ACT, adoptive cell therapy. Data shown are representative of 3 independent experiments.

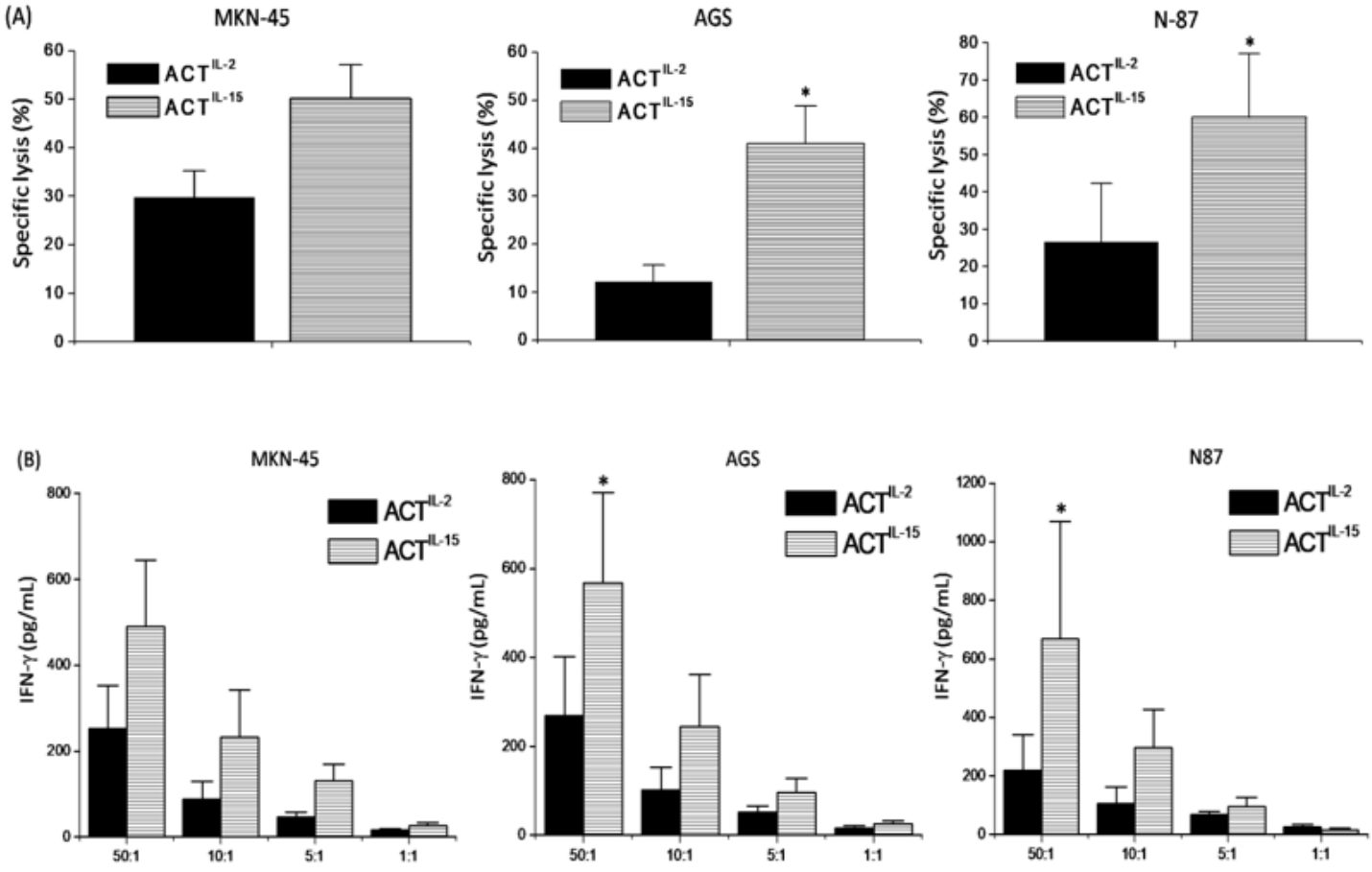

Figure 3

The cytotoxic capacity of the ACTIL-2 and ACTIL-15 cells against the MKN-45, AGS, and N87 cell lines. (A) The cytotoxic capacity of ACTIL-15 cells was significantly higher than that of ACTIL-2 cells against AGS and N87. (B) The secretion of cytokine IFN-γ was significantly increased at effector/target ratio of 50:1. *P < 0.05. ACT, adoptive cell therapy. Data shown are representative of 4 independent experiments.

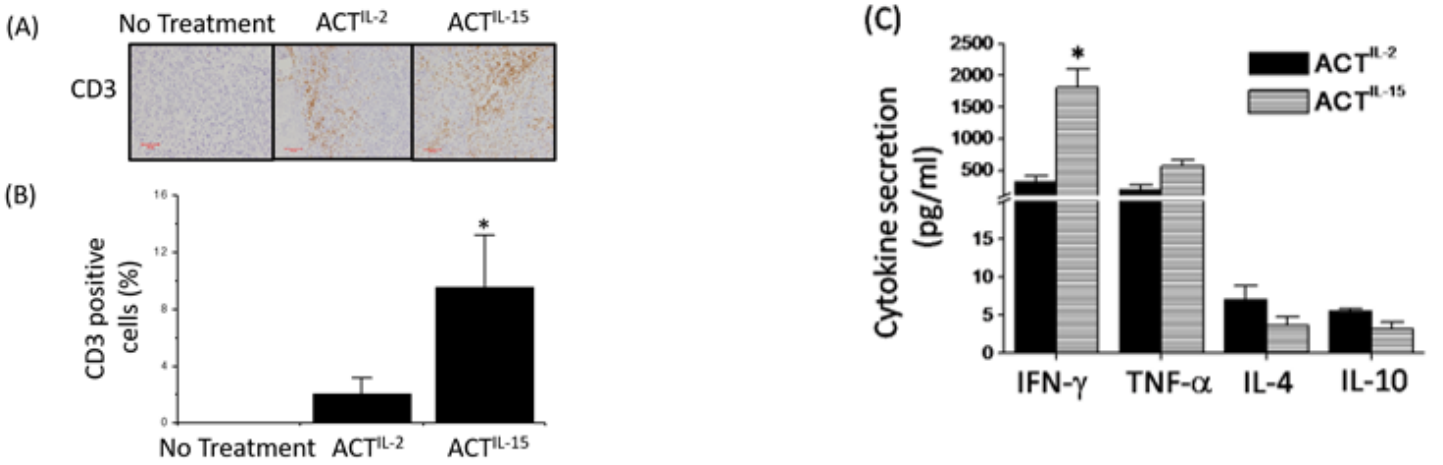

Figure 4

ACTIL-15 cells are significantly enriched in the tumor and possess higher effector activities than the adoptively transferred ACTIL-2. (A) Tumor-infiltrating lymphocytes were identified by immunohistochemical staining. A representative image (200× magnification) from each group is shown. (B) Quantitative analysis of CD3-positive cells in the tumor. Data are mean ± SEM for n = 10 mice with ten fields per animal. (C) Adoptively transferred ACTIL-15 cells produced more IFN-y than did adoptively transferred ACTIL-2. *P < 0.05. ACT, adoptive cell therapy.

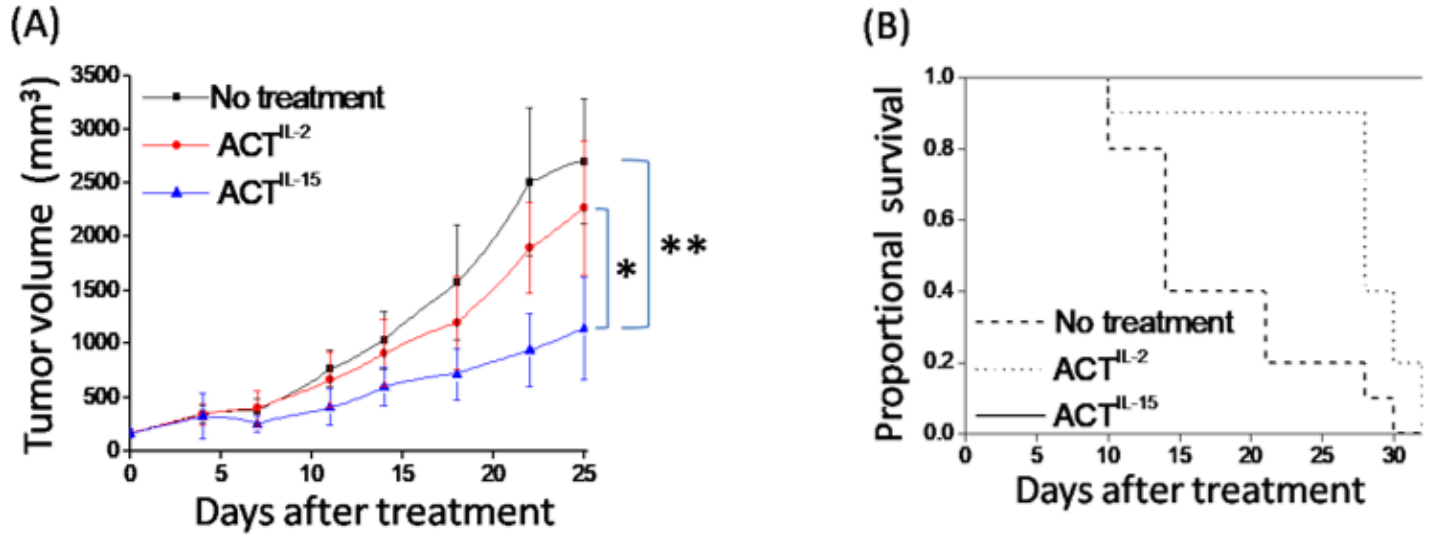

Figure 5

Tumor volume (A) and survival (B) in mice receiving ACTIL-2 or ACTIL-15 cells compared with untreated controls. Results shown are from three independent experiments. *P < 0.05; **P < 0.01. ACT, adoptive cell therapy. Data shown are representative of 3 independent experiments.

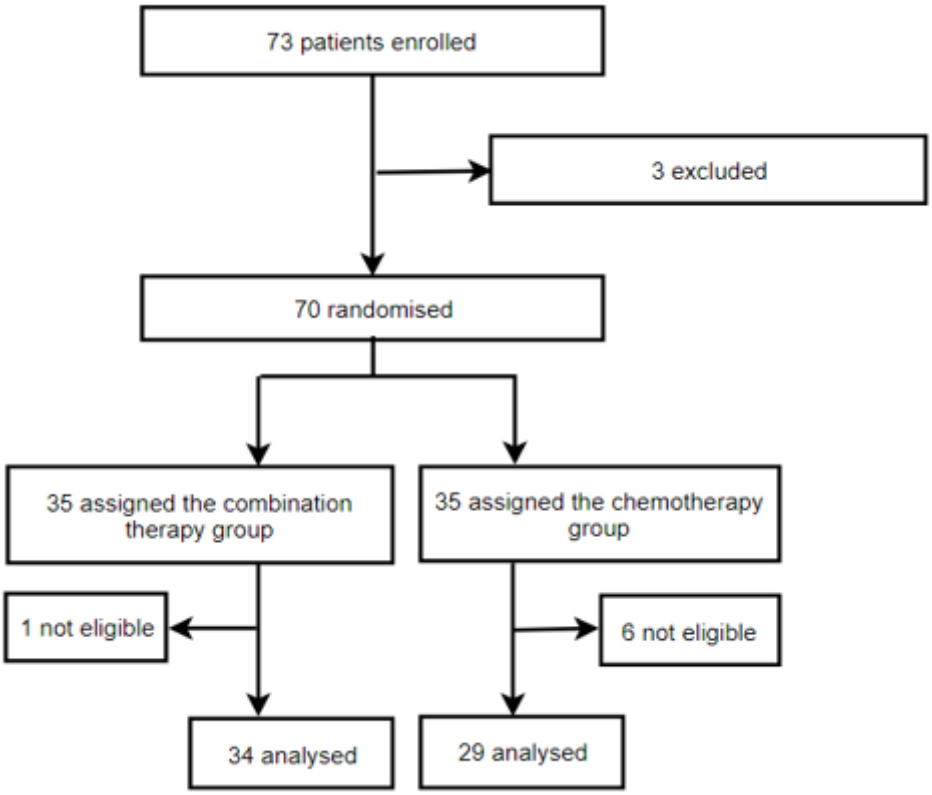

Figure 6

The trial scheme. Seventy-three patients were enrolled, of whom three were excluded, and 35 each were randomly assigned to the combination therapy and chemotherapy group. After the elimination of patients based on nonadherence, 34 remained in the combination group and 29 in the chemotherapy group.

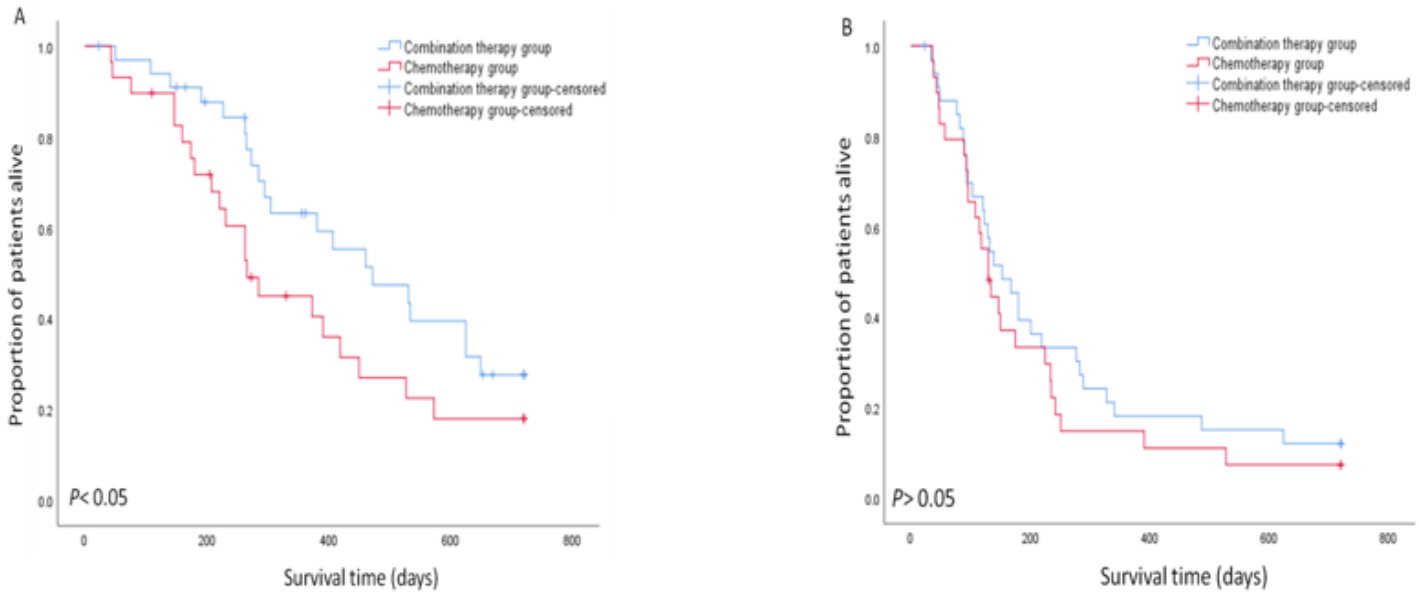

Figure 7

Survival analysis in patients with gastric cancer. Overall survival curves (A) and PFS curves (B) for patients with gastric cancer who received adoptive T-cell immunotherapy combined with chemotherapy (the combination therapy group) or chemotherapy alone (chemotherapy group) are shown. PFS, progression-free survival.