Effects of carrimycin on biomarkers of inflammation and immune function in tumor patients with sepsis: A multicenter double-blind randomized controlled trial

Protocol of Clinical Study

Responsible Unit of Clinical Research:	The First Affiliated Hospital of Harbin Medical University Tianjin Cancer Hospital
Principal Investigator:	Prof. Kaijiang Yu Prof. Donghao Wang
Principal Sponsor:	Cancer Critical Care Medicine Committee of the Chinese Anti-Cancer Association
Statistical analysis of Data Management:	Guangzhou Jeeyor Medical Research Co., Ltd.

Scheme No.: KLMS-2019001

Version No.: V2.0

Version date: 20191202

Statement of Confidentiality

All information related to this study is confidential. All participants in this study are subject to clinical study confidentiality and may not be used for any other purpose except with the written consent of the subject authority.

Catalogue

Declaration and signature of all parties

1. Statement by the bidding entity

We have read and understood the trial protocol (Scheme No.: KLMS-2019001; Version number: V2.0; Version date: December 02, 2019), agrees to the content of the trial protocol, strictly follows the trial protocol, and complies with all the obligations and other relevant requirements of the sponsor in accordance with the Quality Management Practice for Clinical Trials, Declaration of Helsinki, and relevant regulations of the State Food and Drug Administration. We will be responsible for initiating, applying for and organizing this clinical study in accordance with the provisions of the GCP; In case of any injury or death related to the study, we will bear the treatment costs and corresponding financial compensation, and provide legal and economic guarantees to the investigator. We are responsible for the scientific and rational nature of this program.

Sponsor: Cancer Critical Care Medicine Committee of the Chinese Anti-Cancer Association

Director: Kaijiang Yu

2. Statement of Clinical Trial Group Leader (1)

I have read and understood the trial protocol (Protocol No. : KLMS-2019001; Version number: V2.0; Version date: December 02, 2019), the study will be conducted in accordance with the moral, ethical and scientific principles set out in the Declaration of Helsinki and the Practice for the Quality Management of Pharmaceutical Clinical Trials (GCP). I agree to conduct this clinical study in accordance with the design and provisions of this protocol and the relevant laws and regulations of the State Food and Drug Administration.

I will ensure that subjects receive timely treatment in the event of adverse events occurring during the study. I am aware of the requirements for correct reporting of serious adverse events and I will record and report such events as required.

I will ensure the quality of clinical trials by ensuring that the data is recorded accurately, completely and in a timely manner in the original documentation. I will accept the inspection by the supervisors and inspectors dispatched by the sponsoring unit and its designated unit (CRO), as well as the inspection by the drug regulatory department.

The information contained herein and any additional protocol information is confidential. I warrant that this protocol will not be used in other clinical trials, and I will not disclose this protocol content to any other individual or group not associated with the trial.

Clinical trial unit: The First Affiliated Hospital of Harbin Medical University

Principal Investigator: Kaijiang Yu

3. Statement of Clinical Trial Group Leader (2)

I have read and understood the trial protocol (Protocol No.: KLMS-2019001; Version number: V2.0; Version date: December 02, 2019), the study will be conducted in accordance with the moral, ethical and scientific principles set out in the Declaration of Helsinki and the Practice for the Quality Management of Pharmaceutical Clinical Trials (GCP). I agree to conduct this clinical study in accordance with the design and provisions of this protocol and the relevant laws and regulations of the State Food and Drug Administration.

I will ensure that subjects receive timely treatment in the event of adverse events occurring during the study. I am aware of the requirements for correct reporting of serious adverse events and I will record and report such events as required.

I will ensure the quality of clinical trials by ensuring that the data is recorded accurately, completely and in a timely manner in the original documentation. I will accept the inspection by the supervisors and inspectors dispatched by the sponsoring unit and its designated unit (CRO), as well as the inspection by the drug regulatory department.

The information contained herein and any additional protocol information is confidential. I warrant that this protocol will not be used in other clinical trials, and I will not disclose this protocol content to any other individual or group not associated with the trial.

Clinical trial unit: Tianjin Cancer Hospital

Principal investigator: Donghao Wang

4. Statement of data management and statistical analysis units

I have read and understood the test protocol (Scheme No. : KLMS-2019001; Version number: V2.0; Version Date: December 02, 2019), I agree that in accordance with the design and provisions of this protocol, and in accordance with the Guidelines for Planning and Reporting of Drug Clinical Trial Data Management and Statistical Analysis, Guidelines for Electronic Data Acquisition Technology for Clinical Trials, Guidelines for Biostatistics for Drug Clinical Trials and other relevant regulations issued by NMPA, To carry out the data management and statistical analysis of this program.

I will conscientiously fulfill the responsibilities of data management and statistical analysis personnel according to relevant regulations.

The information contained herein and any additional protocol information is confidential. I warrant that this protocol will not be used in other clinical trials, and I will not disclose this protocol content to any other individual or group not associated with the trial.

Data management and statistical analysis Unit: Guangzhou Jeeyor Medical Research Co., Ltd.

Principal: Zhaoshi Xu

5. Statement of Contract Study Organization (1)

I have read and understood the test protocol (Scheme No.: KLMS-2019001; Version number: V2.0; Version Date: December 02, 2019), I agree to perform the relevant responsibilities in accordance with the design and provisions of this scheme, and in strict accordance with Chinese laws, Declaration of Helsinki, NMPA GCP and this research scheme.

I ensure that copies of this protocol are provided to all participants in the study and that I discuss the protocol and relevant information with them to ensure that they fully understand the study drug and how to conduct the study prior to the trial.

In accordance with the requirements of GCP regulations and the contract with the sponsor, I will be responsible for the research center research, project approval, assisting the sponsor to conduct ethical review, assisting the sponsor to communicate the agreement and other related work before the launch of the project. I will establish a quality control and quality assurance system for clinical trials to ensure the smooth start of the project.

The information contained herein and any additional protocol information is confidential. I warrant that this protocol will not be used in other clinical trials, and I will not disclose this protocol content to any other individual or group not associated with the trial.

Contract research Organization: Guangzhou Yushi Medical Technology Co., Ltd.

Principal Investigator: Shengpeng Zhang

6. Statement of Contract Study Organization (2)

I have read and understood the test protocol (Scheme No.: KLMS-2019001; Version number: V2.0; Version Date: December 02, 2019), I agree to perform the relevant responsibilities in accordance with the design and provisions of this scheme, and in strict accordance with Chinese laws, Declaration of Helsinki, NMPA GCP and this research scheme.

I ensure that copies of this protocol are provided to all participants in the study and that I discuss the protocol and relevant information with them to ensure that they fully understand the study drug and how to conduct the study prior to the trial.

I will be responsible for the clinical study agreement signing, project funding management, clinical trial quality control and other related work in accordance with GCP regulations and the requirements of the contract with the sponsor. I will set up a quality control and quality assurance system for clinical trials, and appoint qualified inspectors to inspect the project during the project to ensure the quality of the project.

The information contained herein and any additional protocol information is confidential. I warrant that this protocol will not be used in other clinical trials, and I will not disclose this protocol content to any other individual or group not associated with the trial.

Contract research Organization: Krypton Technology (Beijing) Co., Ltd.

Principal Investigator: Ying Ma

Glossary of Abbreviations

abbreviations	annotation
AE	Adverse events
ALT	Alanine aminotransferase
APACHE	Acute Physiology vs. Chronic health score
AST	Aspartate aminotransferase
BUN	Urea nitrogen
Cr	Creatinine
CTLA4	Cytotoxic T lymphocyte-associated protein 4
EDC	Electronic data acquisition system
FAS	Full analysis set
GCP	Drug clinical trial quality management practices
Hb	Hemoglobin
HCT	Red blood cell volume
ITT analysis	Intentionality analysis
IRT	Interactive response techniques
KLMS	Carrimycin
MDSC	Myeloid inhibitory cells
PD-1	Programmed death receptor 1
PLT	Platelets
PPS	Per-protocol set
PP analysis	Fit scheme set analysis
PT	Prothrombin time
SAE	Serious Adverse Events
Sepsis	Sepsis
SOFA	Sequential organ failure
SS	Safety set (Safety set)
TBil	Total bilirubin
TNF	Tumor necrosis factor
WBC	White blood cells

Protocol Summary

Scheme No.	KLMS-2019001
Version Number	V2.0
Version date	20191202
Research Title	Effects of carrimycin on biomarkers of inflammation and immune function in tumor patients with sepsis: A multicenter double-blind randomized controlled trial
Study Subjects	Patients with tumor sepsis
Purpose of the trial	To evaluate the effect of carrimycin tablets on improving inflammation and immune regulation in patients with tumor sepsis
Trial Design	Multicenter double-blind randomized controlled trial
Total number of cases	120 cases
Subject selection	Inclusion criteria: 1. Informed consent has been signed by subject or legal representative 2. Age ≥18 and ≤75 years old 3. Meet Sepsis3.0 diagnostic criteria 4. SOFA score: 2 to 13 5. Patients with malignant tumors (histological diagnoses) Exclusion criteria: 1. A clear diagnosis of sepsis has taken longer than 48 hours 2. Associated with serious primary diseases that affect survival, including hematologic diseases, hematologic tumors, lymphatic cancers, and HIV 3. Patients with liver or kidney dysfunction with single SOFA score ≥3 4. Patients who have received tumor immunotherapy (such as PD-1/L1, CTLA4, etc.) or commonly used immunomodulators (such as thymus

	facidine, etc.) or inflammatory cytokines regulators (such as ulinastatin) within the past 1 month 5. Organ transplant patients within the last 6 months 6. Cannot be given by gastrointestinal diet, such as in patients with intestinal obstruction 7. Pregnant and lactating women, subjects (including male subjects) have pregnancy plans (including sperm and egg donation plans) or are unable to take effective contraceptive measures in the next 6 months 8. Known to have participated in any other clinical trials within 1 month 9. Patients deemed unsuitable for inclusion by the investigators
Study drugs	Trial drug: Carrimycin tablet (200mg/ tablet) Control drug: Placebo tablet (200mg/ tablet)
Administration regimen	Trial group: Clinical treatment plan + carrimycin tablets (400mg/ day) for 7 days Control group: clinical treatment plan + placebo tablet (400mg/ day), treatment course was 7 days The clinical treatment plan was determined according to China Guidelines for Emergency Treatment of Sepsis/Septic Shock (2018), which could be adjusted by each center according to the actual situation (dosage adjustment and drug addition and subtraction were recorded in detail).
Follow-up time point	Subjects were admitted for observation at 1, 3, 5, and 7 days after administration and followed up at 28 days
Outcome measures	**Primary outcomes:** Immune-related indicators Human leukocyte surface antigen -DR, IL-6, IL-8, IL-10, TNF-α, PD-1, T cell PD-1 expression, MDSC (myeloid inhibitory cells), T cell subsets (CD4+ and CD8+T cells) **Secondary outcomes:** All-cause mortality (28 days) All-cause mortality (7 days) SOFA score

	APACHEII Rating Mechanical ventilation time Length of stay in ICU Duration of CRRT C-reactive protein Procalcitonin **Safety indicators:** Adverse events, serious adverse events
Statistical analysis	1. Statistical analysis software and general methods Perform statistical analysis with SAS 9.4 or above. Unless otherwise stated, all statistical inferences were made using a two-sided test with a level of 0.05 and the estimation of parameters was performed using the 95% confidence interval method. 2. Statistical description The main indicators collected in this study are statistically described. Quantitative indicators were described by means, standard deviation, median, range and other indicators. Qualitative indicators are described by frequency, percentage and other indicators. 3. Primary and secondary outcome analysis The main outcome indicators were immune-related indicators (human leukocyte surface antigen -DR, IL-6, IL-8, IL-10, TNF-α, PD-1, T cell PD-1 expression, MDSC (myeloid inhibitory cells), T cell subsets). Secondary outcome measures included all-cause mortality (28 days), all-cause mortality in both groups (7 days), SOFA score, APACHEII score, duration of mechanical ventilation, length of stay in ICU, and duration of CRRT, C-reactive protein, procalcitonin index. Among the primary and secondary outcome indexes, the quantitative and approximately normal distribution indexes were analyzed by T-test. For

	quantitative indicators that did not obey the normal distribution, the rank sum test was used to compare the two groups. And categorical variables (including ordered categorical variables), which were analyzed using chi-square test or rank-sum test according to the research hypothesis. 4. Security analysis Summarize all reported adverse events, adverse reactions, significant adverse events, and serious adverse events during the study. For all adverse events, the incidence of adverse events was calculated based on the number of subjects in which adverse events occurred and compared between groups. In addition, adverse events occurring after administration that may be (definitely, probably, possibly, possibly, possibly not) related to study medication were analyzed as a subset of adverse events.

Protocol Text

1. Research background

Sepsis is an important clinical problem in acute and critical care medicine. Worldwide, the number of patients with sepsis exceeds 19 million every year, among which 6 million patients die, with a case fatality rate of more than 1/4. Among the surviving patients, about 3 million have cognitive dysfunction[1-3]. In recent years, the research on sepsis at home and abroad continues to deepen, and the clinical practice and evidence continue to increase. It has been clear that the early use of antibacterial drugs is very important for the prognosis of patients with sepsis or septic shock.

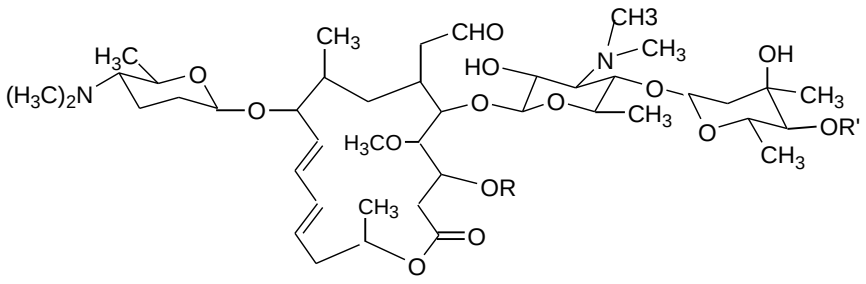
Carrimycin is a physiologic active substance with various medicinal value jointly developed by Shenyang Tonglian Group Co., LTD and Institute of Pharmaceutical Biotechnology, Chinese Academy of Medical Sciences. It belongs to the national 1.1 New drug (National drug approval number H20190029), which has Chinese independent intellectual property rights and is a key research project of "863" and "Tenth Five-Year Plan". It has obtained the special support of "National Major New Drug Creation" from the Ministry of Science and Technology.

Carrimycin belongs to macrolide antibiotics in a broad sense. It is a multi-component antibiotic, which is directly fermented by modified genetically engineered bacteria. It is a bioactive substance with 16-element cyclolide bond. The main components were isovalerylspiramycin (ISP) I, II and III. It inhibits protein synthesis by binding to the large subunit of the bacterial ribosome 50S.In terms of anti-infection, it has inhibitory activity against Gram-positive bacteria, some Gram-negative bacteria, Legionella, especially mycoplasma and chlamydia. The completed phase III clinical trials have confirmed that carrimycin is safe to take, with a small oral dose and a low adverse reaction rate. It has a good effect on respiratory bacterial infection, and there is no complete cross-resistance with similar drugs. The clinical efficacy and safety of carrimycin are better than the control drug azithromycin. In view of the above good clinical efficacy and safety, Carrimycin tablets have been approved for marketing. At the same time, further basic and animal experiments confirmed that carrimycin has the potential to improve inflammation and regulate immunity. Therefore, a series of clinical studies on the treatment of sepsis by carrimycin were initiated by the Cancer Critical Care Medicine Committee of the Chinese Anti-Cancer Association to explore the effects of carrimycin on the improvement of inflammation and immune regulation in patients with tumor sepsis.

1.1 Drug Introduction

Carrimycin is a mixture mainly composed of isovalyl spiramycin III, isovalyl spiramycin II and isovalyl spiramycin I, containing a certain amount of (isobutyryl), propionyl, acetylspiramycin III and (isobutyryl), propionyl, acetylspiramycin II.

The chemical structural formula of the main components is as follows:



Isovalyl spiramycin III: R=COCH2CH3 R '=COCH2CH(CH3)2

Molecular formula: C51H86N2O16 Molecular weight: 982.00

Isovalyl spiramycin II : R=COCH3 R '=COCH2CH(CH3)2

Molecular formula: C50H84N2O16 Molecular weight: 968.00

Isovalyl spiramycin I : R=H R '=COCH2CH(CH3)2

Molecular formula: C48H82N2O15 Molecular weight: 926.00

1.2 Anti-inflammatory mechanism of carrimycin

Carrimycin is a 16-member-ring macrolide antibiotic. Its mechanism of action is to inhibit protein synthesis by binding to bacterial ribosomes. In vitro and clinical trials have shown that it has antibacterial activity against Gram-positive bacteria, mycoplasma, chlamydia, some gram-negative bacteria, and has antibacterial activity and tissue permeability against toxoplasmosis, Legionella, etc.

1.2.1 Carrimycin can inhibit the PI3K-AKT-mTOR pathway

Mammalian target of rapamycin (mTOR) is a downstream molecule of PI3K/Akt/mTOR and other signaling pathways. It plays an important role in cell proliferation, differentiation, metastasis and survival, and has become an important target for cancer therapy.

A549 cells were cultured in vitro and treated with different concentrations of clarithromycin for 48 h. mTOR protein and its upstream and downstream proteins in the cytoplasm were investigated by Western Blot method. The results showed that the expression of mTOR and p-mTOR was inhibited

by carrimycin, while the expression of P70S6K1 (ribosomal protein S6 kinase) and p-P70S6K1 downstream of mTOR was inhibited by carrimycin (Figure 1).

PI3K is a kind of intracellular phosphatidylinositol kinase. The PI3Ks protein family is involved in the regulation of cell proliferation, differentiation, apoptosis and glucose transport and other cellular functions. Increased PI3K activity is often associated with a variety of cancers. AKT (protein kinase B) is the primary downstream effector of PI3K. AKT activates mTOR by phosphorylating mTOR directly or by inactivating TSC2 (nodular sclerosis complex 2). Treatment of A549 cells with carrimycin for 48 h had no significant effect on PI3K, but significantly down-regulated the levels of p-PI3K and downstream p-AKT protein (Figure 1).

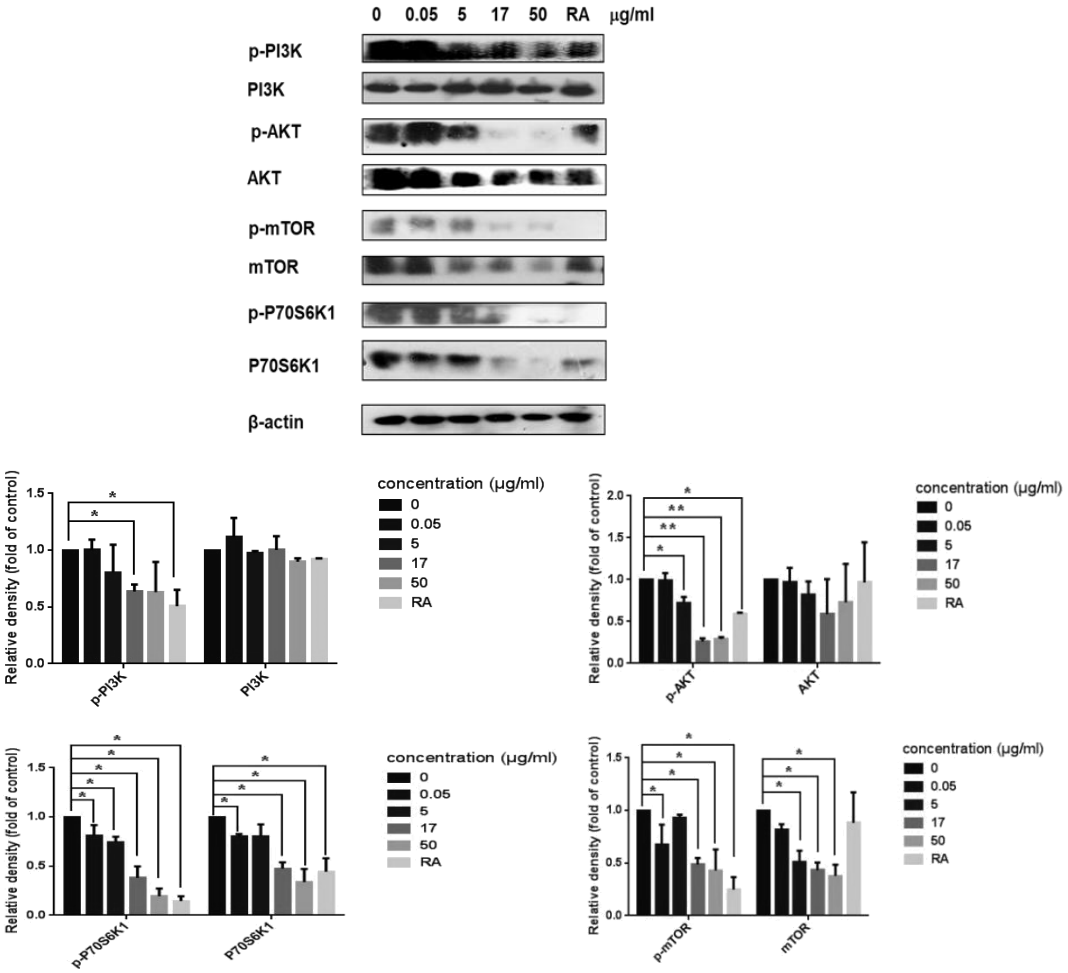


Figure 1: Carrimycin inhibited the PI3K-AKT-mTOR pathway. Western Blot assay was used to detect the expression of p-PI3K, PI3K, p-AKT, AKT, p-mTOR, mTOR, p-P70S6K1, and P70S6K1 proteins. β-actin was used as control.

*p< 0.05 compared with the model group; ** p< 0.01 compared with the model group

The above study showed that the mTOR protein and its upstream and downstream proteins in the

cytoplasm were investigated by Western Blot after being treated with different concentrations of carrimycin for 48 h. The results showed that the expression of mTOR and p-mTOR could be inhibited by carrimycin, and the expression of P70S6K1 (ribosomal protein S6 kinase) and p-P70S6K1 downstream of mTOR could be inhibited by carrimycin. These results suggest that carrimycin can inhibit the mTOR pathway, especially the expression of S6K1 downstream, which may result in the inflammatory factor TNF-α, decreased secretion of IL-6, and enhanced secretion of anti-inflammatory factor IL-10.

1.2.2. Carrimycin can inhibit the expression of PD-L1

PD-L1 molecule plays an important role in inducing tumor-specific T cell apoptosis and tumor immune escape. The brown-yellow particles or clumps of PD-L1 molecules were positively expressed in the membrane of PD-L1 lung cancer cells. The expression of PD-L1 on the cell membrane of A549 was significantly reduced by carrimycin (0.05, 5μg/ml) (Figure 2).

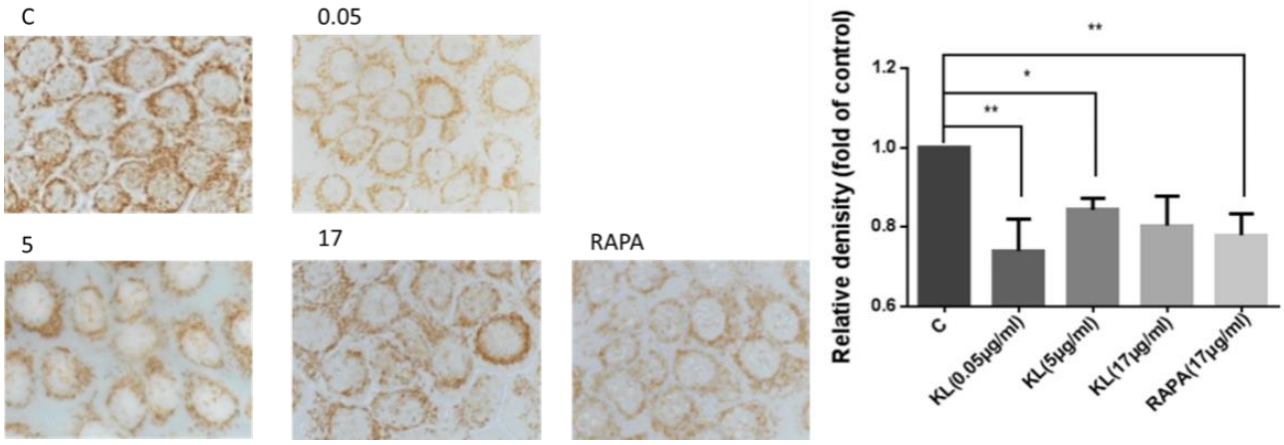


Figure 2: The expression of PD-L1 protein was detected by immunohistochemistry. Brown-yellow particles or clumps of PD-L1 molecules were positively expressed in the membrane of A549 lung cancer cells.

*p< 0.05 compared with the model group; ** p< 0.01 compared with the model group.

1.3 Studies on the immune regulatory mechanism of carrimycin

1.3.1 Effects of carrimycin on phagocytosis of macrophages

Itraconazole was used as positive control. The normal saline group was the placebo control group. The positive control group was itraconazole: 50mg/kg, po. The experimental group was given carrimycin: 50mg/kg, po. for five days.

Experimental results:

Compared with the placebo group (NC), the carrimycin group (Keli) and itraconazole group (Yiqu) enhanced the ability of macrophages to phagocytose chicken red blood cells to some extent.

1.3.2 Detection of the effect of carrimycin on neutrophil function

In mice, carrimycin and itraconazole significantly promoted the migration of neutrophils to the site of inflammation, especially in individual mice. No further enhancement was found after three days of treatment compared with seven days of treatment. In mice, carrimycin and itraconazole significantly increased the number of total T cells (CD3 positive cells), including both CD4 and CD8 positive cells.

1.3.3 Transdifferentiation of differentiated macrophages induced by carrimycin

The results showed that the expression levels of TNF-α and iNos in M2-type macrophages were significantly higher than those induced by LPS+INF-γ, better than itraconazole, and the expression level of Arg-1 in M2-type macrophages was significantly inhibited.

A series of studies carried out by Yiguo Hu et al in West China Hospital suggest that carrimycin can strongly promote the transformation of M2-type macrophages to M1-type macrophages and improve the function of M1-type macrophages, which is mainly manifested in the following three aspects:

(1) RAW246.7 cell lines were cultured in vitro, treated with drugs for 1h, and then differentiated by cytokines. The corresponding RNA levels were detected, and it was found that carrimycin could increase the expression of TNF-α and iNos, and inhibit the expression of Arg-1, suggesting that carrimycin may promote the differentiation and function of M1-type macrophages.

(2) Cultured RAW246.7 cell lines in vitro, cytokine was added first to induce the differentiation of RAW cells into M1-type macrophages, and then corresponding drugs were added to detect the corresponding RNA level. It was found that the expression of TNF-α and iNos induced by LPS+INF-γ was not further enhanced by carrimycin. However, it could significantly inhibit the expression of Arg1 in M1-type macrophages, suggesting that carrimycin could potentially enhance the function of M1-type macrophages.

(3) RAW246.7 cell lines were cultured in vitro. Cytokines were added first to induce the differentiation of RAW cells into M1 macrophages, and then corresponding drugs were added to induce the transformation of cells into M2 macrophages. The corresponding RNA levels were

detected, and it was found that the expression levels of TNF-α and iNos in M2 macrophages were significantly increased by carrimycin. The expression levels of TNF-α and iNos induced by LPS+INF-γ were significantly higher than those of LPS+INF-γ, and the expression level of Arg1 in M2-type macrophages was significantly inhibited.

1.4 Toxicological studies of carrimycin

To evaluate the effect of carrimycin on blood routine in mice: Itraconazole was used as positive control group, 50mg/kg, PO.

The experimental group was carrimycin group: 50mg/kg, po. for five consecutive days.

The blood routine results of normal mice after administration showed no significant changes in the total number of white blood cells, neutrophils, lymphocytes and so on.

	Absolute number / relative ratio		**UNITS**	**control-1**	**control-2**	**itraconazol -2**	**carrimycin -1**	**carrimycin -2**
Total white blood cell count		WBC	10^9/L	5.15	5.63	4.48	4.53	4.35
Neutrophils	#	NEUT	10^9/L	1.65	1.49	1.36	0.99	1.51
Lymphocytes	#	LYMPH	10^9/L	3.29	3.92	2.89	3.3	2.59
Monocytes	#	MONO	10^9/L	0.05	0.06	0.05	0.09	0.04
Eosinophils	#	EOS	10^9/L	0.1	0.07	0.11	0.11	0.09
Basophils	#	BASO	10^9/L	0.01	0.01	0.01	0.01	0.02
Large volume of unstained cells	#	LUC	10^9/L	0.05	0.08	0.06	0.03	0.1
Neutrophils	%	NEUT	%	32.1	26.5	30.4	21.8	34.8
Lymphocytes	%	LYMPH	%	63.8	69.7	64.4	73	59.5
Monocytes	%	MONO	%	1	1	1.1	1.9	1
Eosinophils	%	EOS	%	2	1.2	2.4	2.5	2.1
Basophils	%	BASO	%	0.3	0.2	0.2	0.2	0.4
Large volume of unstained cells	%	LUC	%	0.9	1.4	1.4	0.6	2.3
Red Blood cells		RBC	10^12/L	10.22	10.74	10.53	10.07	10.28
Hemoglobin concentration		HGB	g/L	164	170	161	160	163
Hematocrit		HCT	%	51.1	52.6	51.7	49	49.1
Red blood cell volume		MCV	fL	50	49	49.1	48.7	47.8
Average amount of hemoglobin in red blood cells		MCH	pg	16	15.8	15.3	15.9	15.9
Average red blood cell hemoglobin		MCHC	g/L	320	323	312	326	333

Red blood cell distribution width	RDW	%	13.4	13.8	11.9	13	12.7
Platelets	PLT	10^9/L	892	635	1052	1044	805
Average platelet volume	MPV	fL	9.4	9.1	9.4	9.5	9.1

The experiment confirmed that no obvious hematological toxicity was observed under the condition of continuous administration of carrimycin for five days.

Genotoxicity: The results of the Ames test, the CHL test and the micronucleus test were negative.

Reproductive toxicity: Continuous oral administration of 20 and 500 mg/kg (about 0.3 and 8.3 times of the maximum recommended dose of 0.4g/ day for humans based on body surface area) of carrimycin 60 days before mating in male mice and 14 days before mating to 15 days after conception showed an increase in absorbable fetal rate and no other significant abnormalities.

Pregnant mice were orally given 10, 200 and 1000 mg/kg of carrimycin (0.17, 3.3 and 16.7 times of the maximum recommended dose of 0.4g/ day of human body surface area) for 10 consecutive days. The results showed that the 200 and 1000 mg/kg groups had fetal toxicity, the absorption rate increased, and no teratogenic effect was observed.

From the 15th day of gestation to the 21st day after delivery, the mice were given 20 and 500 mg/kg orally (measured by body surface area, about 0.3 and 8.3 times of the maximum recommended dose of 0.4g/ day for humans). The results showed that, No significant effects were observed on the reproductive function of mice and the survival, development, behavior, reproductive function, movement and learning ability of F1 generation mice, and no appearance deformities or pathological changes in the organs of heart, lung, liver, spleen, kidney and brain of F1 generation mice.

Other toxicities: Rats were given 20, 200 and 500 mg/kg orally for 60 days (measured by body surface area, about 0.5, 5 and 12 times of the maximum recommended dose of 0.4g/ day for humans). The results showed that during the administration of 500 mg/kg group, the animals showed upright hair, increased water intake, and reversible damage to the liver. No significant abnormalities were found in hematology and serum biochemical indexes, urine routine and pathological examination. The toxic reaction dose (NOAEL) of 200mg/kg was not observed in this test. Beagle dogs were orally given carrimycin 75, 150 and 300 mg/kg for 8 weeks (measured by body surface area, about 6, 12 and 24 times of the maximum recommended dose for human 0.4g/ day). The results showed that ALT and

AST were significantly increased in 150 and 300 mg/kg groups, and could recover after withdrawal of the drug. Nausea and vomiting were observed in all groups, which were dose - and time-dependent. In the 300 mg/kg dose group, weight gain was inhibited during administration. No significant abnormalities were observed in hematology and serum biochemical indexes, ECG, urine routine and histopathology.The NOAEL of the test was below 75 mg/kg.

1.5 Summary of the Phase I clinical study of Carrimycin

The Phase I safety and tolerability trial of carrimycin found that carrimycin stimulated the gastrointestinal tract and caused a slight increase in ALT and AST. According to the phase I pharmacokinetic study of carrimycin, the oral absorption of carrimycin tablets was rapid, the drug was widely distributed in the body, and the original drug was rapidly transformed into active metabolites. The elimination of the original drug was slow, and the T1/2β of each component was between 23 and 27h at a single dose.When multiple doses were administered, there was some accumulation in the body.Food had no effect on the substitution of clarithromycin, so in the course of clinical trials, patients took the medicine after meals, which greatly reduced the incidence of adverse reactions.

1.6 Summary of Phase II and III clinical trials of carrimycin

Since 2001, this product has completed 2 phase II and 4 phase III clinical trials. The observed diseases include acute pharyngitis, acute suppurative tonsillitis, acute trachea-bronchitis, mild pneumonia, acute sinusitis, suppurative otitis media, acute skin and skin soft tissue infection. The main observed diseases were acute suppurative tonsillitis, acute trachea-bronchitis, acute sinusitis, acute skin and skin soft tissue infection.

1). Acute sinusitis

A multi-center, randomized, double-blind, double-simulation, and parallel control trial with positive drugs was conducted in 5 grade-III Grade A hospitals according to the unified trial protocol to evaluate the efficacy and safety of carrimycin in the treatment of bacterial sinusitis. Administration methods: On the first day, 400mg of carrimycin tablet was taken orally after breakfast; Once a day for 2 to 7 days, 200mg carrimycin tablet was taken orally; Control on the first day, azithromycin tablet was taken 500mg after breakfast, and azithromycin tablet was taken 250mg once a day for 2 to 7 days. The treatment courses were all 5 to 7 days.

A total of 178 cases of bacterial sinusitis were enrolled in 5 clinical research centers, including

89 cases in the clarithromycin tablet group and 89 cases in the azithromycin tablet group.129 cases were completed, including 65 in the clarithromycin tablet group and 64 in the azithromycin tablet group.

Clinical efficacy evaluation of the two groups: 84 cases and 86 cases of the carrimycin tablet group and the azithromycin tablet group entered the FAS analysis set, respectively, and the clinical cure rates of the two groups were 88.10% and 84.88%, respectively. Statistical analysis showed that the clinical cure rates of the two groups were non-adverse. The clinical cure rates of the two groups were 88.10% and 84.88%, respectively. After statistical analysis, the clinical cure rates of the two groups were established.

Bacteriological efficacy evaluation of the two groups: Among the positive subjects in the carrimycin tablet group, the bacterial clearance rates of 35 cases in MFAS set and 34 cases in MPPS set were 88.57% (31/35) and 88.24% (30/34), respectively; In the 32 patients with positive bacterial culture in the azithromycin tablet group, the bacterial clearance rate of MFAS set and MPPS set was 81.25% (26/32).

Comprehensive efficacy evaluation of the two groups: The comprehensive efficacy of the clarithromycin tablet group and the azithromycin tablet group entered the MFAS concentration with pathogenic bacteria infection, and the recovery rates of the two groups were 88.57% and 71.88%, respectively; the comprehensive efficacy of the two groups entered the MPPS concentration with pathogenic bacteria infection regimen, and the recovery rates of the two groups were 88.24% and 71.88%, respectively.

Safety results showed that 4 of 86 subjects in the carrimycin tablet group had adverse reactions, the incidence of adverse reactions was 4.65% (4/86), including 2 cases of elevated alanine aminotransferase (ALT) (accompanied by elevated AST), 1 case of elevated AST, and 1 case of droopy (accompanied by nausea).Of 87 subjects in the azithromycin tablet group, 5 cases had adverse reactions, the incidence of adverse reactions was 5.75% (5/87), including 1 case of elevated alanine aminotransferase (ALT), 1 case of elevated total bilirubin (TBIL), 1 case of epigastric discomfort, 1 case of diarrhea and 1 case of headache. There was no significant difference between the two groups.

2). Acute trachea-bronchitis

A multi-center, randomized, double-blind, double-simulated, and parallel controlled trial with

positive drugs was conducted in 7 grade-III Grade A hospitals according to the unified trial protocol to evaluate the efficacy and safety of carrimycin in the treatment of acute bacterial trachea-bronchitis.

Administration methods: On the first day, oral administration of carrimycin tablet 400mg after breakfast, once a day for 2 to 7 days, oral administration of carrimycin tablet 200mg; Control drug on day 1, azithromycin tablet 500mg was taken orally after breakfast, and azithromycin tablet 250mg was taken orally once a day from 2 to 7 days. The treatment courses were all 5 to 7 days.

A total of 263 patients with acute bacterial tracheobronchitis were enrolled in 7 clinical research centers, including 130 patients in the clarithromycin tablet group and 133 in the azithromycin tablet group.190 cases were completed, including 97 in the clarithromycin tablet group and 93 in the azithromycin tablet group.

Clinical efficacy evaluation of the two groups: In the FAS analysis set, 104 cases were in the carrimycin group and 105 cases in the azithromycin group, respectively, and the clinical cure rates of the two groups were 88.46% and 90.48%, respectively. After statistical analysis, the clinical cure rate of the two groups was not inferior. There were 104 cases in the PPS analysis group in the clarithromycin tablet group and 104 cases in the azithromycin tablet group, respectively, and the clinical cure rates of the two groups were 88.46% and 90.38%, respectively. After statistical analysis, the clinical cure rate of the two groups was not inferior.

Bacteriological efficacy evaluation of the two groups: Among the 18 patients with positive bacterial culture in the carrimycin tablet group, the bacterial clearance rates of MFAS set and MPPS set were 94.44% (17/18). In the 16 cases of positive subjects in the azithromycin tablet group, the bacterial clearance rate of MFAS set and MPPS set was 87.50% (14/16).

Comprehensive efficacy evaluation of the two groups: the recovery rates of the carrimycin group and azithromycin group were 94.44% and 87.50%, respectively, in the MFAS set infected with pathogenic bacteria. The recovery rates of the carrimycin group and azithromycin group were 94.44% and 87.50% respectively when they entered the MFAS set with pathogenic bacteria infection regimen.

Safety results showed that 5 out of 125 subjects in the carrimycin tablet group had adverse reactions, the total incidence of adverse reactions was 4.00% (5/125), including 3 cases of elevated alanine aminotransferase (ALT), 1 case of elevated total bilirubin (TBIL), and 1 case of epigastric

discomfort.9 of 125 subjects in the azithromycin tablet group had adverse reactions, with a total incidence of 7.20% (9/125), including 3 cases of elevated alanine aminotransferase (ALT) (1 case accompanied by elevated aspartate aminotransferase (AST) and urinary leukocytosis), 4 cases of epigastric or stomach discomfort (1 case accompanied by nausea), 1 case of stomachache (accompanied by diarrhea) and 1 case of headache. There was no significant difference between the two groups.

2. Research purposes

To evaluate the effect of carrimycin tablets on improving inflammation and immune regulation in patients with tumor sepsis.

3. Research design

This study was a randomized, double-blind, controlled, multicenter clinical study. 120 subjects were randomly assigned 1:1 to the experimental group and the control group, and the corresponding random number was assigned according to the enrollment order. After enrollment, the subjects were treated with the clinical treatment plan combined with the experimental drug (control drug) for a course of 7 days, and were visited or followed up at 1, 3, 5, 7 and 28 days after medication.

4.Case selection criteria

4.1 Total samples

A total of 120 patients were included in this study. There were 60 patients in the experimental group and 60 in the control group.

4.2 Inclusion criteria

1) Informed consent has been signed by subject or legal representative;
2) Age ≥18 and ≤75 years old;
3) Meet Sepsis3.0 diagnostic criteria;
4) SOFA score: 2 ~13 points;
5) Patients with malignant tumors (histological diagnosis).

4.3 Exclusion criteria

1) A clear diagnosis of sepsis has taken longer than 48 hours;
2) Severe primary diseases that affect survival, including hematologic diseases, hematologic tumors, lymphatic cancers, and HIV;

3) Liver and kidney dysfunction patients with single SOFA score of liver or kidney ≥3 points;
4) Patients who received tumor immunotherapy (such as PD-1/L1, CTLA4, etc.) or commonly used immunomodulators (such as thymus facatin, etc.) or inflammatory cytokines regulators (such as ulinastatin) within the past 1 month;
5) Organ transplant patients within the last 6 months;
6) Unable to give drugs through gastrointestinal diet, such as patients with intestinal obstruction;
7) Pregnant and lactating women, subjects (including male subjects) have pregnancy plans (including sperm and egg donation plans) in the next 6 months or are unable to take effective contraceptive measures;
8) Have participated in any other clinical trials within the last 1 month;
9) Patients deemed unsuitable for inclusion by the investigator.

4.4 Culling criteria

The following circumstances should be generally excluded:

1) Seriously violate the inclusion and exclusion criteria;
2) Poor adherence to the protocol and the use of prohibited drugs during the trial;
3) Loss of follow-up;
4) Interruption of treatment due to pregnancy.

4.5 Termination criteria

1) The subject may withdraw from the test at any time for any reason;
2) A serious adverse event or an intolerable adverse event;
3) Any of the exclusion criteria occurred during the study period;
4) The investigator may decide whether to withdraw due to medical necessity.

For subjects who withdraw early, the reason for withdrawal should be noted on the CRF. Those who withdrew due to adverse events should try to complete 7±3 days of follow-up.

Assays (clinical assessments, laboratory tests, etc.) that were scheduled to be performed at the end of treatment should be performed as far as possible at the end of treatment.

5 Research groups

Trial group: clinical treatment plan + carrimycin tablets (400mg/ day), 60 randomized subjects.
Control group: clinical treatment plan + placebo tablets (400mg/ day), 60 randomized subjects.

6. Research steps

6.1 Screening period (-7 to 0 days)

1) Sign informed consent form;
2) Demographic data;
3) Medical history and complications were collected and recorded;
4) Vital signs (including respiration, blood pressure, heart rate, temperature);
5) SOFA score;
6) APACHEII score;
7) Mechanical ventilation time;
8) Routine blood tests (hemoglobin, white blood cell count, red blood cell count, red blood cell volume, platelet count); Routine urine tests (protein, red blood cell, white blood cell);Blood biochemistry (ALT, AST, TBIL, Cr, BUN, Glu); C-reactive protein and procalcitonin (to receive ICU rapid test results);
9) Immune-related indicators: human leukocyte surface antigen -DR; IL-6, IL-8, IL-10; TNF alpha; PD-1; Expression of PD-1 in T cells; MDSC (myeloid inhibitory cells); T cell subsets (CD4+ and CD8+T cells);
10) Electrocardiogram;
11) Urine pregnancy tests for women of childbearing age;
12) Record drug combinations;
13) Review the inclusion and exclusion criteria of subjects, and screen qualified subjects for inclusion.

6.2 Randomization

The subjects eligible for screening were randomized into groups.

6.3 Visit 1 (1 day after administration)

1) Vital signs (including respiration, blood pressure, heart rate, body temperature);
2) SOFA score;
3) APACHEII score;
4) Mechanical ventilation time;
5) CRRT duration;
6) Routine blood tests (hemoglobin, white blood cell count, red blood cell count, red blood cell

volume, platelet count); Routine urine tests (protein, red blood cell, white blood cell); Blood biochemistry (ALT, AST, TBIL, Cr, BUN, Glu); C-reactive protein and procalcitonin (to receive ICU rapid test results);

7) Immune-related indicators: human leukocyte surface antigen -DR; IL-6, IL-8, IL-10; TNF alpha; PD-1; Expression of PD-1 in T cells; MDSC (myeloid inhibitory cells); T cell subsets (CD4+ and CD8+T cells);
8) Electrocardiogram;
9) Record drug combination and accompanying treatment;
10) And record adverse events.

6.4 Visit 2 (3 days after administration)

1) Vital signs (including breathing, blood pressure, heart rate, body temperature);
2) SOFA score;
3) APACHEII score;
4) Mechanical ventilation time;
5) CRRT duration;
6) Routine blood tests (hemoglobin, white blood cell count, red blood cell count, red blood cell volume, platelet count); Routine urine tests (protein, red blood cell, white blood cell); Blood biochemistry (ALT, AST, TBIL, Cr, BUN, Glu); C-reactive protein and procalcitonin (to receive ICU rapid test results);
7) Immune-related indicators: human leukocyte surface antigen -DR; IL-6, IL-8, IL-10; TNF alpha; PD-1; Expression of PD-1 in T cells; MDSC (myeloid inhibitory cells); T cell subsets (CD4+ and CD8+T cells);
8) Electrocardiogram;
9) Record drug combination and accompanying treatment;
10) And record adverse events.

6.5 Visit 3 (5 days after administration)

1) Vital signs (including breathing, blood pressure, heart rate, body temperature);
2) SOFA score;
3) APACHEII score;

4) Mechanical ventilation time;
5) CRRT duration;
6) Routine blood tests (hemoglobin, white blood cell count, red blood cell count, red blood cell volume, platelet count); Routine urine tests (protein, red blood cell, white blood cell); Blood biochemistry (ALT, AST, TBIL, Cr, BUN, Glu); C-reactive protein and procalcitonin (to receive ICU rapid test results);
7) Immune-related indicators: human leukocyte surface antigen -DR; IL-6, IL-8, IL-10; TNF alpha; PD - 1; Expression of PD-1 in T cells; MDSC (myeloid inhibitory cells); T cell subsets (CD4+ and CD8+T cells);
8) Electrocardiogram;
9) Record drug combination and accompanying treatment;
10) And record adverse events.

6.6 Visit 4 (7 days after administration)

1) Vital signs (including respiration, blood pressure, heart rate, body temperature);
2) SOFA score;
3) APACHEII score;
4) Mechanical ventilation time;
5) CRRT duration;
6) Routine blood tests (hemoglobin, white blood cell count, red blood cell count, red blood cell volume, platelet count); Routine urine tests (protein, red blood cell, white blood cell); Blood biochemistry (ALT, AST, TBIL, Cr, BUN, Glu); C-reactive protein and procalcitonin (to receive ICU rapid test results);
7) Immune-related indicators: human leukocyte surface antigen -DR; IL-6, IL-8, IL-10; TNF alpha; PD-1; Expression of PD-1 in T cells; MDSC (myeloid inhibitory cells); T cell subsets (CD4+ and CD8+T cells);
8) Electrocardiogram;
9) Record drug combination and accompanying treatment;
10) And record adverse events.

6.7 Visit 5 (28 days ±2 days after administration)

1) All-cause mortality events were recorded;
2) Record adverse events;
3) Follow-up was conducted in the outpatient department or by telephone, and the details of follow-up were recorded.

7 Research medications

7.1 Testing drug

Generic name: carrimycin tablets

Trade name: Bite

Specification: 200mg/ tablet

Usage: 400mg/ day, orally, for 7 days

Storage: sealed in a dry place below 25℃

7.2 Control drug

Generic name: Placebo tablets

Specification: 200mg/ tablet

Usage: 400mg/ day, orally, for 7 days

Storage: sealed in a dry place below 25℃

7.3 Drug Management

Before the start of the test, the sponsor shall send the test drug, control drug and drug test report to each research center, go through the registration procedures and distribute to the research department. The test drug shall be transported and stored according to the requirements of the instructions. Each research center should designate special personnel to keep, distribute and register the drug. The outer packaging and tablets of the experimental drug should be properly kept after use, and all unused drugs should be returned to the sponsor at the end of the study together with the outer packaging and tablets.

The outer label of the experimental drug is identical to that of the control drug. The label style is as follows:

National medicine approval number: Drug number:
Carrimycin tablets for clinical study
(For clinical study use only)
[Functional indications] for the treatment of sepsis
[Usage and dosage] 400mg/ day, oral,
[Treatment course] 7 days
[Gauge] 200mg/ tablet
[Packing] Aluminum plastic packing, 8 pieces/box
[Batch number]
[Valid period] 24 months
[Storage] Closed, under 25℃ in a dry place
Provided by Shanghai Tonglian Pharmaceutical Co., Ltd.
Please return all remaining medications and empty cartridges to the drug keeper at the end of treatment

7.4 Accompanying Treatment

Allow the combined use of supportive, symptomatic treatment. During the trial, any drug combination or treatment should be accurately recorded in the case report form, including the name of the drug (treatment method), start and end dates, usage and dosage, etc.

Subjects shall not use any other anti-infective therapy or treatment other than those prescribed in this protocol and shall not use any other investigational drug.

Subjects will receive clinical treatment according to the Chinese Guidelines for Emergency Treatment of Sepsis/Septic Shock (2018)[4]. The cause, time, process and outcome should be recorded in detail.

8. Study end points

8.1 Primary outcomes

Immune-related indicators

Human leukocyte surface antigen -DR, IL-6, IL-8, IL-10, TNF-α, PD-1, T cell PD-1 expression, MDSC (myeloid inhibitory cells), T cell subsets (CD4+ and CD8+T cells)

8.2 Secondary outcomes

1) All-cause mortality (28 days)
2) All-cause mortality (7 days)
3) SOFA score
4) APACHEII Rating
5) Mechanical ventilation time
6) Length of stay in ICU
7) Duration of CRRT

8) C-reactive protein

9) Procalcitonin

8.3 Security indicators

Adverse events, serious adverse events

Adverse Event evaluation form						
Adverse Reactions	score					Discontinuation indicators
	0	1	2	3	4	
nausea	There is no	Able to eat with normal appetite	Significantly decreased appetite, but able to eat	Inability to eat normally (due to nausea)	-	When baseline score was <2, single ADR score ≥ 3 drug withdrawal; When baseline score ≥ 2, the time of drug withdrawal was determined by the researchers.
vomiting	There is no	1 time /24h	2-5 times /24h	6-10 reps /24h	>10 times /24h for gastrointestinal support	When the baseline score was <2, the single ADR score was ≧3 for drug discontinuation; When baseline score ≥ 2, subjects were not recommended to be enrolled because they could not absorb drugs orally.
diarrhea	There is no	Increase the number of stools 2-3 times a day	Increase the number of stools 4 to 6 times a day, or	Increase the number of bowel movements by 7-9	An increase in the number of stools >10 times per	When the baseline score was <2, the single ADR score was ≧3 for drug discontinuation; When the baseline score was ≧2, the number of stool exceeded the baseline 3 times of drug

			overnight stools or moderate abdominal pain	times per day, or fecal incontinence or severe abdominal pain	day, or significant bloody diarrhea or the need for parenteral support treatment	discontinuation.
Alanine amino transferase was elevated	normal	2.5 ≦ N	2.6 5 x N	5.1-20 x N	> 20 x N	When the baseline score was <2, the single ADR score ≥ 3 was discontinued; When the baseline score ≧2, the transaminase exceeded the baseline value plus 2.5 times the sum of the normal upper limit, and the drug was discontinued.
Aspartate amino transferase increased	normal	2.5 ≦ N	2.6 5 x N	5.1-20 x N	> 20 x N	When the baseline score was <2, the single ADR score ≥ 3 was discontinued; When the baseline score ≧2, the transaminase exceeded the baseline value plus 2.5 times the sum of the normal upper limit, and the drug was discontinued.
Total bilirubin (TBIL	normal	1-1.5 x N	1.5 2.5 x N	2.5 3.5 x N	> 5.0 x N	When the baseline score was <2, the single ADR score ≥ 3 was discontinued; When the baseline score ≥ 2, the total bilirubin

) was elevated						exceeded the baseline value plus 1.5 times the sum of the normal upper limit, and the drug was discontinued.
Decrease of white blood cells	≧ 4.0	3.0-3.9	2.0-2.9	1.0-1.9	< 1.0	When baseline score was <2, single ADR score ≥ 3 drug discontinuation; When baseline score ≥ 2, WBC ≤ 1.0 drug withdrawal.
Neutrophils decreased	≧ 2.0	1.5-1.9	1.0-1.4	0.5-0.9	< 0.5	When baseline score was <2, single ADR score ≥ 3 drug discontinuation; When baseline score ≥ 2, neutrophil ≤ 0.5 drug withdrawal.
Indicators of ineffective discontinuation	Since placebo control group was set in this study, it is completely possible to have an invalid situation before the blindness was uncovered, so no invalid drug withdrawal index was designed					

8.4 Adverse Events

An adverse event (AE) is any adverse medical event that occurs to a subject in a clinical trial study after the signing of the informed consent, whether or not it is causally related to the study drug or study procedure. As such, an adverse event includes adverse drug reactions, significant laboratory outliers, and diseases that occur during the study.

The investigator must report all adverse events in an electronic data acquisition system (EDC). All adverse events that occur during the study are recorded in the EDC form. An adverse event is an adverse change that is now different from the patient's baseline (pre-treatment) condition, including a co-morbidities that occurred after the start of the study, whether or not related to treatment.

The causal relationship between an AE and the study drug was classified as definitely related, very likely related, probably related, probably unrelated, and definitely unrelated.

Definitely related: The occurrence of AE and the use of the study drug have a reasonable time sequence. AE is a known adverse reaction of the study drug, which is alleviated or disappeared after withdrawal of the drug, and repeated after re-use of the drug, which cannot be explained by the subject's own disease.

It is likely to be related to: the occurrence of AE and the use of the study drug have a reasonable time sequence, AE is the known adverse reaction of the study drug, and the reduction or disappearance of the reaction after the study drug withdrawal cannot be explained by the subject's own disease, and the effect after re-use is unknown.

May be related: the occurrence of AE and the use of the study drug have a reasonable chronological order, AE is the known or suspected adverse reaction of the study drug, however, there are other factors that may cause the event, such as disease, drug combination, etc.; Unclear effects after drug discontinuation, unclear or lack of conclusive information.

May be irrelevant: There is a reasonable chronological sequence for the occurrence of AE and the use of the study drug, but the event does not fall into the known type of adverse drug reaction and is most likely caused by the subject's disease or other treatment.

Definitely unrelated: there is no reasonable chronological order for the occurrence of AE and the study drug use, if the event occurred before the study drug use; Is not a known adverse drug reaction; Or the AE is caused by other factors, such as the subject's disease, other treatments, or drug combination.

If the evaluation results in a definite, probable, or probable association, it will be considered an adverse event related to the investigational drug, and depending on its severity, it will be further determined as a serious adverse event.

The severity of AE was classified as mild, moderate, or severe according to the following

criteria.

- Mild: A temporary condition that does not interfere with the subject's daily activities.
- Moderate: Significant symptoms that moderately affect the subject's daily activities.
- Severe: it severely affects the subject's daily activities and is unacceptable.

Any adverse events that occur during the trial (such as adverse drug reactions, symptoms and signs requiring urgent treatment, and new illnesses during the trial) should be recorded in the electronic data acquisition System (EDC).The content of the record should include a specific description of the occurrence of the adverse event; Whether the adverse event existed before the trial and, if so, whether it was aggravated during the trial (e.g. in terms of severity and/or frequency of occurrence);The date of the adverse event, its severity, whether appropriate measures were taken, its relationship with the trial medication, and its outcome. Symptoms, signs and abnormalities in laboratory tests should be described in clear, standard and recognized professional medical terms, and vague and ambiguous colloquial language should be avoided.

8.5 Serious adverse events

A Serious Adverse Event (SAE) is a reaction to one of the following adverse events caused by the use of a product: 1) resulting in death;2) life-threatening;3) Carcinogenic, teratogenic and birth defects;4) cause significant or permanent human disability or organ function impairment;5) resulting in hospitalization or prolonged hospitalization (other than hospitalization for medical insurance reimbursement reasons, elective surgery, or hospitalization without the consent of the study physician).

If serious adverse events occur during the study, the unit undertaking the clinical trial shall immediately take necessary measures to protect the safety of the subjects, and report to the local provincial drug regulatory department, the State Food and Drug Administration, the sponsor and the ethics committee of the unit responsible for clinical research within 24 hours, as well as notify each experimental unit. The sponsor will ensure that all legal and regulatory requirements for reporting procedures are met.

8.6 Treatment of research-related injuries when they occur

In the event of any discomfort, new changes in the subject's condition, or any unexpected situation, whether drug-related or not, the subject shall be notified promptly to the study physician, who will make judgment and medical treatment. If minor adverse reactions occur during the study period, which will not affect the continuation of the clinical study, the subjects are expected to cooperate with the researchers to complete the clinical study.

Treatment plan after adverse reactions:

1. Mild nausea does not need to be treated, and can recover by itself after drug withdrawal; If the subject has severe nausea and vomiting and cannot tolerate it, the drug should be stopped and anti-vomiting symptomatic treatment should be carried out.

2. If the level of transaminase does not exceed the baseline value plus 2.5 times the normal upper limit, it can not be treated, and can recover to normal a few days after the end of medication; If the transaminase exceeds the baseline value and 2.5 times the sum of the normal upper limit, drug withdrawal, liver protection, detoxification and symptomatic treatment (such as compound vitamin B, reduced glutathione, ammonium glycyrrhizate preparation, acetyl cysteine, etc.) can be performed.

3. If the increase of total bilirubin does not exceed the sum of the baseline value plus 1.5 times the normal upper limit, it can not be treated, and it can return to normal a few days after the end of medication;If the increase of transaminase exceeds the baseline value plus 1.5 times the sum of the normal upper limit, drug withdrawal, liver protection, detoxification and symptomatic treatment (such as compound vitamin B, reduced glutathione, ammonium glycyrrhizate preparation, acetyl cysteine, gluconolactone, etc.) can be performed.

The study physician and sponsor will make every effort to prevent and treat any harm that may arise as a result of this study. The Sponsor has purchased clinical trial liability insurance for this clinical study. If an adverse event occurs during the clinical study, it will be determined whether it is related to the study drug.The Sponsor will provide for the cost of treatment and corresponding financial compensation and compensation for research-related damage.

9. Data management

The electronic Data management system was used for data collection in this experiment. The

database was established by the data department of the data management and statistical analysis unit. The database should be managed by system login, data entry, modification, deletion and other data traces. The establishment of the database should adopt the Clinical Data Exchange Standard Association (CDISC) standard as far as possible.

9.1 Data entry and modification

Each user will be given an independent user name and password, and the Research Center will assign personnel to input the original data into the eCRF through the secure network. The system will automatically check the data deviation in the eCRF and generate corresponding prompts, allowing the Research Center personnel to modify the input data.Before the database is locked, the researcher will use an electronic signature to confirm the data and ensure the accuracy of the data recording.

9.2 Verification of raw data

The inspector confirms that all data in the eCRF is consistent with the original record.If there are any errors or omissions, a challenge will be sent to the Research Center staff in the form of an electronic challenge sheet requesting clarification or modification of the data in the eCRF.

9.3 Data Verification

The data management unit reviews the data recorded in the eCRF in accordance with the standard operating procedures (procedural and manual verification).For questions arising in the data, the data management unit will issue questions to the research center personnel in the form of an electronic challenge sheet, and ask the personnel designated by the research Center to reply to the questions and make some necessary modifications to the data.All data changes and related operations need to be recorded with a trace.

9.4 Database Locking

The data manager writes the blind state data review report according to the test scheme, data review standards and database.The clinical project manager will organize sponsors, key researchers, statisticians and data managers to attend the blind state data review meeting to review the data, and the review resolution will be signed by the representatives of the participants.After the parties have approved the data locking, the data manager will organize the data locking according to the relevant SOP.Submit the locked data to the statistician for statistical analysis.

10 Statistical considerations

10.1 Sample size determination

This study was a preliminary test for the clinical effect evaluation study on the treatment of sepsis by carrimycin, which was used to preliminatively evaluate the effects of carrimycin tablets on immunomodulatory function, mortality, inflammatory indicators, and organ function, so as to provide the research data basis for the later study.

In this study, the sample size was set as 120 cases, including 60 cases in the test (carrimycin) group and 60 cases in the control group.

10.2 Randomization method

Patients were enrolled in this study using a competitive enrollment approach.SAS9.4 (or later) statistical software was used to randomize groups (stratified by center), and a random number table was generated in a 1:1 ratio between the experimental group and the control group, and drug coding and IRT systems were configured.

10.3 Analyze the population statistically

The following analytical populations will be defined in this study for statistical analysis:

Full analysis population (FAS): included all patients who were randomly enrolled, received at least one study medication at baseline, and evaluated for efficacy at least once;

Protocol eligible population (PPS, active cases): a part of the FAS set, including cases in the FAS set with no major regimen violations, effective baseline values, and good medication compliance;

Safety population (SS): includes all patients who received at least one study medication and had at least one post-baseline safety evaluation.

The main effective indicators will be analyzed in the FAS and PPS populations respectively. If the two conclusions are inconsistent, the conclusion of FAS analysis will be the final conclusion. Safety indicators will be analyzed in the SS population.

10.4 Statistical analysis software and general methods

Perform statistical analysis with SAS9.4 or later.

Unless otherwise noted, all statistical inferences in this study were performed using a two-side test with a test level of 0.05 and the estimation of parameters was performed using a 95% confidence

interval method.

10.5 Statistical description

The main indicators collected in this study are described statistically. Quantitative indicators were described by means, standard deviation, median, range and other indicators. Qualitative indicators are described by frequency, percentage and other indicators.

10.6 Outcome analysis

The primary outcome indicators were immune-related indicators (human leukocyte surface antigen -DR, IL-6, IL-8, IL-10, TNF-α, PD-1, T cell PD-1 expression, MDSC (myeloid inhibitory cells), T cell subsets (CD4+ and CD8+T cells)). Secondary outcome measures included all-cause mortality (28 days), all-cause mortality in both groups (7 days), SOFA score, APACHEII score, duration of mechanical ventilation, duration of ICU stay, duration of CRRT, C-reactive protein, and procalcitonin index.

Among the primary and secondary outcome indicators, the quantitative and approximately normal distribution indicators were used to analyze the difference between the two groups. For quantitative indicators that did not obey the normal distribution, the rank sum test was used to compare the two groups. And categorical variables (including ordered categorical variables), which were analyzed using chi-square test or rank-sum test according to the research hypothesis.

10.7 Security analysis

Summarize all reported adverse events, adverse reactions, significant adverse events, and serious adverse events during the study. For all adverse events, the incidence of adverse events was calculated based on the number of subjects in which adverse events occurred and compared between groups. If multiple adverse events occur in the same subject, they will be calculated as 1 case. In addition, adverse events occurring after administration that may be (definitely, probably, possibly, possibly, possibly not) related to the study medication were analyzed as a subset of adverse events.

Describe the distribution of the severity of adverse events.

Provide a list of cases in which adverse events have occurred.

11. Ethics

11.1 Subject information and informed consent

Informed consent to participate in the study will be obtained from subjects or their families who will be provided with information related to the study purpose and possible consequences. A copy of the signed informed consent will be given to the subject or the subject's family for retention.

11.2 Confidentiality of the subject's privacy

The subject's personal information will be treated as confidential. All subject data obtained from the subject's case will be kept as confidential. Subjects will be identified by their initials and the number provided at the time of study selection. Subjects will be made aware of the anonymity of this data and their rights to privacy. However, they must also be aware of the fact that the data will be submitted to the subject authority or government regulatory body, and may also be submitted to an agency such as the Ministry of Health for inspection and evaluation. The participating physician will keep a list of subjects' personal data (subject information and corresponding subject name) for confirmation of records.

11.3 Risks and benefits

The drugs observed in this study have been conducted in multiple clinical studies at home and abroad, and their clinical efficacy and safety have been verified. However, all therapeutic drugs are likely to cause side effects, so this study still carries certain risks. If a serious adverse event occurs during the study, it will be handled in accordance with the national regulations on the handling of medical malpractice.

The study has a doctor in charge of the study to guide and record the drug use of the subjects, which may lead to the optimal treatment of the subjects.

By participating in the study, subjects will receive several free investigational medications and investigation-related laboratory tests.

By participating in this study, it is possible to help future patients, that is, the information obtained from the subject's participation in this study can benefit other patients with the same disease in the future.

12. Research management

12.1 List of research centers

Center Numbers	The hospital	PI
1	The First Affiliated Hospital of Harbin Medical University	Kaijiang Yu
2	Tianjin Cancer Hospital	Donghao Wang
3	Affiliated Cancer Hospital of Harbin Medical University	Changsong Wang
4	Henan Cancer Hospital	Dongmin Zhou
5	Sun Yat-Sen University Cancer Prevention Center	Gang Ma
6	Affiliated Cancer Hospital of Guangzhou Medical University	Lewu Xian
7	Hubei Cancer Hospital	Li Zhang
8	Hunan Cancer Hospital	Jianghong Zhao
9	Sichuan Cancer Hospital	Song Lu
10	Zhejiang Cancer Hospital	Kaizhong Liu
11	Hangzhou Cancer Hospital	Ming Zhang
12	Shandong Cancer Hospital	Zhijun Guo
13	The Fourth Hospital of Hebei Medical University	Zhenjie Hu
14	Chongqing Cancer Hospital	Zhengying Jiang
15	Peking University Cancer Hospital	Hongzhi Wang
16	Cancer Hospital, Chinese Academy of Medical Sciences	Xuezhong Xing
17	Affiliated Cancer Hospital of Fudan University	Biao Zhu

12.2 The sponsor

Sponsor: Tumor Intensive Care Medicine Committee of the Chinese Anti-Cancer Association

Director: Kaijiang Yu

Address: No.23, Youzheng Street, Nangang District, Harbin

Tel: 021-37682940 Fax: 021-57741390 Postcode: 150001

12.3 CRO

Guangzhou Yushi Medical Technology Co., Ltd.: Preparation before project launch

Krypton Technology (Beijing) Co., Ltd.: Quality control

12.4 Use of research results

All information related to the research that is not published is confidential, and the full ownership of the subject leader of the group leader's unit must be retained. The participating physician will agree to use this information only as necessary to carry out the study and not for any other purpose except with the written consent of the project leader.

The project leader will be responsible for reviewing and confirming the final report of this study.

13. Research schedule

September 2019: program discussion and finalization; Center selection

November 2019: Center ethics approval and clinical study started

Dec. 2019 -- Jun. 2020: Enrollment, follow-up, supervision and quality control

May 2020: Interim analysis

August 2020: Final data analysis and review

14. Responsibilities of parties and other relevant provisions

The sponsor, the researcher, the research responsible unit and the research participating unit shall undertake corresponding responsibilities in accordance with the provisions of the GCP and this program.

Article publication regulations: After the end of the trial, the responsible unit of clinical research has the right to publish the summary report of the clinical research in the form of a paper, and the researchers of each research unit enjoy the right of authorship of the paper. Each research unit may publish its own test report.

15. References

[1] Perner A, Cecconi M, Cronhjort M, et al. Experts tatement for the management of hypovolemia in sepsis[J]. Intensive Care Medicine, 2018, 44(6): 791-798.

[2] Prescott HC, Angus DC. Post sepsis Morbidity[J]. JAMA, 2018, 319(1):91.

[1] Reinhart K, Daniels R, Kissoon N, et al. Recognizing Sepsis as a Global Health Priority-A WHO Resolution[J]. NEngl J Med, 2017, 377(5):414-417.

[2] Chinese Guidelines for Emergency Treatment of Sepsis/Septic Shock (2018).

Annex 1

Investigator Signature Page

Study Title: Evaluating the effect of carrimycin tablets on biomarkers of inflammation and immune function in patients with tumor sepsis: a multicenter randomized double-blind controlled clinical study

I have read and understood the trial protocol (Protocol No.: KLMS-2019001; Version number: V2.0; Version date: December 02, 2019). I will conscientiously fulfill my duties as a researcher according to GCP regulations. This research will be conducted in accordance with the moral, ethical and scientific principles stipulated in the Declaration of Helsinki and the Chinese GCP.I agree to conduct this clinical study in accordance with the protocol design and regulations.

I will be responsible for making clinically relevant medical decisions to ensure that subjects receive timely and appropriate treatment if adverse events occur during the study. I am aware of the requirements for proper reporting of serious adverse events and I will record and report such events as required.

I guarantee that the data will be loaded into the case report form truthfully, accurately, completely, in a timely and lawful manner. I will accept the inspectors or inspectors dispatched by the sponsor and the drug supervision and administration department to ensure the quality of clinical research.

I will provide a curriculum vitae to the Ethics Committee and possibly the drug administration for review before the study begins.

I have read this protocol and agree to carry out this study according to the protocol.

Investigator Signature: Date:
Block Name:
Research Center name/Address:

Annex 2

Clinical Study Flow Chart

Time window / Observation items	Baseline and time after medication (days)					
	Baseline (-7 -- 0d)	**1**	**3**	**5**	**7**	**28 (±2)**
Vital signs	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
SOFA Scoring	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
APACHEII rating	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Mechanical ventilation time	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
CRRT duration		**Square root**	**Square root**	**Square root**	**Square root**	
Length of stay in ICU	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Blood routine tests	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Routine urine tests	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Blood Biochemistry	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
C-reactive protein	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Procalcitonin	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Human Leukocyte Surface Antigen -DR	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
IL-6, IL-8, IL-10	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
TNF alpha	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
PD-1	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
T cell PD-1 expression	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
MDSC	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	

T cell subsets	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Electrocardiogram	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	
Drug delivery	**Square root**					
Record drug combinations	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**
Record adverse events	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**	**Square root**

Note: ● Vital signs include: breathing, blood pressure, heart rate, temperature

● Routine blood tests include: WBC, RBC, Hb, HCT, PLT

● Urine routine tests include: WBC, RBC, Pro

● Blood biochemical tests include: ALT, AST, TBIL, Cr, BUN, Glu

● Administration: Random administration on day 0, followed by oral administration of either clarithromycin or placebo for 7 consecutive days.