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Efficacy of allogeneic hematopoietic stem cell transplantation with cocktail conditioning regimen for the treatment of pediatric patients with chronic active Epstein-Barr virus: A retrospective observational study

Shifen Fan[1,*], Jiao Chen[1,*], Zhouyang Liu[1], Juan Xiao[1], Fan Jiang[1], Xiaomei Liu[1], Yuan Sun[1]

[1]Department of Hematology, Beijing Jingdu Children's Hospital, Beijing 102208, China

* These authors contributed equally to this work.

Running title: CCR-allo-HSCT for pediatric patients with CAEBV

Corresponding author:

Yuan Sun, PhD, Professor,

Department of Hematology, Beijing Jingdu Children's Hospital

No. 308, Huilongguan East Street, Changping District, Beijing 102208, China

Tel:+86 10 69787777

Fax: +86 10 69787777

Email: sy@jdetyy.com

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Abstract

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was considered as an only therapeutic strategy for chronic active Epstein-Barr virus (CAEBV) infection with few exceptions, while efficacy of various allo-HSCT conditioning regimens for CAEBV has not been fully investigated yet. This study aimed to compare the effectiveness of cocktail conditioning regimen (CCR)-allo-HSCT with reduced-intensity conditioning regimen (RICR)-allo-HSCT for pediatric patients with CAEBV. Data of a total of 54 children with CAEBV from July 2015 to December 2020, were retrospectively analyzed. Among them, 32 patients received VP16, total body irradiation (TBI), busulfan, fludarabine, cyclophosphamide, and anti-thymocyte globulin (ATG) (CCR1 group), 10 patients received VP16, ara-C, TBI, busulfan, fludarabine, cyclophosphamide, and ATG (CCR2 group), and the remaining 12 patients received VP16, busulfan or melphalan, fludarabine, and ATG with or without ara-C (RICR group). The overall survival (OS), hematopoietic engraftment, the incidence of severe graft-versus-host disease (GVHD), and other parameters were analyzed. After adjusting for potential confounders, CCR1 (hazard ratio (HR): 0.023; 95% confidence interval (CI): 0.001-0.448; *P*<0.02) and CCR2 (HR: 0.028; 95%CI: 0.002-0.457; *P*<0.02) were associated with a longer OS than RICR. The use of CCR could markedly improve the engraftment success rate and OS rate compared with RICR for pediatric patients with CAEBV.

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Introduction

Epstein–Barr virus (EBV) belongs to the gamma–herpesvirus family that consists of double-stranded DNA viruses, which is ubiquitously present and carried by more than 90% of adults worldwide [1]. EBV infection mainly occurs in childhood and is always asymptomatic, while the prevalence of infectious mononucleosis and self-limiting lymphoproliferative disease (LPD) ranges from 25-74% if the EBV infection occurs in adolescents or young adults [2]. Moreover, EBV is associated with a variety of malignancies and LPDs, including Burkitt lymphoma, Hodgkin lymphoma, nasopharyngeal carcinoma, gastric carcinoma, and immunodeficiency-associated lymphoproliferative disorders [1]. Furthermore, persistent EBV infection could cause the progression of B-cell LPD and T-cell/natural killer cell (T/NK cell) LPD.

Chronic active EBV (CAEBV) infection is a prototype of the EBV-positive T/NK-LPD, which was originally proposed as a disorder of sustained inflammation, similar to infectious mononucleosis [3,4]. At present, the prevalence of CAEBV in Asia, South and Central America and Mexico is higher than that in other regions [5]. The diagnosis of CAEBV is based on clinical manifestations and EBV in tissues or peripheral blood samples, and the two characteristics of CAEBV include systemic inflammation and neoplastic disease. Moreover, the main clinical manifestations of systemic inflammation include fever, lymphadenopathy, liver dysfunction, hepatosplenomegaly, and an abnormal complete blood count (CBC) and erythrocyte sedimentation rate [6]. The prognosis of CAEBV is poor, and the majority of patients will die within 15 years after the onset of the disease, mainly of hepatic failure, cardiac failure, hemophagocytic syndrome, malignant lymphoma, opportunistic infections, or bleeding [7,8].

At present, allogeneic hematopoietic stem cell transplantation (allo-HSCT) is considered as an effective treatment to eradicate EBV-infected T or NK cells. However, allo-HSCT was not highly advised for all patients, especially for those in a generally poor condition [9-11]. Moreover, a significant difference was detected in CAEBV between patients undergoing allo-HSCT compared with those receiving different conditioning regimens. Therefore, clarifying the optimal conditioning regimen for patients with CAEBV undergoing allo-HSCT is of great significance. Therefore, the present study aimed to compare the therapeutic outcomes of cocktail conditioning regimen (CCR)-allo-HSCT (CRST) and reduced-intensity

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conditioning regimen (RICR)-allo-HSCT to identify the optimal conditioning regimen for pediatric patient with CAEBV.

Methods

Patients

A total of 54 pediatric patients with CAEBV were admitted to Beijing JingDu Children's Hospital from July 2015 to December 2020. The eligible patients were selected according to the following criteria [12,13]: (1) persistent or recurrent infectious mononucleosis-like symptoms (e.g., fever, liver dysfunction, lymphadenopathy, hepatosplenomegaly, hydroa vacciniforme, and hypersensitivity to mosquito bites) with the disease course of more than 3 months; (2) EBV antibodies (EBV-CA and EBV-EA) were detected in tissues or peripheral blood samples, EBV-encoded small RNAs in tissues, or EBV-DNA in plasma and $>10^{2.5}$ copies/ml in the whole blood; and (3) clinical manifestations could not be explained by other known diseases. Furthermore, the diagnosis of hemophagocytic lymphohistiocytosis (HLH) was undertaken based on the HLH-04 criteria proposed by the International Histiocyte Society [14]. Informed consent was obtained from study subjects' parents, and the study protocol was approved by the Ethics Committee of Beijing Jingdu Children's Hospital (China, Approval No. 2020019).

Data collection

The eligible patients' data were retrospectively collected, including their mean age at the time of undergoing allo-HSCT, gender, clinical manifestations, laboratory findings, time from the onset of the disease to the date of undergoing HSCT, graft selection, conditioning regimens, recovery after HSCT, graft-versus-host disease (GVHD), chimera state, and survival outcomes.

Disease status

Disease status before allo-HSCT was assessed based on the clinical manifestations, and it was consequently classified as either active or inactive. Active disease was defined as the presence of fever, hepatitis, lymphadenopathy, hepatosplenomegaly, and/or progressive skin lesions.

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Conditioning regimens

The cocktail conditioning regimen 1 (CCR1) consisted of VP16, total body irradiation (TBI), busulfan, fludarabine, cyclophosphamide, and anti-thymocyte globulin (ATG); besides, CCR2 involved VP16, ara-C, TBI, busulfan, fludarabine, cyclophosphamide, and ATG; RICR included VP16, busulfan or melphalan, fludarabine, and ATG with or without ara-C. The details of conditioning regimens are presented in Table 1.

Origin of grafts

The patients received matched sibling donor-HSCT (MSD-HSCT) and matched unrelated donor- HSCT (MUD-HSCT) based on the human leukocyte antigen (HLA) class (e.g., HLA-A, -B, -C, DR, and –DQ). Granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells were collected as the source of stem cells from both unrelated donors and matched siblings. G-CSF-mobilized bone marrow and peripheral blood stem cells were considered as the sources of stem cells in all haplo-identical donors.

GVHD and infection prophylaxis

Cyclosporine and methylprednisolone were applied to prevent the occurrence of acute GVHD. Moreover, patients who underwent haplo-HSCT also received mycophenolate mofetil (MMF) and basiliximab, and MMF and methotrexate were used for patients with GVHD who underwent MUD-HSCT. All patients were kept in single laminar airflow rooms until the success of neutrophil engraftment. Besides, they received ganciclovir from the first day of conditioning until day −1 and acyclovir from day 0 for cytomegalovirus and herpes simplex virus (HSV) prophylaxis. Voriconazole or caspofungin were administered for antifungal prophylaxis, depending on preexisting fungal infections and organ dysfunction. Cotrimoxazole was prescribed for pneumocystis carinii pneumonia when caspofungin was not used. Ceftazidime was given for prophylaxis. All patients received immunoglobulin intravenously.

Engraftment and chimerism

Neutrophil engraftment was defined as neutrophil count exceeded 0.5×10^9 cells/L for 3 consecutive days. Platelet engraftment was considered if platelet count exceeded 20×10^9 cells/L for 7 consecutive days without platelet infusion. Chimerism analyses were performed using DNA isolated from peripheral blood samples collected on the time of neutrophil engraftment on 100 days, 6 months, and 1 year post-transplantation.

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Conventional polymerase chain reaction (PCR) was used for short tandem repeat (STR) typing, and the PCR products, consisting of amplified STR loci (amplicons), were electrophoresed with a DNA analysis device. Complete donor chimerism (CDC) was defined as the presence of more than 95% donor-derived cells in the whole blood, while mixed donor chimerism (MDC) was defined as the presence of more than 5% and less than 95% host-derived cells.

Statistical analysis

The data were presented as median (range) and frequency (proportion) for continuous and categorical variables, respectively. The one-way analysis of variance (ANOVA) was applied to compare differences between each pair of groups for continuous variables, while the Chi-square test was employed to compare differences between each pair of groups for categorical variables. The overall survival (OS) rate was assessed using the log-rank test, and the Cox proportional-hazards model was utilized to calculate hazard ratio (HR) with 95% confidence interval (CI) to compare the CCR with RICR on the survival outcome. All the tests were two-sided, and the level of significance was set at 0.050. The statistical analyses were conducted using the SPSS 24.0 software (IBM, Armonk, NY, USA).

Results

Patients' demographic and clinical characteristics at baseline

Of 54 patients, 32, 10, and 12 patients were in CCR1, CCR2, and RICR groups, respectively. Patients' median age at the time of undergoing allo-HSCT was 8.5 years old, and the median follow-up time after allo-HSCT was 9.25 months. A total of 16 patients died during the follow-up. Patients' demographic and clinical characteristics at baseline in CCR1, CCR2, and RICR groups are shown in Table 2. There were no significant differences among the three groups in terms of median age (P=0.21), gender (P=0.40), limited to liver, spleen, lymph nodes, or skin (P=0.08), and inactive disease at the time of undergoing allo-HSCT (P=0.15), while significant differences were found among the groups in terms of the incidences of hemophagocytic syndrome (P<0.02) and EBV-DNA in plasma (>500copies/ml) at the time of undergoing allo-HSCT (P<0.05).

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The characteristics of graft

The characteristics of graft are summarized in Table 3. A total of 35 patients underwent haplo-HSCT, 9 underwent MSD-HSCT, and the remaining 10 underwent MUD-HSCT. There were no significant differences among the groups in terms of haplo-HSCT (*P*=0.73), MSD-HSCT (*P*=0.79), and MUD-HSCT (*P*=0.43). Moreover, no significant differences were identified among the groups for G-CSF-mobilized peripheral blood mononuclear cells (*P*=0.333) and CD34+ cell dose (*P*=0.95).

Engraftment, GVHD, and chimerism

The details of engraftment, GVHD, and chimerism for the three groups are presented in Table 4. The success rate of engraftment in 54 patients was 90.7% (49/54), and the engraftment success rate in CCR1, CCR2, and RICR groups was 100.0%, 90.0%, and 66.7%, respectively. We noted that the engraftment success rate in CCR1 and CCR2 groups was higher than that in RICR group (*P*<0.005). There were no significant differences among the three groups regarding the number of days for neutrophil engraftment (*P*=0.28) and platelet engraftment (*P*=0.10). Furthermore, no significant differences were detected among the groups in terms of the incidence of acute GVHD (*P*=0.78) and chronic GVHD (*P*=0.58). Finally, there were no significant differences among the groups regarding the incidence of chimerism in cases who underwent allo-HSCT on 100 days, 6 months, and 1 year post-transplantation (*P*>0.050).

OS

During 9.25 months of follow-up, 6, 3, and 7 patients died in the CCR1, CCR2, and RICR groups. The results of the log-rank test revealed that there were significant differences among the three groups in terms of the OS (P<0.05; Figure 1). The univariate Cox proportional-hazards model indicated that CCR1 was associated with a longer OS than RICR (HR: 0.247; 95%CI: 0.083-0.736; *P*<0.02), while CCR2 was not associated with a remarkable OS compared with RICR (HR: 0.412; 95%CI: 0.106-1.599; *P*=0.20). After adjusting for potential confounders, we noted that CCR1 (HR: 0.023; 95%CI: 0.001-0.448; *P*<0.02) and CCR2 (HR: 0.028; 95%CI: 0.002-0.457; *P*<0.02) were associated with a longer OS compared with RICR.

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Discussion

The clinical manifestations of CAEBV involve all tissues and organs, and numerous clinicians are not aware of the disease, which caused misdiagnosis and poor prognosis. Without radical treatment, patients with CAEBV may encounter high mortality due to life-threatening complications, including uncontrollable HLH, followed by distributive shock or multi-organ failure, inflammatory cell infiltration resulting in organ failure, or disease progression [7,15,16]. Although allo-HSCT is the radical treatment strategy for CAEBV, a standard treatment approach has not been presented yet. In the present, 54 patients with diverse clinical characteristics received allo-HSCT and the outcomes of the patients preconditioned with CCR with RICR were compared. It was found that the use of CCR allo-HSCT was associated with a higher incidence of engraftment success rate and a prolonged overall survival compared with RICR allo-HSCT for pediatric patients with CAEBV.

Several studies have concentrated on the therapeutic influences of allo-HSCT on patients with CAEBV. Sawada et al. retrospectively analyzed 17 patients who underwent RICR and allo-HSCT, and the overall survival rates for RICR plus bone marrow transplantation and RICR followed by cord blood transplantation were 92.9% and 93.3%, respectively [17]. In another study, 5 patients with CAEBV were treated with RICR followed by allo-HSCT, and all patients were alive without serious toxicities within the follow-up of 16 months [18]. Nakagawa et al. reported a 56-year-old woman with CAEBV who was treated with RICR, followed by stem cell transplantation using cryopreserved cord blood with a poor prognosis, and the use of EBV-seronegative CB cells was effective and well tolerated [19]. In a recently conducted research, 29 patients with CAEBV were treated with myeloablative conditioning or RICR allo-HSCT, and it was revealed that RICR followed by allo-HSCT could be a promising therapeutic approach for CAEBV [10]. However, the above-mentioned studies concentrated on the treatment efficacy of RICR followed by allo-HSCT, and no new conditioning regimen was reported.

The common treatment strategies for CAEBV patients include immunotherapy, multi-drug chemotherapy, and allo-HSCT [20]. In the current study, all the patients received immunotherapy prior to allo-HSCT to ensure that the disease retained inactive. However, the use of multi-drug chemotherapy for CAEBV has still remained controversial [21]. Therefore, multi-drug chemotherapy was used for patients with active disease after

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immunotherapy, while allo-HSCT was directly given after immunotherapy for patients with inactive disease. Allo-HSCT was used to eradicate residual infected cells and to reconstitute normal immunity. The OS rate of RICR, consisting of busulfan/melphalan, fludarabine and ATG, was only 41.7%, which was lower than that reported previously [10,22,23], which may be related to the lack of the standard treatment guidelines. These patients presented longer duration from the disease onset to allo-HSCT, the risk of organ failure was elevated, and the tolerance of conditioning regimen was reduced.

The present study indicated that the use of CCR could improve engraftment rate and OS rate compared with RICR. Moreover, the OS rate still remained remarkable when CCR was used without ara-C. These results suggested that CCR was superior to RICR for patients with CAEBV. The potential reason for this conclusion could be related to the facts that the CCR with a lower dose could reduce the toxicity, increase the tolerance, eliminate the cells infected with EBV, and improve engraftment rate. Moreover, the proportion of major complications, such as hemagophagocytic syndrome and EBV-DNA in plasma >500copies/ml at the time of undergoing allo-HSCT, in the RICR group was higher than that in the CCR-based groups, which could be related to engraftment rate and survival outcomes. Finally, 35 of 54 patients were treated with haplo-HSCT, and the results indicated that the use of haplo-HSCT could be regarded as an alternative option for patients with CAEBV, in case of the lack of matched donors.

Several shortcomings of the present study should be acknowledged: (1) due to the retrospective observational nature of the study, the bias in participants' selection, recall, and confounders was inevitable; (2) the duration of CAEBV was not considered, and the severity of the disease might differ among CCR1, CCR2, and RICR groups, which might affect the prognosis of CAEBV; (3) the sample size in different groups was not balanced, and only a smaller number of patients were included, which caused the conclusion of this study to be relatively unreliable; and (4) no stratified analysis according to patients' characteristics was conducted owing to the small sample size.

Conclusion

our findings revealed that CCR could markedly increase the engraftment success rate and prolong overall survival compared with RICR for pediatric patients with CAEBV. Moreover,

the incidence rates of acute and chronic GVHD among the three groups were similar. However, further large-scale prospective study should be performed to verify the findings of this study and to explore whether the treatment efficacy of CCR differs in patients with specific clinical characteristics.

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The authors declared that they have no conflict of interest.

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None.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Table 1. Conditioning regimens for CAEBV[a]

CCR1[b]	VP16 600mg/m^2 (day-10 to -14)
(n=32)	TBI[c] 6GY[d] divided into 3 times (day-9 to -7)
	busulfan 3.2mg-4.8mg/kg (day-6)
	fludarabine 90mg/m2 (day-5 to -3)
	cyclophosphamide 20mg/kg (day-5 to -4)
	ATG[e] 7.5-8.6mg/kg when the donor is not MSD[f] (day-5 to -2), 2.5mg/kg when the donor is MSD (day-4 to -3)
	plasmapheresis twice (after the first TBI and ATG)
CCR2[g]	VP16 600mg/m^2 (day-10 to -14)
(n=10)	ara-C 6g/m2 (day-10 to -14)
	TBI 6GY divided into 3 times (day-9 to -7)
	busulfan 3.2mg-4.8mg/kg (day-6)
	fludarabine 90mg/m2 (day-5 to -3)
	cyclophosphamide 20mg/kg (day-5 to -4)
	ATG 7.5-8.6mg/kg when the donor is not MSD (day-5 to -2), 2.5mg/kg when the donor is MSD (day-4 to -3)
	plasmapheresis twice (after the first TBI and ATG)
RICR[h]	VP16 600mg/m^2 (day-9 to -13)
(n=12)	with/without ara-C 6g/m2 (day-9 to -13)
	busulfan 9.6mg-14.4mg/kg or melphalan 180mg/m2 (day-6 to -8)

	fludarabine 120~160mg/m2 (day-5 to -2)
	ATG 7.5-8.6mg/kg when the donor is not MSD (day-5 to -2), 2.5mg/kg when the donor is MSD (day-4 to -3)
	plasmapheresis twice (after the first TBI and ATG)

Footnotes: a, chronic active Epstein-Barr virus; b, cocktail conditioning regimen 1; c, total body irradiation; d, total body irradiation; e, anti-thymocyte globulin; f, matched sibling donor; g, cocktail conditioning regimen 2; h, reduced-intensity conditioning regimen.

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Table 2. Baseline characteristics of CAEBV[a] patients before undergoing allo-HSCT[b]

	CCR1[c] (n=32)	CCR2[d] (n=10)	RICR[e] (n=12)	*P* value
Median age at HSCT[f], y (range)	10 (2.1–17.3)	7.8 (3.5–15.1)	7.8 (1.7–11.9)	0.211
Sex (male/female)	14/18	5/5	8/4	0.400
Limited to liver, spleen, lymph nodes, skin	10 (31.3%)	7 (70%)	4 (33.3%)	0.082
Complicated with hemagophagocytic syndrome	9 (28.1%)	1 (10%)	8 (66.7%)	0.012
Non-active disease at HSCT	6 (18.8%)	3 (30%)	0 (0%)	0.151
EBV[g]-DNA in plasma >500 copies/ml at HSCT	6 (18.8%)	2 (20%)	7 (58.6%)	0.028

Footnotes: a, chronic active Epstein-Barr virus; b, allogeneic hematopoietic stem cell transplantation; c, cocktail conditioning regimen 1; d, cocktail conditioning regimen 2; e, reduced-intensity conditioning regimen; f, hematopoietic stem cell transplantation; g, Epstein-Barr virus.

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Table 3. The characteristics of graft

	CCR1[a](n=32)	CCR2[b] (n=10)	RICR[c] (n=12)	*P* value
Graft relation to patient				
Haplo	20 (62.5%)	6 (60%)	9 (75%)	0.732
Matched sibling	6 (18.8%)	1 (10%)	2 (16.7%)	0.790
Unrelated	6 (18.8%)	3 (30%)	1 (8.3%)	0.427
Graft source				
Bone marrow+Peripheral blood	20 (62.5%)	6 (60%)	9 (75%)	0.732
Peripheral blood	12 (37.5%)	4 (40%)	3 (25%)	0.512
Graft cell dose				
Median mononuclear cell dose 10^8/kg(range)	8.8 (4.2–10.9)	8.6 (6.3–15.1)	9.2 (5.7–15.5)	0.333
Median CD34+ cell dose 10^6/kg(range)	5.5 (1.8–6.2)	5.9 (2.7–7.0)	4.8 (3.9–5.5)	0.946

Footnotes: a, cocktail conditioning regimen 1; b, cocktail conditioning regimen 2; c, reduced-intensity conditioning regimen.

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Table 4. The details of engraftment, GVHD[a], and chimerism for the three groups

	CCR1[b] (n=32)	CRC2[c] (n=10)	RICR[d] (n=12)	*P* value
Engraftment				
Engraftment success rate (%)	32 (100.0%)	9 (90.0%)	8 (66.7%)	0.003
Number of days for neutrophil engraftment	11.5 (8–21)	11.0 (9–15)	12.5 (9–20)	0.276
Number of days for platelet engraftment	16.0 (7–134)	14.0 (7–133)	39.0 (10–120)	0.095
GVHD				
Acute GVHD	7 (21.9%)	2 (22.2%)	4 (50.0%)	0.783
Chronic GVHD	13 (40.6%)	3 (33.3%)	3 (37.5%)	0.583
Chimerism rate				
At allo-HSCT[e]	25/32 (78.1%)	8/8 (100.0%)	7/8 (87.5%)	0.313
100 days	25/28 (89.3%)	7/7 (100.0%)	6/6 (100.0%)	0.472
6 months	18/18 (100.0%)	7/7 (100.0%)	5/5 (100.0%)	-
1 year	16/16 (100.0%)	7/7 (100.0%)	3/3 (100.0%)	-

Footnotes: a, graft-versus-host disease; b, cocktail conditioning regimen 1; c, cocktail conditioning regimen 2; d, reduced-intensity conditioning regimen; e, allogeneic hematopoietic stem cell transplantation.

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Figure legends:

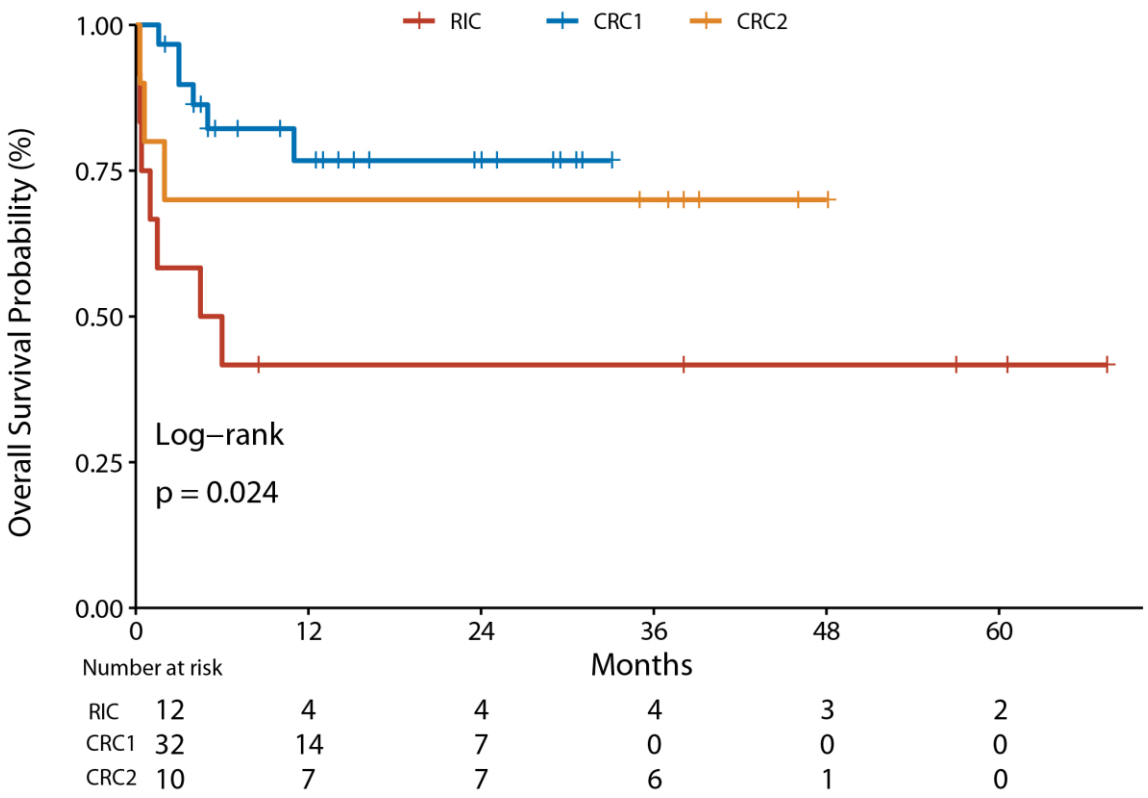

Figure 1. The overall survival rate in CCR1, CCR2, and RICR group