A combination of Granulocyte-macrophage colony-stimulating factor with the R2 regimen in elderly high-risk B cell lymphoma maintenance therapy improves survival by modulating natural killer cells

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Research Article

Keywords: maintenance therapy, B-cell lymphoma, lenalidomide, rituximab, rhGM-CSF

Posted Date: May 4th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1524082/v2

Abstract

Background

The clinical outcome of B-cell lymphoma has improved dramatically due to hematopoietic stem cell transplantation (HSCT) and biological agents. However, treatment of high-risk B cell lymphoma in the elderly remains a challenge because of their ineligibility for HSCT, high mortality and relapse rates. Maintenance therapy to improve the prognosis of B cell lymphoma in the elderly might be feasible.

Methods

We analyzed the efficacy of a combination of human recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF) with the R2 regimen in maintenance therapy in high-risk B cell lymphoma in the elderly of our center. A total of 83 elderly patients, who were ineligible for auto-HSCT and had a response after 6 cycles of induction therapy above a partial response, were divided into two groups: observation (n = 44) and R2 + GM-CSF (n = 39) by enrollment time.

Results

The clinical data, survival outcome, and the number of peripheral blood mononuclear cells were analyzed and compared. The numbers of lymphocytes (1.19×10^9/L *vs*. 1.03×10^9/L, $P = 0.0062$) and leukocyte (6.46×10^9/L *vs*. 4.85×10^9/L, $P = 0.0048$) increased after receipt of maintenance therapy, particularly the number of natural killer cells (0.131×10^9/L *vs*. 0.061×10^9/L, $P = 0.0244$). Patients receiving R2 + GM-CSF for maintenance had longer-term remission (duration of response (DOR): 18.9 months *vs*. 11.3 months, $P = 0.001$), and longer-term progression free survival (PFS) (not reached (NR) *vs*. 31.7 months, $P = 0.037$), and overall survival (OS) (NR *vs*. NR, $P = 0.015$). The new R2 regimen was safe and well tolerated. The elderly, high-risk, and high tumor burden seemed to have tendency to be independent prognostic factors for a better PFS ($P = 0.060$, 0.012, 0.005).

Conclusions

The new R2 regimen prolonged progression-free survival and overall survival among elderly patients with high-risk B cell lymphoma, appeared to lead to improve response without compromising its tolerability profile in elderly patients who are ineligible to receive HSCT. The number of patients in this trial was limited and the conclusions should be verified in larger, prospective, multicenter studies.

Retrospectively registered:

The ethical committee of Huadong Hospital (approval number: 2021K186).

Background

High-risk aggressive B cell lymphoma predominantly affects older individuals, with a median age at diagnosis of 66. The 5-year overall survival (OS) of elderly patients with advanced-stage B cell lymphoma has reached 60%.[1] Unfortunately, about 40% of patients eventually fail to achieve complete remission or experience relapsed after front-line rituximab-chemotherapy.[2] Numerous studies have documented that high-dose therapy (HDT) and consolidative hematopoietic stem cell transplantation (HSCT) could produce durable remission and prolong OS in young patients with chemotherapy-sensitive high-risk lymphomas,[3] while most elderly patients are not eligible for the intensive therapy.[4] Moreover, few studies have provided an optimal strategy to protect elderly patients from disease progression and recurrence. Recently, maintenance therapy, as a strategy to delay relapse, has successfully prolonged the OS of certain patients with mantle cell lymphoma (MCL), but it is not beneficial to patients with diffuse large B cell lymphoma (DLBCL).[5–8] Only lenalidomide maintenance therapy after standard immune chemotherapy showed promising results: It significantly prolonged progression-free survival (PFS) in elderly patients with DLBCL, but was generally unsuccessful in improving OS.[9–11]

Lenalidomide is widely used to treat B cell lymphoma in nowadays. It is an oral immunomodulatory drug that is effective against lymphoma and immunological effects. It stimulated T cell and natural killer (NK) cell-mediated cytotoxicity in experimental models.[12–14] Lenalidomide treatment enhanced NK cell-induced cytotoxicity against several rituximab-treated NHL cell lines; the effects of lenalidomide-induced NK cell cytotoxicity and ADCC may be mediated indirectly via IL-2 production by T cells or Fc-γ receptors bound to rutiximab.[15, 16] Antineoplastic and antiproliferative effects as well as increased NK cell numbers and activity were observed after lenalidomide treatment in malignant B cell lymphoma, especially against DLBCL, follicular lymphoma (FL), and MCL cells.[12] The successful administration of lenalidomide to improve PFS in elderly patients with DLBCL as maintenance therapy hinted at the importance of modulating the tumor microenvironment.

Cellular components of the tumor microenvironment contribute to anti-tumorigenic functions, survival, and proliferation.[17] NK cells are functionally impaired in patients with high-risk B cell lymphoma [18]. Some studies explored that B cell lymphoma (BCL) inhibits NK cell function by reducing cytoplasmic granulation[19] and by reducing their ability to form immunological synapse[20]. Another scholar believed that BCL reduces the number of NK cells[21]. Thus, how to further modulate and improve tumor environment using lenalidomide and other biological agents remains a challenge. In previous studies and our small cohort of study, we found that human recombinant granulocyte-macrophage colony-stimulating factor (rhGM-CSF) could increase the number of NK cells.[22, 23] GM-CSF belongs to a family of hematopoietic cytokines that stimulate a number of autocrine-mediated effects on immune and non-immune cells and act in a paracrine manner following its release from cells.[24, 25] It increases granulocyte proliferation and phagocytosis, promotes antibody-dependent cellular cytotoxicity (ADCC) of macrophages and monocytes, and enhances monocyte differentiation into dendritic cells, which are known as potent antigen-presenting cells.[24, 26, 27] The amplification of NK cells provides a potent anti-tumor response. The ADCC induced by rituximab relies on NK cells, and experimental data suggested that

rituximab, with minimal toxicity[28], induced apoptosis, complement-dependent cytotoxicity (CDC), and phagocytosis.[26, 28] Lenalidomide increased the ADCC of rituximab both *in vitro* and *in vivo* via an NK cell-mediated mechanism. Perhaps combination of rhGM-CSF and lenalidomide and rituximab could modulate the lymphoma microenvironment and improve survival further.

Then we hypothesized that the combination of rhGM-CSF and the R2 regimen, a maintenance therapy regimen, might provide a foundation to improve the complete response (CR) rate and prolong PFS and OS. We carried out this retrospective cohort study of maintenance therapy following first-line chemoimmunotherapy and evaluated its efficacy in elderly patients with high-risk B cell lymphoma.

Methods

Patients

Patients were enrolled from January 2013 to October 2020 from the Department of Hematology, Huadong Hospital (Shanghai, China). A total of 83 patients were diagnosed with high-risk B cell lymphoma, meeting the 2016 WHO diagnostic guidelines for hematopoietic and lymphoid tissue tumors, and all of them underwent pathological type classification by biopsy of lymph nodes and other extranodal tissues. Patients had no vital organ dysfunctions, and their liver, kidney, and heart functions were normal or nearly normal. All patients were intermediate- or adverse-risk (International Prognostic Index (IPI) ≥ 2). For note, 22 patients with IPI = 2 were recurrent, or had cytogenetic aberrations (double-hit lymphoma (*MYC/Bcl-2, MYC/Bcl-6*) or triple-hit lymphoma (*MYC/Bcl-2/Bcl-6*) or double-protein expression (MYC/Bcl-2) or triple-protein expression (MYC/Bcl-2/Bcl-6)) and we included these patients into patients with high-risk B cell lymphoma. There were 51 males (61.4%) and 32 females (38.6%). Their median age was 65.9 ± 11.79 years. This study was approved by the ethical committee of Huadong Hospital (approval number: 2021K186) and all patients signed informed consent forms prior to participating in the study.

Treatment

Patients were given chemotherapy regimens recommended by the National Comprehensive Cancer Network (NCCN) guidelines based on pathological classification, staging, and general conditions. We adjusted the dose of chemotherapy drugs and/or the dosing cycle according to the side effects of treatment and patient tolerance. The first-line chemotherapy for B cell lymphoma is R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone). The second-line regimen is RICE (rituximab, ifosfamide, cisplatin, etoposide), R-ESHAP (rituximab, etoposide, methylprednisolone, cisplatin, cytarabine). If patients couldn't achieve CR after 6 cycles of R-CHOP chemotherapy, we would choose second-line therapy such as R-ICE or R-ESHAP with modified dose for another two cycles for the elderly fit while ineligible to autologous transplantation. A total of 83 patients who demonstrated an objective response to induction therapies were finally included. Patients who achieve CR or PR after induction therapy from 2013 to 2017 did not receive any maintenance therapy (in observation group), and those from 2018 to 2021 received maintenance therapy (in R2+GM-CSF group). Patients in the R2+GM-CSF

group were mostly elderly patients who were not suitable or refused to receive autologous HSCT. They continued to receive the R2 regimen combined with rhGM-CSF (Lenalidomide, 10 mg/d, orally on days 6–15; rituximab 375 mg/m^2, intravenous injection on day 6; rhGM-CSF (Amoytop Biotech co) 150 μg/d, subcutaneously on days 1–8) every three months after reaching remission by induction chemotherapy. The patients in the observation group were mostly elderly patients or those who refused to receive autologous HSCT. Patients in the observation group would no longer receive any drugs for maintenance after reaching remission under induction chemotherapy. The flowchart of a clinical trial protocol is shown in Figure 1. There were 39 cases in the R2+GM-CSF group and 44 cases in the observation group.

PBMC collection and Flow cytometry Analysis

Blood was collected from patients in maintenance therapy group and PBMC and immune cells was analyzed before and after each cycle of the maintenance, then compared the difference of WBC, lymphocytes, monocytes, B cells, T cells and NK cells. Eight different surface markers were used in the flow cytometric studies. List mode reanalysis was performed using gating for lymphocytes, monocytes, and myeloid populations, based on side scatter-CD45 histograms.[29] The monoclonal antibodies include isotype controls, and those recognizing CD45, CD3, CD4, CD8, CD19, CD56/16, CD5, and CD7 (BD Horizonä, BD Biosciences, San Jose, CA, USA). The samples were analyzed performed on a Coulter Flow Cytometer (Beckman Coulter, Indianapolis, IN, USA) equipped with a 488 nm Argon laser. The results were reanalyzed using FlowJoä software (FlowJO LLC, Ashland, OR, USA). Flow cytometric data were compared with patient characteristics, including blood counts.[29] The detailed protocol and materials of the flow were shown in Supplemental materials.

Endpoint and follow-up

According to the 2016 NCCN recommended LUGANO evaluation criteria, the efficacy evaluation is divided into complete remission (CR), partial remission (PR), stable disease (SD), and progressive disease (PD). The objective response rate (ORR) is the proportion of CR and PR. All patients were evaluated for efficacy every three cycles during induction treatment period, mainly through positron emission tomography computed tomography (PET/CT) examination of the whole body, ultrasound evaluation or CT scan. For those who did not reach the response of PR or with stable disease or disease progression, the regimen would be changed to the second line therapy. If patients could achieve CR or PR, they would enroll into this study. Patients from 2013 to 2017 were into the observation group (they just stopped chemo and followed up), patients from 2018 to 2021 were into the maintenance therapy group (they just received new R2 regimen as maintenance therapy). The enrolled patients were followed every three months in the first year, every six months in the second year and once a year in the third year. The patients did PET/CT every half a year (every other cycle of maintenance therapy) in the first and the second year and once a year in the third year. For other time, patients received CT scan and ultrasound to follow up. The duration of response (DOR) was defined as the time from the first evaluation of efficacy as complete remission (CR) or partial remission (PR) to the first evaluation of disease progression (PD) or death from any cause.

The follow-up period of this study ended on November 30, 2021. We recorded the data based on the last hospitalization record, out-patient review, and telephone follow-up. OS was defined as the time from the start of randomization to the date of death of the patient due to any cause or the date of the last follow-up. Progression free survival (PFS) was defined as the time from randomization to the date of disease progression or death or last follow-up of patients. In this study, OS and PFS were calculated on a monthly basis. The 83 patients were followed up for a median time of 21.1 ± 14.81 months.

Statistical analysis

The patients' characteristics were summarized using the mean and standard deviation for numerical variables and as frequencies with percentages for categorical variables. Differences in patients' characteristics among the two groups were assessed using Student's *t* test for numerical variables and with Fisher's exact test for categorical variables, including F-tests and Chi-squared tests. The Kaplan–Meier (KM) method was used to estimate unadjusted probabilities of OS and PFS. Unadjusted OS and PFS between subgroups were compared using a Log-rank test. The joint effects and prognostic factors of patient covariates and treatment arms on OS and PFS were assessed using Cox regression models. SPSS 20.0 software (IBM Corp., Armonk, NY, USA) was used to perform all the statistical analyses. A value of P 0.05 was considered statistically significant.

Results

Patient characteristics

A total of 83 elderly patients were enrolled and treated after six cycles of induction therapy. 62 patients (32 cases in observation group and 30 cases in R2+GM-CSF group) achieved CR after first-line induction therapy and 21 patients (12 cases in observation group and 9 cases in R2+GM-CSF group) achieved above PR after first-line induction therapy, then received second-line therapy. The patients were ineligible for HSCT, had a response above PR, and entered this retrospective cohort study of receiving R2+GM-CSF (new R2) group as maintenance therapy or observation, by the patients' intention. All patients provided signed the consent. Thirty-nine patients received R2+GM-CSF and 44 patients received observation. Most of the 83 patients with high-risk B cell lymphoma were newly diagnosed (75 cases, 90.4%) and the main pathological type was DLBCL (70 cases, 84.3%; germinal center B-cell (GCB) 9 cases, Non-GCB 61 cases). Additionally, the majority of patients were elderly with good physical status and were at the advanced stage of lymphoma at the time of diagnosis. Although the number of patients who achieved CR before maintenance therapy was higher in the R2+GM-CSF group (61.5% *vs*. 47.7%), there was no significant difference between the two groups (P = 0.208). Table 1 shows the patients' baseline characteristics. The mean age, sex, disease isotype, lactate dehydrogenase (LDH) level, beta-2 microglobulin (beta2-MG) level and metaphase cytogenetics showed no significant differences between the two treatment groups. Furthermore, Ann Arbor stage, IPI score, and Eastern Cooperative Oncology Group (ECOG) score at diagnosis also showed no significant differences. All the details are presented in Table 1.

New R2 regimen combined with rhGM-CSF increased the number of NK cells

Peripheral blood mononuclear cells (PBMCs) and immune cells were analyzed by flow cytometry in patients before maintenance therapy and after each cycle of maintenance therapy. We found rhGM-CSF plus R2 regimen may upregulate NK cell numbers about for 3 months, that's why we determine new R2 regimen administration for three months each cycle, but detailed data was not shown in this paper. Table 2 only shows the data before and after the first cycle of maintenance therapy. We observed a statistically significant increase in leukocytes (6.46×10^9/L vs 4.85×10^9/L, $P = 0.0048$), hemoglobin (126.36 g/L *vs*. 119.08 g/L, $P < 0.001$), and erythrocytes (4.18×10^{12}/L *vs*. 3.96×10^{12}/L, $P = 0.0002$). Additionally, Table 2 and Figure 2 show that after R2+GM-CSF treatment, the number of lymphocytes (1.19×10^9/L *vs*. 1.03×10^9/L, $P = 0.0062$) and NK cells (0.131×10^9/L vs. 0.061×10^9/L, $P = 0.0244$) increased significantly. Thus, R2+GM-CSF affected the levels of certain immune cells, and in particular, the number of NK cells increased. We found the number of immune cells was increased after R2+GM-CSF (Figure 2), but not significantly. All the details are presented in Table 2 and Figure 2.

The new R2 regimen combined with rhGM-CSF maintained long-term remission of patients with high-risk B cell lymphoma

After receiving different maintenance treatments, the remission status of patients were statistically different. The median duration of remission (DOR) of the 83 patients was 15.0 ± 10.67 months. The DOR in the R2+GM-CSF maintenance group was longer than that in the observation group (18.8 ± 9.32 months *vs*. 11.3 ± 10.72 months, $P = 0.001$). Although the recurrence rate of patients in observation group was 1.6-fold higher than that of patients in the R2+GM-CSF group (20.5% *vs*. 12.8%), the difference was not statistically significant ($P = 0.242$).

The new R2 regimen combined with rhGM-CSF prolonged PFS and OS of patients with high-risk B cell lymphoma

The median PFS of the 83 patients was 48.9 ± 11.38 (95% confidence interval (CI): 26.55–71.18) months. The R2 regimen combined with rhGM-CSF maintenance treatment enabled patients to achieve longer-term PFS ($P = 0.037$, Figure 3B). The 1-year PFS rates and 5-year PFS rates of the R2+GM-CSF group and observation group were 100% and 84.1%, 76.9% and 72.7%, respectively, and the differences were statistically significant ($P = 0.003$, $P = 0.037$).

However, the difference in OS between two groups was statistically significant ($P = 0.015$, Figure 3A). The 1-year OS rate of R2+GM-CSF group and observation group were 100% and 95.5%, and there was no significant difference ($P = 0.165$). However, the 5-year OS rates were 97.4% and 86.4%, respectively, and the difference was statistically significant ($P = 0.015$).

Prognostic Factors

For elderly patients (> 60 years old), the R2 regimen combined with rhGM-CSF as a maintenance regimen was very helpful to prolong the long-term PFS. If the elderly patients did not continue to be given the corresponding treatment after induction of remission, the disease was progressed easily, which was

manifested in a shorter PFS time, although there was no significant difference [*P* = 0.060, hazard ratio (HR) 0.368 (95% CI 0.129–1.044), Figure 4].

Additionally, a single-factor stratification study found that compared with the observation group, the R2 regimen combined with rhGM-CSF as a maintenance regimen was helpful to prolong the long-term PFS rate, especially for patients who stage III or IV disease [*P* = 0.005, HR 0.221 (95% CI: 0.078–0.628)], patients with an IPI score ≥ 3 [*P* =0.012, HR 0.275 (95% CI: 0.101–0.752)], and patients with bone marrow involvement [*P* = 0.023, HR 0.077 (95% CI: 0.009–0.698)]. All the details are presented in Figure 4 and the Supplementary Table 1.

Safety

Safety during maintenance therapy was assessed for 39 patients who undertook at least one new R2 regimen combined with rhGM-CSF. We collected all immediate responses during analyzing side effects. All adverse events and serious adverse events were collected and graded according to the Common Terminology Criteria for Adverse Events (CTCAE-Version 4.0). All adverse events are shown in Table 3. Hematological toxicities that occurred more frequently in the R2+GM-CSF group included anemia (13/39), lymphocytopenia (9/39), and leukopenia (9/39). Of note, all the hematological adverse events were graded 1–2 (16/39), and none were graded 3 or 4. Besides, common treatment-related non-hematological adverse events were of grade 1–2 (16/20), with most frequent ones being nausea or vomiting (6/39). Only one patient had fever because of rhGM-CSF. Notably, only one patient died (1/39) during the study. In short, the side effects so mild that none of them were managed through dose interruptions and/or reductions and other support, and no serious or fatal adverse events (graded above 3) occurred.

Discussion

Maintenance therapy, including rituximab monotherapy[30], rituximab in combination with lenalidomide[31], and obinutuzuamb plus bendamustine[32], has evolved as a successful strategy to prolong PFS and OS in some mantle cell lymphoma clinical settings[5]; however, it has had little success in high-risk B cell lymphoma. Two phase Ⅱ multicenter studies on lenalidomide maintenance therapy first revealed that lenalidomide could improve PFS in high-risk patients with relapsed DLBCL who were ineligible for transplantation[33, 34]. In addition, the phase Ⅲ REMARC trial showed that there was no superiority in prolonging OS (lenalidomide versus placebo) in elderly patients following front-line R-CHOP induction[11]. To date, no clinical studies have reported whether maintenance therapy has an important role in high-risk B cell lymphoma.

This retrospective cohort study focused on the new R2 regimen (GM-CSF + R2) following front-line chemoimmunotherapy and evaluated its efficacy in elderly patients with high-risk B cell lymphoma. The results showed that the new R2 regimen for maintenance therapy prolonged PFS and OS significantly compared with observation, delayed the time to next anti-lymphoma chemotherapy and the duration of response. New R2 regimen was well tolerated, with some mild adverse events, without exit of treatment. The New R2 regimen seemed to improve the PFS in the elderly, high-risk, high tumor burden patients who

were ineligible for auto-HSCT; therefore, the new R2 regimen might represent a new choice for these patients.

Lenalidomide is an oral immunomodulator with direct anti-tumor activity and indirect antineoplastic actions. It has effects on multiple targets in the tumor microenvironment. Lenalidomide recruits NK cells to tumor sites and stimulates the proliferation and activation of NK cells (and other immune cells) to modify the tumor microenvironment.[35] Lenalidomide maintenance to prolong PFS in elderly patients with DLBCL suggested the importance of modulating the tumor microenvironment. However, lenalidomide has many side effects including myelosuppression.[36, 37] The most common grade 3 or 4 adverse events associated with lenalidomide are neutropenia (57%) and fatigue (13%) in elderly patients with DLBCL.[11, 33, 37] RhGM-CSF is used to prevent neutropenia and febrile neutropenia in patients with non-Hodgkin lymphoma. RhGM-CSF can increase the numbers and cytotoxicity of effector cells, and the combined biological effects of rhGM-CSF and rituximab appear promising in that they might enhance a patients innate immune response against non-Hodgkin lymphoma.[24] We further followed up immune cells in the peripheral blood of the patients and found that rhGM-CSF in combination with R2 increased the numbers of NK cells and lymphocytes significantly. Thus, the new R2 regimen enhanced and augmented NKmediated cytotoxicity and antibody-dependent cellular cytotoxicity.

The significant improvement of OS and PFS in the GM-CSF + R2 group hinted that high risk B cell lymphoma, especially in the elderly high-risk, high tumor burden might obtain a survival benefit from maintenance therapy using the new R2 regimen. A limitation of this study was the small number of patients. We only found the combination of R2 with rhGM-CSF regimen improved the OS and PFS in high-risk elderly B cell lymphoma patients, while lack of comparison between R2 alone and GM-CSF alone made the mechanism and effect of each drug unclear. Another limitation was the lack of NK cell function detection. Therefore, the conclusions should be verified in prospective, randomized, multicenter clinical trials, more work would make the combination regimen explicit and consummate.

Conclusion

Maintenance therapy to improve the prognosis of B cell lymphoma in the elderly might be feasible. Our current study involving 83 elderly patients, provided evidence that patients receiving R2 + GM-CSF for maintenance had longer-term remission, and longer-term progression free survival, and overall survival without compromising its tolerability profile in elderly patients who are ineligible to receive HSCT. Besides, we found that the numbers of lymphocytes and leukocyte increased after receipt of maintenance therapy, particularly the number of natural killer cells.

List Of Abbreviations

HSCT	hematopoietic stem cell transplantation
rhGM-CSF	human recombinant granulocyte-macrophage colony-stimulating factor
DOR	duration of response
PFS	progression free survival
NR	not reached
OS	overall survival
HDT	high-dose therapy
MCL	mantle cell lymphoma
DLBCL	diffuse large B cell lymphoma
NK cell	natural killer cell
ADCC	antibody-dependent cellular cytotoxicity
FL	follicular lymphoma
BCL	B cell lymphoma
CDC	complement-dependent cytotoxicity
CR	complete response
PR	partial remission
SD	stable disease
PD	progressive disease
ORR	objective response rate
IPI	International Prognostic Index
R-CHOP	rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone
RICE	rituximab, ifosfamide, cisplatin, etoposide
R-ESHAP	rituximab, etoposide, methylprednisolone, cisplatin, cytarabine
PBMC	Peripheral blood mononuclear cell
WBC	White blood cell
PET	positron emission tomography computed tomography
GCB	germinal center B-cell
LDH	lactate dehydrogenase
beta2-MG	beta-2 microglobulin

ECOG	Eastern Cooperative Oncology Group score

Declarations

Ethics approval and consent to participate

The study protocols were approved by the ethical committee of Huadong Hospital (approval number: 2021K186). The patients provided written informed consent. This clinical investigation was conducted according to the principles of the Declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by the Shanghai Science and Technology Committee [grant number

21Y11909000 and 19DZ1910300], Shanghai Health Committee [grant number 202140518], the Elite Project of Huadong hospital (HD1439), and the Project of Huadong Hospital (20191c014).

Author's contributions

Jiexian Ma and Yanhui Xie was responsible for developing the conceptual framework of the study, designing and writing the protocol. Jiexian Ma, Wulipan fulati and Shunrong Sun collected and analyzed data, conducted experiments, wrote and reviewed the paper. Pingping Chen, Mingyue Chen, Lin Shen, Min Wu, Wensi Qian, Yu Xv, Yingwei Hu and Hongdi Zhang conducted experiments. Zilan Huang contributed to the technical support. The authors read and approved the final manuscript.

Acknowledgements

We thank the patients and their families who participated in this study. And we also thank all physicians, nurse and other patient care providers involved in the care of these patients.

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Tables

Table 1
Baseline Clinical Characteristics of Patients Receiving the Two Regimens

Characteristics	Observation	R2 + GM-CSF	Statistic	*P* value
Mean age, years	64.5 ± 11.76	67.5 ± 11.76	−1.163	0.248
Male, n (%)	27 (61.4%)	24 (61.5%)	0.000	0.987
WHO type, n (%)	40 (90.9%)	30 (76.9%)	3.062	0.080
DLBCL	4 (9.1%)	9 (23.1%)		
B-cell lymphoma, unclassifiable				
Relapse/Refractory lymphoma, n (%)	3 (6.8%)	5 (12.8%)	0.855	0.465
Ann Arbor stage at diagnosis, n (%)	7 (15.9%)	7 (17.9%)	0.061	0.804
I, II	37 (84.1%)	32 (82.1%)		
III, IV				
IPI score at diagnosis, n (%)	31 (70.5%)	30 (76.9%)	0.444	0.505
≥ 3	13 (29.5%)	9 (23.1%)		
= 2				
ECOG score at diagnosis, n (%)	10 (22.7%)	17 (43.6%)	4.100	0.060
≥ 3	34 (77.3%)	22 (56.4%)		
< 3				
Fever, n (%)	14 (31.8%)	20 (51.3%)	3.239	0.072
B syndrome	25 (56.8%)	26 (66.7%)	0.846	0.358
EBV infection with positive, n(%)	4 (9.1%)	9 (23.1%)	3.062	0.080
Bone marrow involvement, n (%)	18 (40.9%)	14 (35.9%)	0.219	0.640
Metaphase cytogenetics, n (%)	38 (86.4%)	31 (79.5%)	0.697	0.404
Normal	6 (13.6%)	8 (20.5%)		
Abnormal				
LDH n(%)	17 (38.6%)	16 (41.0%)	0.049	0.824
Normal	27 (61.4%)	23 (59.0%)		
Abnormal				

DLBCL, diffuse large B cell lymphoma; EBV, Epstein-Barr virus; IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; CR, complete response; PR, partial response.

Characteristics	Observation	R2 + GM-CSF	Statistic	*P* value
Beta2-microglobulin, n(%)	23 (52.3%)	23 (59.0%)	0.376	0.540
Normal	21 (47.7%)	16 (41.0%)		
Abnormal				
Medium cycle	6.0 ± 2.18	5.8 ± 1.47	0.386	0.700
Induction therapy			0.1926	0.801
First-line chemotherapy	32(72.7%)	30(76.9%)		
Second-line chemotherapy	12(27.3%)	9(23.1%)		
Response before maintaining treatment, n(%)	21 (47.7%)	24 (61.5%)	1.589	0.208
CR	23 (52.3%)	15 (38.5%)		
PR				

DLBCL, diffuse large B cell lymphoma; EBV, Epstein-Barr virus; IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; CR, complete response; PR, partial response.

Table 2
The change of number of immune cells before and after R2 + GM-CSF maintenance therapy

	Before R2 + GM-CSF	After R2 + GM-CSF	t	*P* value
RBC ($\times 10^{12}$/L)	3.96	4.18	4.082	0.0002
HGB (g/L)	119.08	126.36	4.581	< 0.001
PLT ($\times 10^{9}$/L)	190.42	181.46	1.341	0.1863
WBC ($\times 10^{9}$/L)	4.85	6.46	2.951	0.0048
NEUT ($\times 10^{9}$/L)	3.13	4.49	2.806	0.0072
LYM ($\times 10^{9}$/L)	1.03	1.19	2.862	0.0062
MONO ($\times 10^{9}$/L)	0.47	0.52	0.8458	0.4018
EOS ($\times 10^{9}$/L)	0.19	0.23	1.087	0.2823
BASO ($\times 10^{9}$/L)	0.0281	0.0283	0.04485	0.9644

RBC: red blood cell; HGB: hemoglobin; PLT: platelet; WBC: white blood cell; NEUT: neutrophil; LYM: lymphocyte; MONO: monocyte; ESO: basophilic granulocyte; BASO: eosinophilic granulocyte.

Table 3
treatment-emergent adverse events in patients receiving R2 + GM-CSF.

Adverse events	N (n = 39)	
	Graded 1–2	Graded 3 or 4
Hematological adverse events	23	0
Anemia	13	0
Leukopenia	9	0
Neutropenia	2	0
Lymphocytopenia	9	0
Thrombocytopenia	2	0
Non-hematological adverse events	6	0
Diarrhea	0	0
Constipation	1	0
Cough	3	0
Fatigue	1	0
Pyrexia	3	0
Upper respiratory tract infection	2	0
Headache	0	0
Infusion-related reaction	0	0
Asthenia	0	0
Decreased appetite	0	0
Muscle spasms	0	0
Peripheral edema	0	0
Abdominal pain	1	0
Pruritus	0	0
Nausea or vomiting	6	0
Dyspnea	0	0

* Safety during maintenance was assessed for patients who undertook at least one visit. All adverse events, defined as any adverse change from the patient's baseline condition, whether considered related to treatment or not, were collected and graded according to the Common Terminology Criteria for Adverse Events 4.0 grading system.

Adverse events	N (n = 39)	
Rash	1	0
Tumor flare	0	0
Alanine aminotransferase increased	3	0
Influenza	0	0
Back pain	2	0
Nasopharyngitis	0	0

* Safety during maintenance was assessed for patients who undertook at least one visit. All adverse events, defined as any adverse change from the patient's baseline condition, whether considered related to treatment or not, were collected and graded according to the Common Terminology Criteria for Adverse Events 4.0 grading system.

Figures

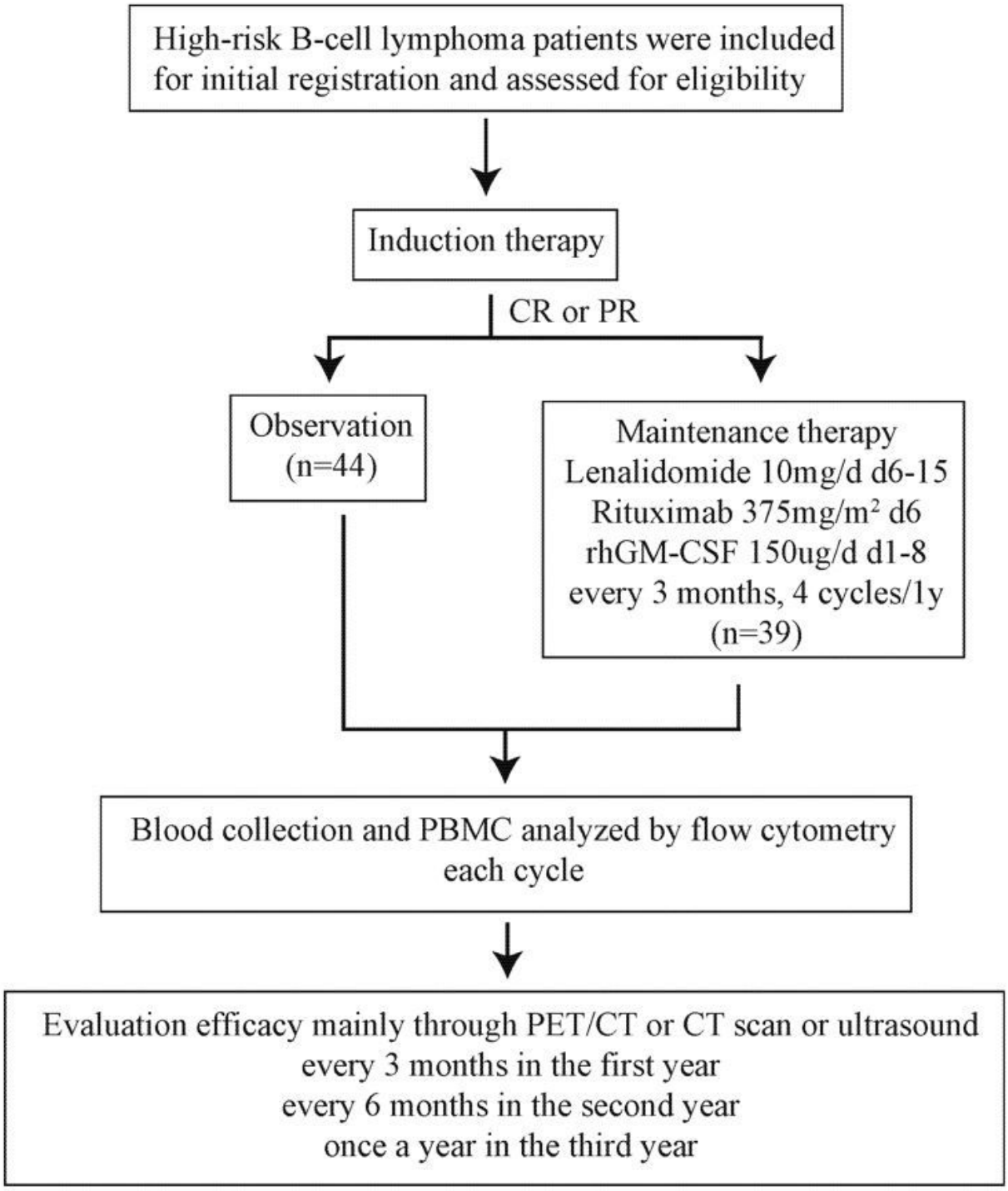

Figure 1

The flowchart of treatment regimen in observation group and R2+GM-CSF group.

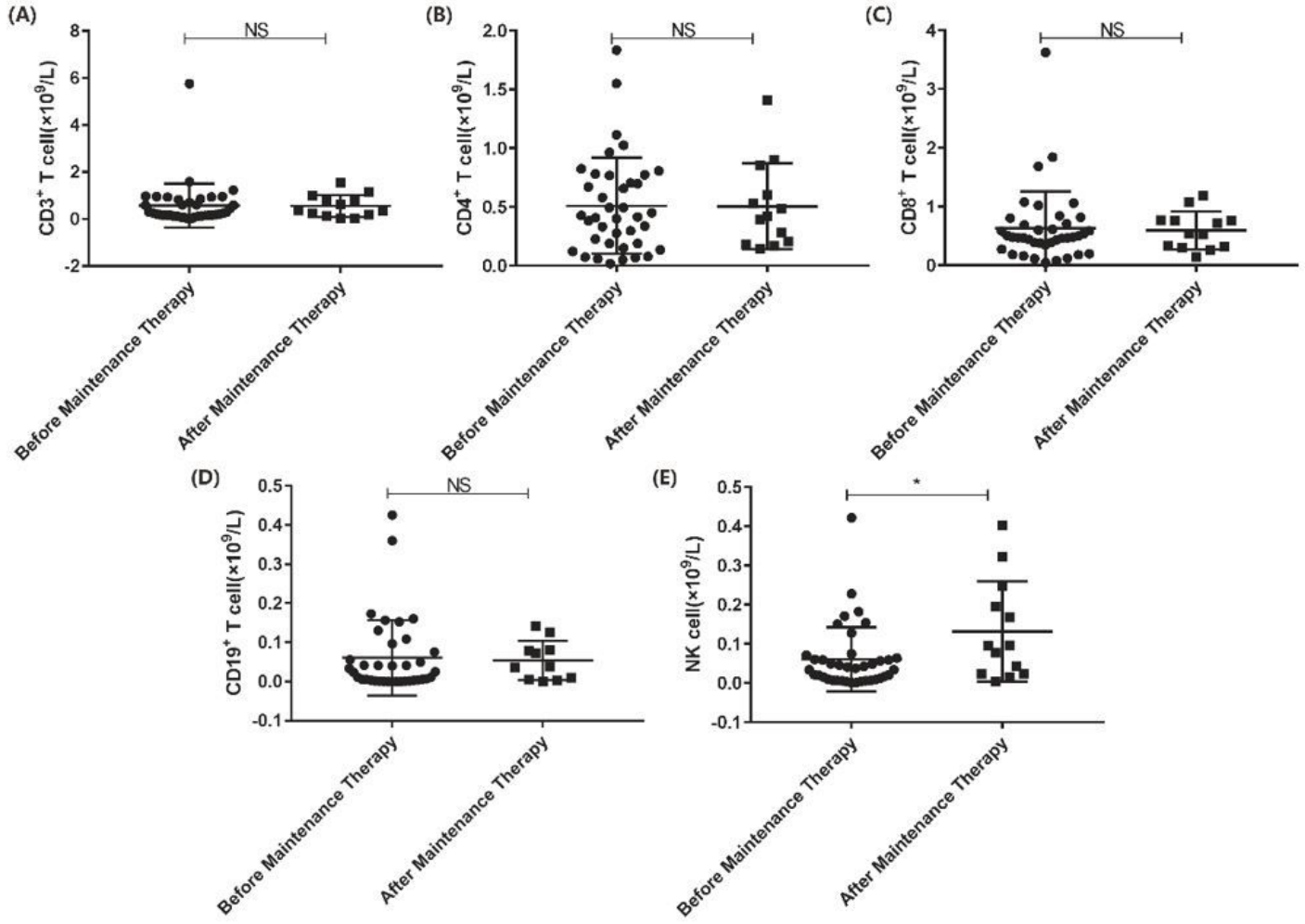

Figure 2

The number of immune cells in peripheral blood before and after R2+GM-CSF as maintenance therapy. Symbols represent mean ± SEM and all statistical significance determined by paired student *t* test or unpaired student *t* test with $P < 0.05$. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

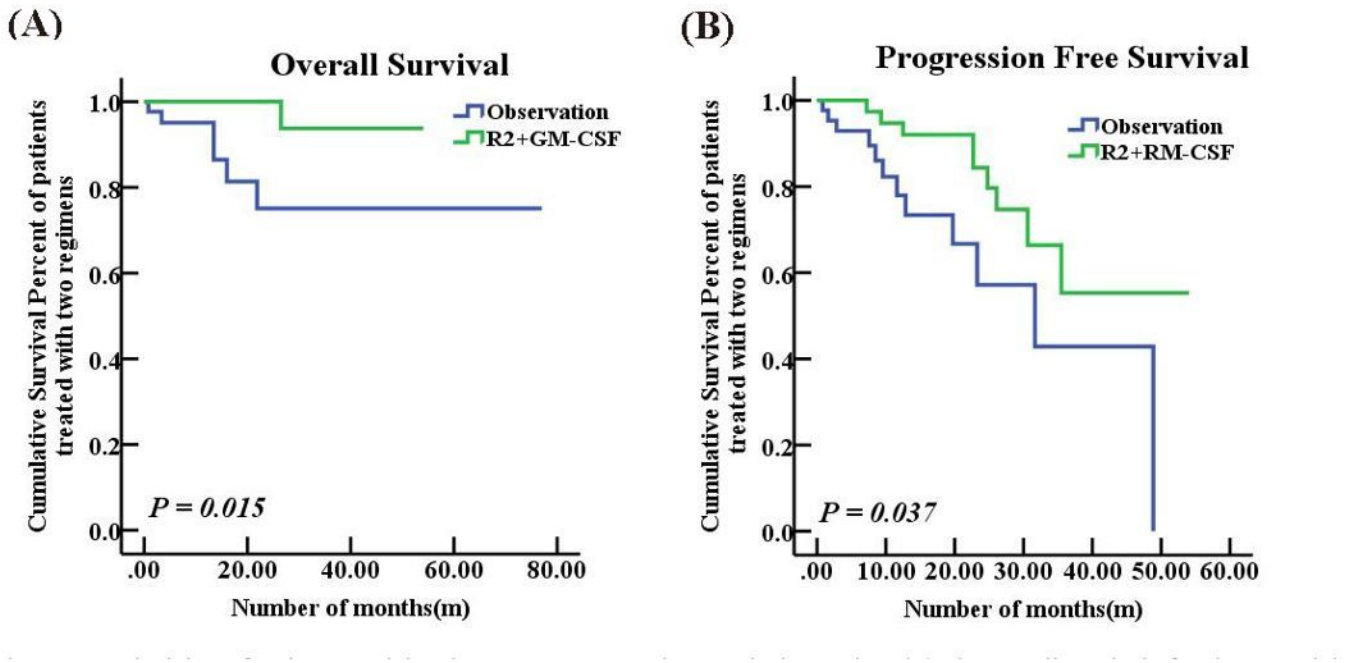

Figure 3

Survival data of patients receiving the R2+GM-CSF regimen and observation. (A) The overall survival of patients receiving the two regimens (P = 0.015). Patients in R2+GM-CSF group and observation group did not reach the median OS time, but the difference was statistically significant (χ^2 = 5.924, P = 0.015). (B) The progression-free survival of patients receiving the two regimens (P = 0.037). The patients in R2+GM-CSF group did not reach the median PFS time, but the median PFS time of patients in observation group was 31.7 ± 9.07 (95% Confidence Interval: 13.89-49.45) months. The difference was statistically significant (χ^2 = 4.355, P = 0.037)

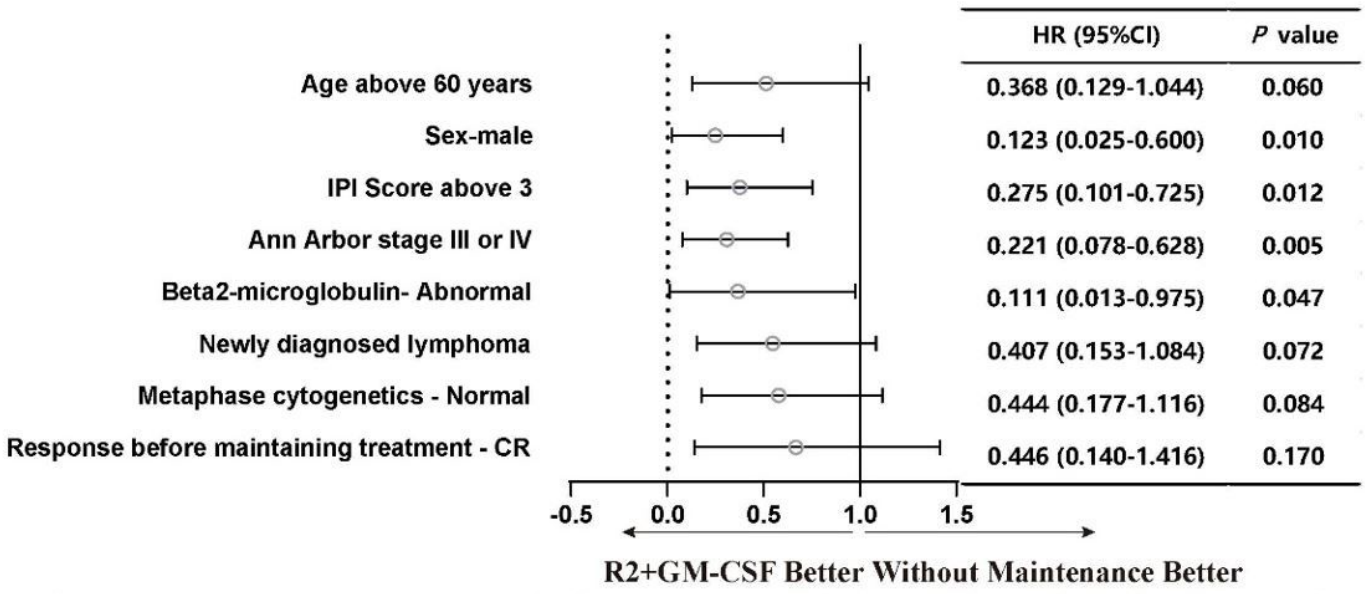

Figure 4

Subgroup analyses of progression-free survival. Metanlysis (Forest) plot shows the results of subgroup analysis of progression-free survival among 83 patients. The dashed vertical line indicates a harzard ratio of 0, and the sloid vertical line indicates a harzard ration of 1.0.

Supplementary Files

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