# Specific KIR-HLA genotypes predict outcomes of refractory or recurrent primary central nervous system lymphoma

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## Abstract

# Purpose

An effective salvage regimen for re-induction of remission is lacking for refractory or recurrent primary central nervous system lymphoma (r/r PCNSL). This study aimed to evaluate the efficacy and safety of high dose cytarabine plus temozolomide in treating r/r PCNSL and explore the prognostic factors

# Methods

A single-center retrospective cohort study was conducted to access the efficacy and safety of high dose cytarabine and temozolomide (AT) in r/r PCNSL patients. KIR and HLA genotyping were performed from peripheral blood sample of each patient.

# Results

Thirty PCNSL patients receiving AT regimen (cytarabine 3g/$m^2$ for 2 days combined with temozolomide 150mg/$m^2$ for 5 days) in our institution were analyzed. The median age was 65 years (range 25–79 years). 43.4% of patients (13/30) achieved overall response with a median follow up of 16 months (95% confidence interval [CI]:11–23 months). The median PFS and OS of the cohort were 1.75 months (95% CI:1–4 months) and 19.5 months (95%CI:11 months to not calculable), respectively. Patients harboring KIR3DL1/HLA-B genotypes predicting low affinity had a higher response rate (p = 0.042) and longer median PFS (3 months) than those with KIR3DL1/HLA-B genotypes predicting high affinity (1 month) (p = 0.0047). Cox regression analysis indicated that KIR/HLA-B genotypes were independently associated with the PFS (p = 0.042). But it had no impact on the OS of the cohort. Toxicity of AT treatment was mild and manageable.

# Conclusion

AT regimen was well tolerated and patients with specific KIR-HLA genotypes may benefit from it.

## Introduction

Primary central nervous system lymphoma (PCNSL) is an uncommon subtype of lymphoma and takes up to 6.4% of extranodal diffuse large B cell lymphoma (DLBCL) cases in China [1]. RCHOP (rituximab, cyclophosphamide, vincristine, doxorubicin and predinisone) as the standard treatment for DLBCL has shown poor efficacy for PCNSL as most of these drugs cannot penetrate blood brain barrier [2]. Although the prognosis of PCNSL has been greatly improved with high dose methotrexate (MTX) based treatment,

the 5-year overall survival rate (OS) of PCNSL is only 24%-44% for European countries [3–4]and 26.9% for China [1], which is much lower than that of systemic DLBCL.

As previously reported, the overall response rate (ORR) of high does MTX based therapies was 50%-80% [5–7]. The most common regimens used along with high dose MTX includes temozolomide [8], cytarabine [9], rituximab [10], etc. Although a high response rate has been achieved since the application of MTX, up to 29% of patients were chemoresistant to MTX and 16.5% of patients were identified relapsed after treatment [11]. The median OS calculated from disease progression was only 7.2 months in patients with refractory disease [12]. Autologous stem cell transplantation (ASCT) has been applied as consolidative treatment for PCNSL with a 5-year OS rate of 70% and as salvage treatment with an ORR of 85% [13]. However, due to the fragile physical conditions of patients caused by disease and ages, ASCT is not applicable to everyone. Some other regimens have been evaluated in refractory or recurrent PCNSL (r/r PCNSL). For example, high dose cytarabine presented an ORR of 36% in r/r PCNSL with a median progression free survival (PFS) of 3 month [14] and premetrexed was reported to have an ORR of 64.7% and a median PFS of 5.8 months [15]. Other regimens including lenalidomide [16], pomalidomide [17], ibrutinib [18]and checkpoint inhibitors [19] have also been evaluated. These new regimens were not routinely used and the efficacy needs to be further evaluated.

As known, natural killer (NK) cells play an important role in the tumor immune surveillance. The activity of NK cells is determined through signals transduced by activating receptors and membrane inhibitory receptors such as killer cell immunoglobulin-like receptor (KIR) family, which recognizes human leukocyte antigen class I (HLA-I) [20]. The binding affinity between donor KIR and recipient HLA-I had an impact on the outcome of myeloid neoplasms receiving allogeneic stem cell transplantation [21, 22], in which NK cells were educated by weak inhibition HLA ligands. Such effects were also reported in autologous stem cell transplantation settings, in which patients haboring KIR along with low affinity HLA ligands were associated with lower relapse [23].

As for non-hematopoietic stem cell transplantation settings, NK cell activity was found to be correlated with the outcome of immunotherapy because anti-tumor effects of immunotherapy are partly due to antibody dependent cell-mediated cytotoxicity (ADCC) exerted by NK cells. For example, follicular lymphoma patients with KIR3DL1/HLA-Bw4 genotypes could benefit from rituximab maintainance therapy [24]. Patients with neuroblastoma harbouring specific KIR3DL1/HLA-B genotypes associated with non-interaction pattern had imroved OS and PFS compared with those with strong or weak interaction pattern[25].

In this report, we retrospectively evaluated the efficacy of high dose cytarabine combined with temozolomide (AT) treatment in patients with r/r PCNSL at our institution. KIR and HLA-I genotypes were analyzed. The results indicated that specific KIR and HLA-I pairs could, to some level, predict the outcome. It could be inferred that NK cell activity, which might be augmented partially by AT treatment, contributed to disease control.

# Materials And Methods

Patient cohort

Patients with histologically confirmed PCNSL at our institution from June 2017 to June 2020 were screened eligibility. Patients were included if they demonstrated disease progression after methotrexate ($3.5g/m^2$ ~ $8.0g/m^2$) based treatments and received high dose cytarabine and temozolomide. All chosen patients must have measurable disease with contrast-enhanced MR scans. Those who had previously used high dose cytarabine or temozolomide were excluded. Medical records of each patient were collected and checked by two physicians. Written informed consent was obtained from each patient. The study protocol was approved by the ethic committee of Huashan Hospital, Fudan University (ChiCTR2100054482).

Treatment and assessment

During the treatment period, patients received cytarabine $3.0g/m^2$ at day 1–2 and temozolomide $150mg/m^2$ at day 1–5. Cytarabine was administered as a 3-h infusion and temozolomide was given orally. The dosage of cytarabine was reduced by 70% for patients who were 70ys or older. In the case of meningeal involvement, intrathecal injection of cytarabine (50mg) was added at each chemotherapy phrase. Intravitreal methotrexate therapy was applied in addition to the base treatment if patients were diagnosed with intraocular lymphoma. The treatment was administrated every 4 weeks. Treatment response was assessed by the outcome of gadolinium enhanced brain MRI scans three weeks after previous AT treatment in accordance with international guidelines [26]. In brief, patients achieving complete remission (CR) or partial remission (PR) were categorized as responders, and those with stable disease (SD) or progressive disease (PD) were regarded as non-responders. The responders would continue to receive 8 cycles of treatment until disease progression whereas non-responders would be transferred to receive other chemotherapy or whole brain radiation therapy. All patients were subjected to perform a follow-up program with MRI controls every 3 months for 2 years. Toxicity of treatment was assessed according to Common Toxicity Criteria (CTCAE, version 5.0).

KIR and HLA class I genotyping

Genomic DNA was extracted from peripheral blood sample. KIR genotyping was performed using PCR with sequence specific primers [27] and HLA class I genotyping was performed using sequencing-based typing [28]. HLA-Bw4 alleles are supposed to have high affinity with KIR3DL1 receptors if an isoleucine is at position 80 (HLA-Bw4-80I); on the other hand, if a threonine is at position 80 (HLA-Bw4-80T), it can predict that HLA-Bw4 alleles have low affinity with KIR3DL1 receptors. HLA-C1 alleles are supposed to have low affinity with KIR2DL2/3 whereas HLA-C2 alleles have high affinity with KIR2DL1. HLA-A*23:01, HLA-A*24:02, and HLA-A*32:01 alleles are considered high affinity KIR3DL1 ligands if HLA-Bw4-80I alleles are present [23]. Binding affinities between specific KIR and corresponding HLA-I ligands were obtained from the Immuno Polymorphism Database [29]. We developed a scoring system predicting the affinity between KIR3DL1 and HLA-B. HLA-Bw6 genotype (predicting mismatch) were scored 0; HLA-Bw4-

80T genotype (predicting low affinity interactions) were scored 1 and HLA-Bw4-80I genotype (predicting high affinity interactions) were scored 2. Patients were categorized as HLA-B high affinity group if the score of two HLA-B alleles was ≥ 2 and HLA-B low affinity group if the score of two HLA-B alleles was < 2.

# Statistical analysis

PFS was defined as the time from AT treatment to disease progression, or death of any cause if progression was not determined. OS was calculated from the date of AT treatment to death of any cause or last date of follow-up. Survival was estimated using Kaplan-Meier method. Treatment response was compared between each group using Fisher's exact test. The univariate impact of KIR/HLA genotypes, age, sex, ECOG, numbers of lesion, location of lesions, tumor volume and CSF involvement on PFS or OS was assessed using the log-rank test. Multivariate analysis was conducted by cox regression analysis. P value less than 0.05 was considered statistically significant. The statistical analysis was performed using Graphpad Prism 9.0 software package and STATA 15 software package.

# Results

Patients characteristics

A total of 41 patients with PCNSL received AT treatments from June 2017 to June 2020 at our institution. 9 patients whose blood samples were not available and 2 patients who were lost to follow-up were excluded from the study. Finally, the analysis included 30 patients (Table 1). Two of these patients were lost to follow-up after disease progression and were deleted from the cohort when calculating OS. As shown, the median age and ECOG performance status of 30 patients was 65 years (range 25–79) and 3 (range 1–4). 60% of patients were male (18/30). There were 80% of patients (24/30) switched to AT therapy due to disease progression after high dose MTX based therapy, and the rest 20% of patients (6/30) received AT therapy as they relapsed after having achieved CR. Based on the medical records, 33.3% of patients (10/30) presented two or more lesions in the brain prior to AT treatment, and 66.7% of them (20/30) had tumors involved in deep structures including periventricular regions, basal ganglia, corpus callosum, brainstem and cerebellum. The median of longest lesion diameter on MR images was 2.3cm (range 0.5–5.1). Furthermore, 16.7% of patients (5/30) had lymphoma cells in the cerebral spinal fluid (CSF), and 3.3% (1/30) had lymphoma cells in vitreous fluid. All patients had received a median of 9g MTX at least once prior to AT treatment. Some patients had received other regimens including idarubicin, rituximab, premetrexed and lenalidomide previously. Other than chemotherapy, 6.7% of patients (2/30) had received whole brain radiotherapy previously.

Table 1
patients characteristics

| characteristics | All the patients (n = 30) |
|---|---|
| Age | 65 (25–79) |
| Sex | 18 (60%) |
| Male | 12 (40%) |
| Female | 0 (0%) |
| ECOG PS | |
| 0 | |
| 1 | 2 (6.6%) |
| 2 | 11 (36.7%) |
| 3 | 14 (46.7%) |
| 4 | 3 (10.0%) |
| Disease state | |
| Progression | 24 (80%) |
| Relapse | 6 (20%) |
| Numbers of lesion | 10 (33.3%) |
| Multifocal lesions | 20 (66.7%) |
| Single lesion | |
| Lesion location | |
| Deep | 20 (66.7%) |
| Superficial | 10 (33.3%) |
| Longest lesion diameter | 2.3 (0.5–5.1) |
| CSF involvement | 5 (16.7%) |
| Vitreous fluid involvement | 1 (3.3%) |

| characteristics | All the patients (n = 30) |
|---|---|
| Previous treatment | 9 (3–18) (unit: gram) |
| MTX | 7 (23.3%) |
| Rituximab | 17 (56.7%) |
| Idarubicin | 1 (3.3%) |
| Premetrexed | 1 (3.3%) |
| Lenalidomide | 2 (6.7%) |
| Whole brain radiotherapy | |

Treatment assessment and toxicity

43.4% of patients (13/30) achieved overall response (8 with complete remission or complete remission unconfirmed and 5 with partial remission). 40% of patients (12/30) developed disease progression. Median follow up was 16 months (95%CI:11–23 months). The median PFS was 1.75 months (95%CI: 1–4 months; Fig. 1A) and the median OS was 19.5 months (95%CI: 11 months to not calculable; Fig. 1B). The response assessment to AT treatment based on patients' characteristics were listed in Table 2. Patients in HLA-B low affinity group had a higher response rate ($p = 0.042$). Other factors such as KIR/HLA-A or KIR/HLA-C pairs, age, sex, ECOG performance status, tumor characteristics including numbers, location or volume of lesions, had no impact on treatment response. The side effect of AT treatment was mild and manageable (Table 3). In brief, 23.3% of patients (7/30) displayed neutropenia, and three of them developed agranulocytosis; all of these patients recovered soon with or without applying granulocyte-colony stimulating factors. 20% of patients (6/30) developed thrombocytopenia; among them, two patients developed grade 3 thrombocytopenia. All recovered without platelet transfusion. 13.3% of patients (4/30) were diagnosed with anemia; only one patient received red blood cell transfusion. 10% of patients (3/30) who had infection (diarrhea, urinary tract infection and sepsis, respectively) all recovered after antibiotics administration.

Figure 1. Kaplan-Meier curve of PFS (A) and OS (B) of total cohort

Table 2
Response to AT treatment according to patients' characteristics

| | Responders(CR + PR) | Non-responders(SD + PD) | |
|---|---|---|---|
| KIR and HLA-B pairs | 1 | 8 | P = 0.042 |
| HLA-B high affinity | | | |
| HLA-B low affinity | 12 | 9 | |
| KIR and HLA-A pairs | | | |
| HLA-A high affinity | 1 | 4 | P = 0.355 |
| HLA-A low affinity | 12 | 13 | |
| KIR and HLA-C pairs | | | |
| KIR2DL1 + and HLA-C2+ | 5 | 6 | P = 1.000 |
| KIR2DL2/3 + and HLA-C1+ | 8 | 11 | |
| Age | | | |
| Age ≥ 70y | 4 | 5 | P = 1.000 |
| Age < 70y | 9 | 12 | |
| Sex | | | |
| Male | 5 | 13 | P = 0.061 |
| Female | 8 | 4 | |
| ECOG PS | | | |
| ECOG ≥ 3 | 9 | 8 | P = 0.283 |
| ECOG < 3 | 4 | 9 | |
| Numbers of Lesion | | | |
| Multifocal lesions | 7 | 3 | P = 0.056 |
| Single lesion | 6 | 14 | |
| Deep involvement | | | |
| Yes | 11 | 9 | P = 0.119 |
| No | 2 | 8 | |
| Tumor volume | | | |
| longest diameter ≥ 3cm | 5 | 7 | P = 1.000 |

| | Responders(CR + PR) | Non-responders(SD + PD) | |
|---|---|---|---|
| longest diameter < 3cm | 8 | 10 | |
| CSF involvement | | | |
| Yes | 2 | 3 | P = 1.000 |
| No | 11 | 14 | |

Table 3
Toxicity of treatment

| | All cohort | Grade 1 | Grade2 | Grade3 |
|---|---|---|---|---|
| Neutropenia | 7(23.3%) | 3(10.0%) | 1(3.3%) | 3(10.0%) |
| Anemia | 4(13.3%) | 2(6.7%) | 1(3.3%) | 1(3.3%) |
| Thrombocytopenia | 6(20.0%) | 0 | 4(13.3%) | 2(6.7%) |
| Infection | | | | |
| sepsis | 1(3.3%) | 0 | 0 | 1(3.3%) |
| diarrhea | 1(3.3%) | 0 | 0 | 1(3.3%) |
| urinary tract | 1(3.3%) | 0 | 0 | 1(3.3%) |

Impact of KIR and HLA-I pairs on PFS and OS

KIR and HLA-I alleles were listed in Supplementary Table 1. Patients in HLA-B low affinity group appeared to have a longer median PFS (3 months) than those in HLA-B high affinity group (1 month) (p = 0.0079; Fig. 2A). In patients possessing KIR3DL1 and specific HLA-A alleles such as HLA-A*23:01, HLA-A*24:02 and HLA-A*32:01, which were predicted to have high affinity with KIR3DL1, did not have an impact on PFS (p = 0.092; Fig. 2B). No significant difference of PFS was found between patients with KIR2DL1 and HLA-C2 alleles (high affinity) and those possessing KIR2DL2/3 and HLA-C1 alleles (low affinity) (p = 0.72; Fig. 2C). HLA-B (p = 0.91), HLA-A (p = 0.64) and HLA-C (p = 0.59) alleles had no impact on the OS of the cohort.

Patients benefitting from AT regimens

Age had no impact on PFS (p = 0.21; Fig. 3A) or OS (p = 0.83; Fig. 3B). In addition, there is no sex related impact on PFS (p = 0.62) or OS (p = 0.64) observed. ECOG ≧ 3 was found to be an independent unfavorable prognostic marker for PCNSL [30]. However, it did not demonstrate shorter PFS (p = 0.83; Fig. 3C) or OS (p = 0.80; Fig. 3D) in this cohort. Interestingly, patients with multifocal lesions, which were considered as an unfavorable factor, developed longer median PFS (3.5 months) than those with single lesion (1 month) (p = 0.019; Fig. 3E). There was no significant difference of median OS between

multifocal-lesion group and single-lesion group (p = 0.15; Fig. 3F). Patients with deep involvements including periventricular regions, basal ganglia, corpus callosum, brainstem and cerebellum, which were regarded as an unfavorable factor, had longer PFS (2.5 months) than those with pallium involvement only (1 months) (p = 0.013; Fig. 3G), however, no significant longer OS was observed between two groups (p = 0.10; Fig. 3H). Patients with larger tumor volume had similar median PFS (p = 0.41; Fig. 3I) or OS (p = 0.15; Fig. 3J) with the counterpart. Cerebral spinal fluid involvement showed no further impact on the PFS (p = 0.61) or OS (p = 0.26) in this cohort. Multivariate analysis by COX regression indicated that KIR3DL1/HLA-B pairs predicting low affinity (score 0 or 1) were independently associated with the longer PFS (p = 0.042; Table 4). There was no significant difference of distribution of KIR3DL1/HLA-B pairs between each subgroups (supplementary Table 2).

Table 4 Multivariate analysis by COX regression for PFS

| Factors | P value | Hazard Ratio[95%CI] |
|---|---|---|
| HLA-B low affinity (score 0 or 1) | 0.042 | 2.56(1.03-6.33) |
| Age≥70y | 0.97 | 1.02(0.46-2.25) |
| Multifocal lesions | 0.18 | 0.51(0.20-1.35) |
| Deep involvement | 0.22 | 0.56(0.22-1.41) |
| Longest lesion diameter | 0.48 | 1.32(0.61-2.88) |

# Discussion

The prognosis of PCNSL has been improved since the application of high dose MTX. But for those who were resistant to MTX or too fragile to tolerate high dose MTX, treatment options are limited. Salvage radiotherapy has been reported to achieve an ORR of 80% and 2-year OS of 57% for refractory or recurrent PCNSL [31], but neurocognitive complications need to be carefully considered especially for the elderly [32, 33]. Radiotherapy is not a priority, especially when other non-MTX regimens that can penetrate blood brain barrier have been developed. Cytarabine is a classical chemotherapeutic agent, which can penetrate blood brain barrier in large doses. It has proven efficacy in combination with high dose MTX in newly diagnosed PCNSL [34]. But severe infectious complications partly caused by 4 doses of cytarabine occurred in up to 28% of patients in the study. In an another retrospective study, cytarabine was administered as a single agent for 4 doses and resulted in severe toxicities such as thrombocytopenia and neutropenic fever although granulocyte-monocyte colony stimulating factor had been applied routinely [14]. In order to reduce the toxicities, cytarabine was administered for 2 doses in our study. As a result, this dose was well tolerated even in elderly patients without routine administration of granulocyte colony stimulating factor. Temozolomide, as an oral alkylating agent, has shown efficacy and safety in the treatment of newly diagnosed PCNSL when combined with high dose MTX and radiotherapy [35] or as consolidation treatment when combined with etoposide [36]. As for r/r PCNSL, temozolomide combined with bruton's kinase inhibitor ibrutinib achieved an ORR of 55% and a median PFS of 5.3 months [37]. Cytarabine plus temozolomide regimen produced an ORR of 43.3% in our study, which was similar to single administered cytarabine as previously reported, and the median PFS was not satisfactory.

Some studies have reported that chemotherapy reagents contributed to initiate tumor cell death by boosting natural killer cells [38, 39]. In this study, patients with KIR3DL1 and HLA-Bw6 or HLA-Bw4-80T genotypes predictive of mismatch or low affinity benefited from AT regimen with a higher response rate and a median PFS of 3 months, longer than that of patients with KIR3DL1 and HLA-Bw4-80I genotypes predictive of high affinity. The prognostic effect of KIR and HLA-I genotypes has been evaluated in other studies [40, 41]. NK cells exert anti-tumor activity when corresponding HLA-I ligand for KIR is missing. As found in the hematopoietic stem cell transplantation (HSCT) settings, donor-recipient KIR3DL1/HLA-B combinations predictive of week inhibition or mismatch were associated with lower relapse [42]. To our knowledge, the impact of KIR and HLA genetic polymorphisms on non-immune cytotoxic chemotherapy have not been evaluated in non-HCST settings before. The results of this study suggested that cytarabine combined with temozolomide killed tumor cells not only by cytotoxicity, but also by boosting NK cell activity simultaneously. Patients who harbouring KIR3DL1/HLA-B pairs predictive of mismatch or low affinity would benefit from NK cell activation. As for those who had high affintiy KIR and HLA-B pairs, tumor cells could escape from NK cell killing by augmenting HLA-B expression. Multifocal lesions and deep involvement, which are supposed to be unfavorable factors of PCNSL, were found to be associated with slightly longer PFS, suggesting that AT regimen's effect, to some extent, was not dependent on cytotoxicity. This was also suggested by the results that elderly ages, high ECOG and high tumor burden were not associated with shorter PFS as previously indicated.

KIR and HLA genotypes showed no significant impact on the OS of the cohort in this study. Patients who did not respond to AT regimen would continue to receive other chemotherapy or whole brain radiation therapy. The results suggested that KIR3DL1/HLA-B pairs may not be associated with the prognosis of PCNSL. We have not observed KIR3DL1/HLA-A or KIR2DL1/HLA-C2 pairs had any impact on the PFS or OS of this cohort. It could be inferred that KIR3DL1/HLA-B may have specific roles in chemotherapy induced immune response.

The sample size is small in this study, so the conclusion needs to be further confirmed by a larger sample size in future. Interestingly, only four types of immune cells-B cells, T cells, macrophages and dentritic cells has been identified in the microenvironment of PCNSL up to now [43]. If NK cells did not reside in the tumor microenvironment, how could they exert anti-tumor effect? Is there possibility that NK cells migrate into central nervous system from peripheral blood after chemotherapy, which means AT regimen could boost NK activity and promote NK cells penetrating blood brain barrier? The hypothesis remains to be elucidated.

# Abbreviations

PCNSL, primary central nervous system lymphoma; DLBCL, diffuse large B cell lymphoma; MTX, methotrexate; OS, overall survival; ORR, overall response rate; ASCT, autologous stem cell transplantation; PFS, progression free survival; NK cells, natural killer cells; KIR, killer cell immunoglobulin-like receptor; HLA-I, human leukocyte antigen class I; ADCC, antibody dependent cell medicated cytotoxicity; AT, high dose cytarabine and temozolomide; CR, complete remission; PR, partial remission; SD, stable disease; PD,

progressive disease; HLA-Bw4-80I, HLA-Bw4 with an isoleucine at position 80; HLA-Bw4-80T, HLA-Bw4 with a threonine at position 80; HSCT, hematopoietic stem cell transplantation.

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# Figures

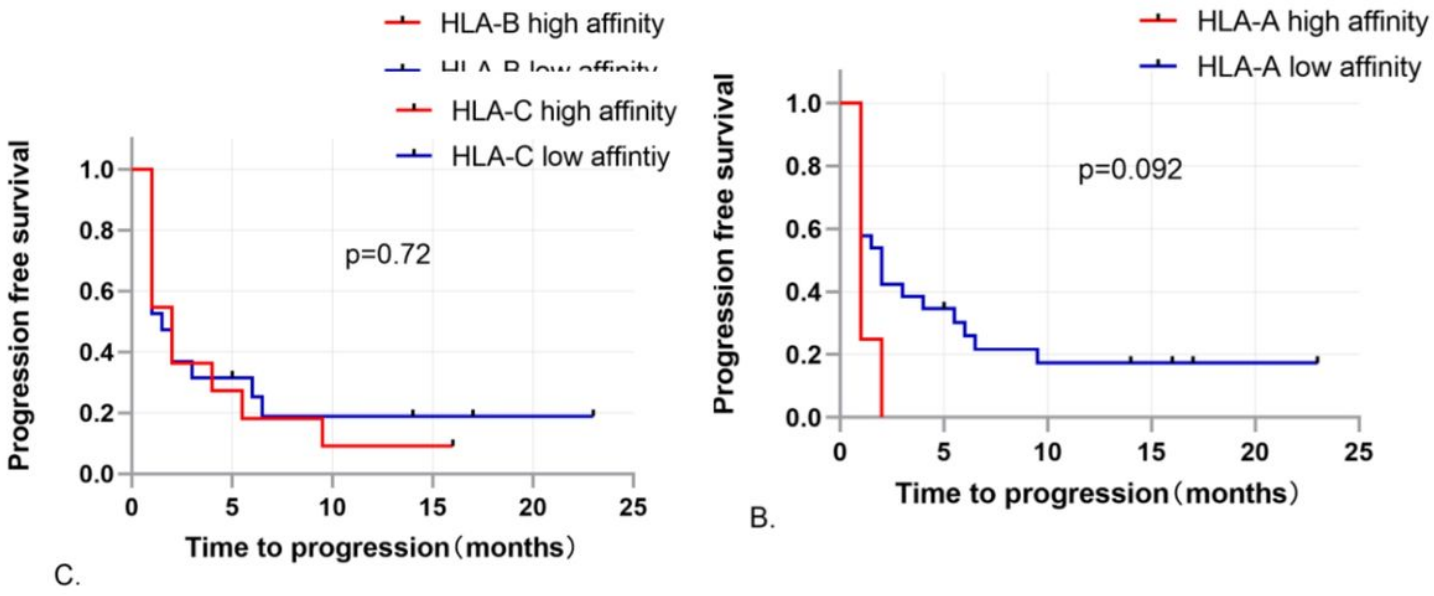
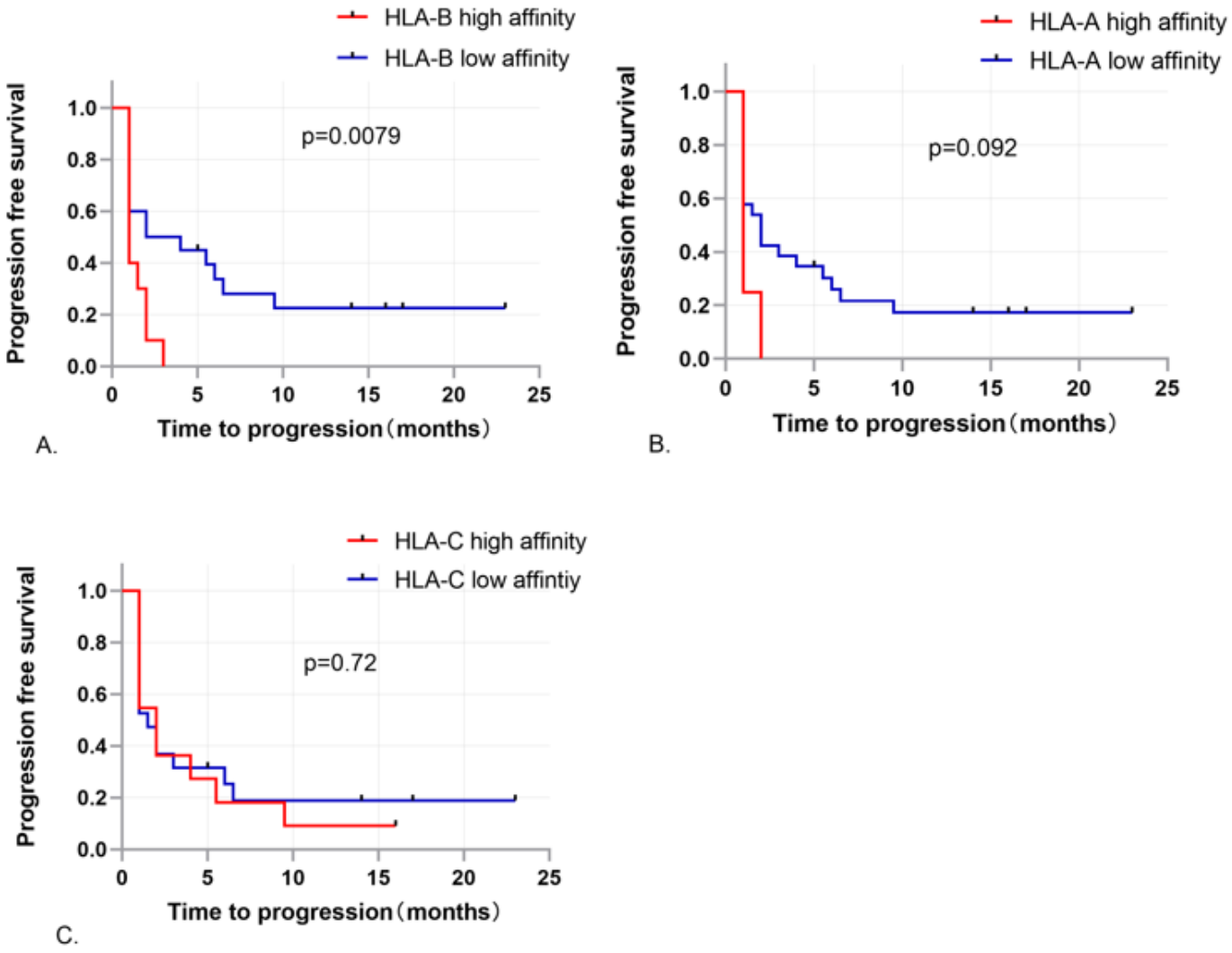


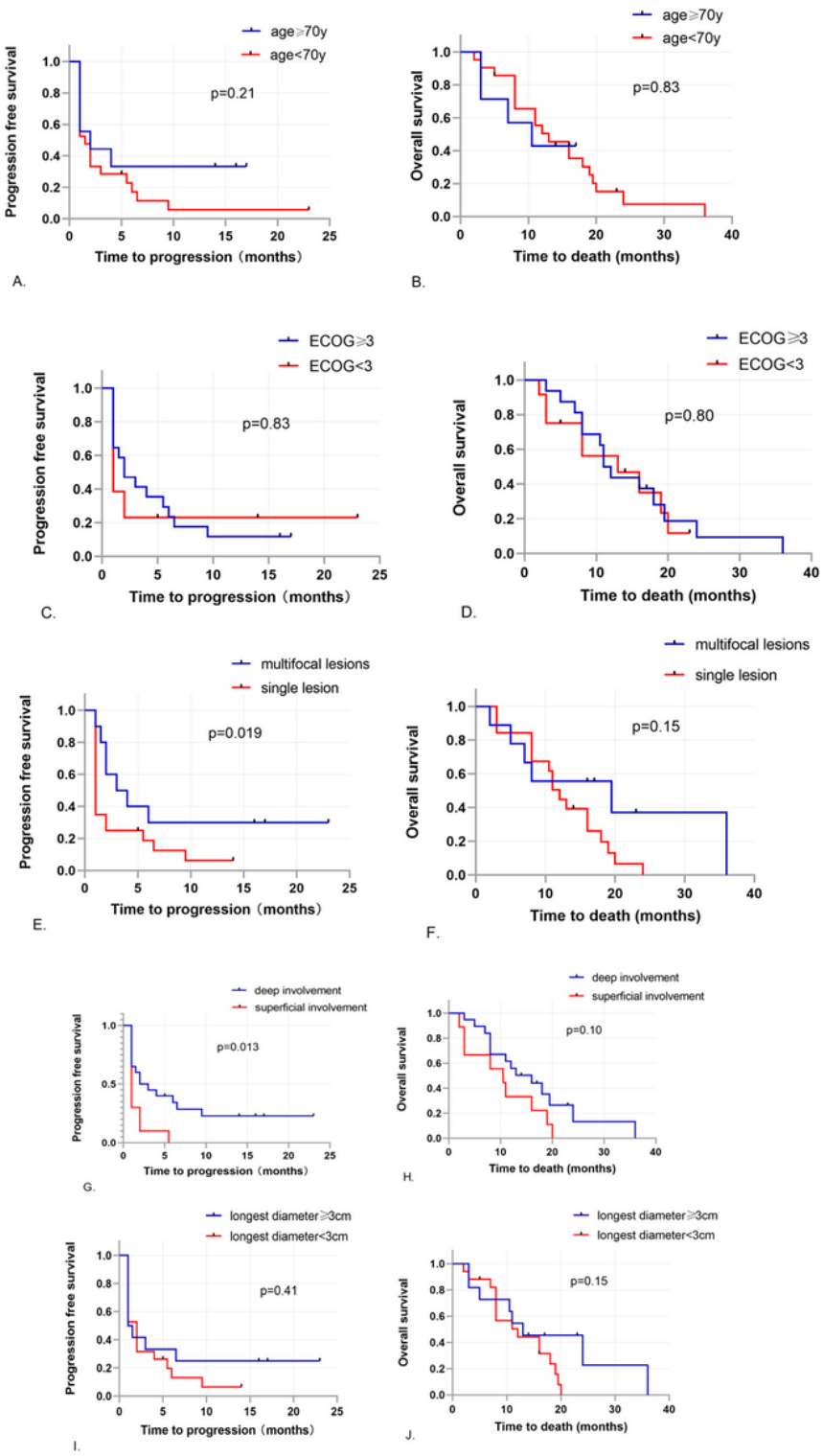

**Figure 1**

Kaplan-Meier curve of PFS (A) and OS (B) of total cohort




## Figure 2

Kaplan-Meier curve of patients with specific KIR-HLA genotypes groups (A) PFS of patients with KIR3DL1 and HLA-B (high affinity) and patients with KIR3DL1 and HLA-B (low affinity) (B) PFS of patients with KIR3DL1 and HLA-A (high affinity) and patients with KIR3DL1 and HLA-A (low affinity) (C) PFS of patients with KIR2DL1 and HLA-C2 (high affinity) and patients with KIR2DL2/3 and HLA-C1 (low affinity)




## Figure 3

Kaplan-Meier curve of patients with specific characteristics (A) PFS of patients≧70ys and patients<70ys (B) OS of patients≧70ys and patients <70ys (C) PFS of patients with high ECOG (≧3) and patients with low ECOG(<3) (D) OS of patients with high ECOG (≧3) and patients with low ECOG(<3) (E) PFS of patients with multifocal lesions and patients with single lesion (F) OS of patients with multifocal lesions and patients with single lesion (G) PFS of patients with deep involvement and patients with superficial

involvement (H) OS of patients with deep involvement and patients with superficial involvement (I) PFS of patients with largest lesion diameter≧3cm and patients with largest lesion diameter<3cm (J) OS of patients with largest lesion diameter≧3cm and patients with largest lesion diameter<3cm

## Supplementary Files

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