The Outcome of HyperCVAD Combined with Alemtuzumab for the Treatment of Aggressive T-Cell and NK-Cell Neoplasms

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Abstract We report our experience in using six cycles of hyperCVAD in combination with alemtuzumab for the treatment of aggressive T-cell and NK/T-cell neoplasms. Seven females and six males with the median age of 41 (range 18–60) diagnosed with T-cell acute lymphoblastic lymphoma and peripheral T-cell and NK/T-cell neoplasms ($n_{\text{PTCL}} = 6$, $n_{\text{T-cell ALL}} = 3$, $n_{\text{NK/T-cell neoplasms}} = 4$) from 2006 to 2008 were treated with alemtuzumab–hyperCVAD regimen. A total of nine patients (69%) responded to the regimen, with seven achieved complete remission and two achieved partial remission. The median progression free survival and overall survival duration among the responders with complete remission were 12.9 and 24.9 months respectively. The incidence of relapse among the responders was 44% and the overall survival rate was 23%. Only four (31%) patients completed the six cycles of alemtuzumab–hyperCVAD. Others were stopped earlier due to progressive disease ($n = 2$), cytomegalovirus (CMV) reactivation and/or disease ($n = 3$), death not due to disease ($n = 2$), and patient's refusal to continue alemtuzumab ($n = 2$). The incidence of death not due to disease, CMV reactivation and recurrent CMV reactivation were 50, 50 and 17%, respectively. This study shows that alemtuzumab in combination with hyperCVAD regimen is a feasible regimen but with high toxicity. The toxicity might be

reduced with the incorporation of filgrastim and use of valganciclovir as CMV prophylaxis.

Keywords HyperCVAD · Alemtuzumab · T-cell · NK-cell

Introduction

The mature or peripheral T-cell and Natural Killer (NK)/T-cell neoplasms are rare, representing 12% of NHL worldwide [1]. Within this minority of diseases, peripheral T-cell lymphoma (PTCL), not otherwise specified (NOS) is the commonest, accounting for 25.9% of them. Majority of the subtypes of T-cell and NK-cell neoplasms represent less than 1% of NHL [2]. In general, T-cell and NK-cell neoplasms are more common in Asia [1–3] probably due to the higher prevalence of viruses such as the Human T-Lymphotropic Virus-1 (HTLV-1) which is associated with the adult T-cell leukemia/lymphoma, and Epstein Barr Virus (EBV) which is associated with the extranodal NK/T-cell lymphoma, nasal type, aggressive NK leukemia and the pediatric EBV T-cell and NK/T-cell lymphomas [4].

An anthracycline-containing regimen like CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone), which is the standard therapy for diffuse large B-cell lymphoma (DLBCL) when combined with rituximab, is commonly used for PTCL and NK/T-cell neoplasms. However, the clinical outcome is poor with this therapy except for anaplastic large-cell lymphoma (ALCL), ALK positive type [2]. Large prospective series did indicate a higher relapse rate and a poorer survival for PTCL than DLBCL [2, 5–13]. Various chemotherapy regimens have been tried out—such as MegaCHOP (intensive or higher dose of CHOP) with or without etoposide [14, 15],

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epirubicin substituted for doxorubicin and addition of bleomycin (CEOP-B) [16], CMED (cyclophosphamide, etoposide, methotrexate, and dexamethasone) [17], CHOP or MegaCHOP/ESHAP (etoposide, cisplatin, cytarabine and prednisone) [18, 19] and VCAP-AMP-VECP (vincristine, cyclophosphamide, doxorubicin, and prednisone (VCAP); doxorubicin, ranimustine, and prednisone (AMP); vindesine, etoposide, carboplatin, and prednisone (VECP)) [20]; fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with high doses of methotrexate and cytarabine (hyperCVAD) [21, 22]—with no superior efficacy to CHOP except CMED at the expense of higher treatment toxicity. Gemcitabine-based chemotherapies are emerging and seem quite compromising [23–27].

Since there is no standard treatment for PTCL and NK-cell neoplasms, it is worth attempting adding a monoclonal antibody to chemotherapy. Alemtuzumab appears to be the most reasonable choice. It is a humanized monoclonal antibody that targets CD52, a cell surface protein present at high density on most normal and malignant B and T lymphocytes. As a single agent, alemtuzumab has been used to treat pretreated cutaneous/PTCL with overall response rate (ORR) of 36–60% [28–30]. A higher ORR of 50% was observed in the treatment of mycoses fungoides [30]. Successful treatment with alemtuzumab has been reported in the subtypes of mature T-cell neoplasms, such as Lennert's lymphoma [31], hepatosplenic gamma/delta T-cell lymphoma [32], angioimmunoblastic T-cell lymphoma [33] and primary cutaneous CD30-negative CD8-positive cytotoxic large T-cell lymphoma [34]. Data of its application in aggressive NK-cell leukemia/lymphoma is scanty with possible potential efficacy [35, 36]. However, its combination with chemotherapy is more likely leading to more sustain and higher response rate in view of the aggressiveness of the disease.

Alemtuzumab has been used in combination with different chemotherapy regimens to improve treatment outcome of PTCL and NK/T-cell lymphoma, such as fludarabine containing regimen in PTCL, NOS [37], DHAP for relapse PTCL, NOS [38], gemcitabine in enteropathy-type T-cell lymphoma [39], cladribine [40] or fludarabine [41] in hepatosplenic gamma-delta T-cell lymphoma. Larger combination trials on alemtuzumab with CHOP [42–44] or EPOCH (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) [45] are still ongoing.

Fractionated cyclophosphamide, vincristine, doxorubicin and dexamethasone (hyperCVAD) alternating with high doses of methotrexate and cytarabine has been used in treating PTCL [21] but did not show favorable outcome than CHOP [22]. However, alemtuzumab in combination with hyperCVAD has not been evaluated before. Therefore, our group pioneered the use of alemtuzumab in

combination with hyperCVAD in the treatment of aggressive T-cell and NK/T-cell neoplasms. The objective of this study was to evaluate the toxicity and effectiveness of this treatment regimen among a selected cohort of patients with aggressive T-cell and NK/T-cell neoplasms in our center.

Materials and Methods

Patient Selection

All patients referred to our centre from April 2006 to May 2008 meeting the inclusion criteria were recruited into the study. The inclusion criteria for this study were as followed: (1) patients aged 18 years old and above, (2) newly diagnosed or relapsed aggressive T-cell and NK/T-cell neoplasms of the following subtypes based on the WHO classification [46]: ALCL, ALK-, NK/T-cell NHL, including nasal and extranasal, PTCL, relapsed angioimmunoblastic T-cell lymphoma (AITL), T-cell lymphoblastic lymphoma (T-ALL), aggressive NK-cell leukaemias, (3) Eastern Cooperative Oncology Group score ≤ 2 , (4) complete work up for baseline evaluation and measurement, (5) adequate hematological, heart, renal and hepatic function, and (6) patient's written informed consent.

Patients with following exclusion criteria were excluded from the study: (1) mature T-cell and NK/T-cell neoplasms of the following subtypes: mycosis fungoides and Sezary syndrome, subcutaneous panniculitis, and ALCL, T/null cell, anaplastic lymphoma kinase (ALK) positive, (2) a known hypersensitivity to murine proteins or to any component of alemtuzumab, (3) patients with central nervous system metastasis, (4) serologic evidence of human immunodeficiency virus (HIV) exposure, (5) history of impaired cardiac status or myocardial infarction, (6) baseline serum creatinine $\geq 158 \mu\text{mol/l}$, bilirubin ≥ 1.5 times upper limit of normal range, aspartate transaminases or alanine transaminases ≥ 3 times upper limit of normal range, unless due to tumor involvement, (7) presence of active uncontrolled infection, active non-malignant gastric or duodenal ulcer, uncontrolled diabetes mellitus or other severe medical conditions which would preclude aggressive cytotoxic chemotherapy, (8) pregnant or lactating women, (9) serious medical or psychiatric illness which prevent informed consent, (10) patients who are likely to lost to follow up (e.g. unwilling or difficult to return, cannot be contacted), and (11) previous treatment with alemtuzumab or hyperCVAD regimen.

Study Design and End Points

This is a Phase II, open label trial. The cohort of patients was followed up until the secondary endpoints were

achieved or the termination of the study. The primary endpoints of this study are complete remission (CR) rate, partial remission (PR) rate, and overall response rate (ORR). The secondary endpoints are progression free survival (PFS) and overall survival (OS).

Treatment Regimen

The treatment regimen consisted of hyperCVAD A alternates with hyperCVAD B every three to four weeks depending on the white cell recovery and general condition. In total, hyperCVAD A and hyperCVAD B were given three times, respectively. Alemtuzumab was added on the first day of every chemotherapy cycle. In the last three patients recruited, additional dose of alemtuzumab was given on day 4 of every cycle after reviewing the first 10 patients and obtaining ethical approval from Medical Research and Ethic Committee (MREC), Ministry of Health (MOH) Malaysia. The detail of the regimen is as follows:

(1) Alemtuzumab-hyperCVAD A

- Alemtuzumab 30 mg subcutaneously on day 1 (and day 4 for the last three patients)
- Cyclophosphamide 300 mg/m² twice a day intravenously over 3 h on day 1–3
- Doxorubicin 50 mg/m² intravenously over 2 h, to be given 12 h after last dose of cyclophosphamide on day 4
- Vincristine 1.4 mg/m² (max 2 mg) bolus, to be given intravenously 12 h after last dose of cyclophosphamide on day 4 and 11
- Mesna 450 mg/m² intravenous infusion over 24 h, starts 1 h before cyclophosphamide on day 1–4
- Intrathecal methotrexate 12 mg on day 2
- Intrathecal cytarabine 100 mg on day 7
- Dexamethasone 40 mg/day intravenously or orally on day 1–4 and day 11–14

(2) Alemtuzumab-hyperCVAD B

- Alemtuzumab 30 mg subcutaneously on day 1 (and day 4 for the last three patients)
- Methotrexate 200 mg/m² intravenously over 2 h, then 800 mg/m² intravenously over 22 h on day 1
- Cytarabine 3 g/m² twice a day intravenously over 2 h on day 2
- Folinic acid rescue 60 mg intravenously, starts from 42 h of methotrexate start time, then 15 mg intravenously six hourly for five doses, or more if therapeutic drug monitoring methotrexate level is high
- Intrathecal methotrexate 12 mg on day 2
- Intrathecal cytarabine 100 mg on day 7

- Filgastrim 5 mcg/kg daily subcutaneously starting on day 4 (24 h after last dose intravenous cytarabine) till total white cell count has recovered

Intravenous diphenhydramine 50 mg and oral acetaminophen 500 mg were administered 30 min before each alemtuzumab dose. Patients with severe reactions were premedicated with intravenous hydrocortisone 50–100 mg with or without pethidine 20–30 mg. The consolidative local radiotherapy was given when deemed appropriate for locally invasive diseases and bulky disease at or more than 10 cm. All patients received oral acyclovir 200 mg thrice daily as anti-viral prophylaxis. Other prophylaxis agents included oral trimethoprim/sulfamethoxazole 80/400 mg two tablets twice a week and fluconazole 200 mg daily throughout the study and were continued for three months post protocol. The physician in-charge of the patient would treat the patient according to their practice in any other circumstances that arose, for example during neutropenic period.

Pretreatment Assessment

The tissue biopsies of all enrolled lymphoma cases were reviewed at a central pathology registry for confirmation of diagnosis. Two hematopathologists from our centre reviewed the bone marrow aspirate (BMA) and trephine biopsy.

Pretreatment evaluations includes physical examination involving examination of all the superficial lymph node groups, full blood count and differential, chemistry profile including lactate dehydrogenase, liver and kidney function, serology testing for HIV, chest X-ray, computerized tomography (CT) scan of the base of skull (for cases involving nasal or paranasal sinuses region), neck, thorax, abdomen and pelvic region in lymphoma cases, and electrocardiography. A negative urine pregnancy test was required for all women of childbearing potential. Quantitative polymerase chain reaction (PCR) for cytomegalovirus (CMV) deoxyribonucleic acid (DNA) was included in pretreatment evaluation starting from second patient due to high incidence of CMV reactivation and/or disease based on the recommendations from the published papers [47–50]. Positivity of the test will exclude patient from the study. However, pretreatment CMV DNA PCR was negative for all the screening subjects. Other additional tests were performed on individual cases according to the needs of patient management and discretions of the treating physician.

Response and Safety Assessment

Patients were monitored for response and complications with clinical evaluation and investigations as in

pretreatment assessment before each cycle. As surveillance for CMV reactivation, CMV DNA PCR test was performed before each cycle and monthly for one year during the follow-up period. Gancyclovir was given to the patients with CMV reactivation or clinically suspect CMV disease while waiting for CMV DNA PCR result. Patient with first episode of asymptomatic CMV reactivation was allowed to continue the study.

The response assessment of lymphoma and the leukemia cases were made according to the revised response criteria for malignant lymphoma [51] and acute myeloid leukemia [52] respectively. In lymphoma cases, restaging CT scans were performed before fourth cycle (hyperCVAD B2), after sixth cycle (hyperCVAD B3) and every six months for two years thereafter. In leukemia cases or lymphoma cases with evidence of bone marrow infiltration, BMA and trephine were repeated before each hyperCVAD A.

Toxicities during treatment were graded according to WHO toxicities criteria. The patients who had completed the study were followed up for the evidence of progressive disease.

Stopping Rule

The patients who show evidence of progressive disease at any time during the study were discontinued from the study. Other stopping rules for the study were occurrence of a serious infection or unacceptable toxicity and patient preference.

Operational Definition and Statistical Analysis

The ORR is the sum of CR and PR. The PFS is calculated from the date of diagnosis based on either the date of BMA or tissue biopsy whichever is relevant, to the date of confirming the disease relapse, based on either the date of BMA or imaging study. The OS is calculated from the date of diagnosis to the date of death. Death not due to the disease is defined as any death that was not caused directly by the effect of disease irrespective of the disease progression status. This includes death due to neutropenia secondary to chemotherapy.

The difference between two continuous data was tested using Mann–Whitney U test. The overall survival curves were calculated using the Kaplan–Meier method. All statistical analysis was carried out using Statistical Package for Social Science (SPSS) software version 15.0. The significant level was set at 0.05 unless otherwise specified.

Ethical Consideration

This study has obtained ethical approval from MREC, MOH Malaysia. All patients recruited into the study were

given patient information sheet as well as the detailed verbal explanation on the conduct of the study. The patients provided voluntary, written informed consent before participation in the study. The study was conducted in accord with the declaration of Helsinki and the International Conference on Harmonization for Good Clinical Practice.

Results

A total of 13 patients (11 new cases and two relapse cases) were recruited into the study. The characteristics of the 13 patients are reported in Table 1. There are four cases of NK/T-cell neoplasms, three cases of T-cell acute lymphoblastic lymphoma (ALL), and six cases of PTCL. One of the lymphoma cases has bone marrow involvement (a 54 years old lady with T-cell ALL). The study had stopped recruiting patient since June 2008 and terminated prematurely due to poor survival outcome attribute from the high death rate and CMV reactivation or disease rate. We followed up the cohort of patients till 31st October 2009 and the outcomes are summarized in Table 2.

Therapeutic Outcomes

Nine patients (69%) responded to the alemtuzumab–hyperCVAD regimen, with seven achieved CR and two achieved PR. Two patients diagnosed with aggressive NK-cell leukemia and two relapse cases did not respond to the regimen. If these patients were to be excluded from the

Table 1 Characteristics of all study subjects

Characteristic	Value
Age, median (range)	41 (18–69)
Gender, <i>n</i> (%)	
Male	6 (46)
Female	7 (54)
Histologic subtypes, <i>n</i> (%)	
Aggressive NK leukaemia	2 (15)
Precursor T-ALL	3 (23)
T-prolymphocytic leukaemia	1 (8)
Anaplastic T-cell lymphoma, ALK negative, primary cutaneous type	2 (15)
ATLL	1 (8)
Anaplastic T-cell lymphoma, ALK negative, primary systemic type	2 (15)
NK/T-cell lymphoma, extranodal, nasal type	2 (15)
Elevated LDH, <i>n</i> (%)	6 (46)
Albumin <30 g/l, <i>n</i> (%)	3 (23)

Table 2 Subjects' response to treatment using alemtuzumab in combination with hyperCVAD regimen (the sequence of patient does not follow the sequence of enrollment)

Patient	Age	Sex	Histopathology subtype	Total dose of alemtuzumab	Total cycle of hyperCVAD	Death	Death not due to disease	Response parameter	PFS (months)	OS (months)	CMV reactivation ^a	CMV disease ^a
1 ^b	33	F	Aggressive NK leukemia	4	2	Y	N	PD	0	7.4	N	N
2	36	M	Aggressive NK leukemia	4	6	Y	N	PD	0	9.0	NA	NA
3	26	M	Pre T-ALL ^c	2	4	Y	Y	PD	0	7.5	Y (1)	Y (1)
4	41	F	Pre T-ALL	4	4	Y	Y	CR	4.2	4.2	Y (1)	N
5	54	F	Pre T-ALL	6	6	Y	N	CR	12.7	14.2	Y (1)	Y (1)
6	53	M	Anaplastic T-cell lymphoma, ALK negative, primary cutaneous type	6	6	Y	N	CR	12.4	25.4	N	N
7	69	M	Anaplastic T-cell lymphoma, ALK negative, primary cutaneous type ^c	2	2	Y	N	PD	0	9.8	N	N
8	52	F	ATLL	6	6	Y	Y	PR	12.4	18.4	N	N
9 ^b	51	M	Anaplastic T-cell lymphoma, ALK negative	7	5	Y	Y	PR	7.8	7.8	Y (2)	N
10 ^b	22	F	Anaplastic T-cell lymphoma, ALK negative	8	5	N	NA	CR	26.5	26.5	Y (1)	Y (1)
11	18	F	NK/T-cell lymphoma, extranodal, nasal type	5	6	Y	Y	CR	15.5	15.5	N	N
12	49	F	NK/T-cell lymphoma, extranodal, nasal type	5	5	N	NA	CR	34.3	34.3	Y (2)	N
13	36	M	T-PLL	3	6	N	NA	CR	11.3	40.1	N	N

^a Values in parentheses represent number of episode(s) with CMV complications^b The last three patients recruited in the study and received additional dose of alemtuzumab 30 mg subcutaneously on day 4 of every cycle^c Relapse case

All stated outcomes are as of 31st October 2009

PD indicates progressive disease, CR complete remission, PFS progression free survival, OS overall survival, F female, M male, N no, Y yes, NA data not available

study, the ORR of alemtuzumab-hyperCVAD in our study achieved 100% with CR and PR rate of 78 and 22% respectively. If only the two relapse cases were excluded from the study, the overall response rate was 73%. Four responded patients relapsed (44%) and the OS rate was only 23%. The median PFS and OS among the respondents with CR were 12.4 and 25.4 months respectively. The survival curve of all patients is shown in Fig. 1a, b.

We also analyzed the impact of alemtuzumab to the treatment outcome by looking at the ratio of total alemtuzumab to the total cycle of hyperCVAD regimen. Patients received alemtuzumab at the ratio of ≥ 1 seems to do better with longer PFS (median = 12.4 months) and OS (median = 14.2 months) as compared to those who received less (median PFS = 5.7 months and median

OS = 12.3 months) although the differences are not statistically significant (P value of 0.433 and 1, respectively).

The last three recruited patients indicated in Table 2 (patient number 1, 9 and 10) were given additional dose of alemtuzumab on day 4 of every cycle in order to increase efficacy after observed the non-sustaining remission from the patient with aggressive NK-cell leukemia who progressed upon completing two cycles of alemtuzumab-hyperCVAD. This attempt has improved treatment efficacy but also increased treatment-related toxicity.

Toxicity

Only four patients (31%) managed to complete the total six cycles of alemtuzumab-hyperCVAD regimen. Others were

Fig. 1 **a** Kaplan–Meier estimate of the overall survival of all patients. **b** Kaplan–Meier estimate of the progression free survival among the patients without progressive disease

stopped earlier due to progressive disease (two patients), CMV reactivation and/or disease (three patients), death not due to disease (two patients), and patient's refusal to continue alemtuzumab as part of the treatment (two patients). Among those patients terminated earlier due to CMV reactivation and/or disease, one died of neutropenic sepsis after the fifth cycle of hyperCVAD and two survived and in remission phase. These two survivors have received five cycles of alemtuzumab-hyperCVAD prior to termination. The cause of death not due to disease in the two patients was neutropenic sepsis, which developed after the fourth cycle of alemtuzumab-hyperCVAD. The three patients who refused to continue alemtuzumab as part of the treatment did however completed six cycles of hyperCVAD.

All patients developed grade three or four neutropenia after each chemotherapy cycle forcing delay of subsequent chemotherapy to four weekly intervals with filgrastim rescue. Severe neutropenia also contribute to treatment mortality of which three patients died of neutropenic sepsis from the alemtuzumab-hyperCVAD regimen. One of the deaths occurred after hyperCVAD A, and two occurred after hyper CVAD B. Two of these three patients achieved CR and PR respectively during their interim assessment.

The mortality rate in this study was 77%. The incidence of death not due to disease among all death was 50% (five patients). The causes of deaths not due to disease were progressive lung fibrosis, neutropenic sepsis after etoposide peripheral stem cell mobilization and neutropenic sepsis after chemotherapy.

The incidence of CMV reactivation and recurrent CMV reactivation in our patients were 50 and 17%, respectively. Half of the patients who have CMV reactivation developed CMV disease. The CMV reactivation and/or disease occurred in patients with as low as two doses of alemtuzumab. The incidence of CMV reactivation is 40% among the patients who received four or lower doses of alemtuzumab and 57% among those who received more than four doses ($P = 0.621$). CMV related complication is the major reason for termination of therapy especially among the three patients who have received additional dose. Two of them developed CMV reactivation after three cycles of day 1 and 4 alemtuzumab-hyperCVAD. Although alemtuzumab was reduced to only day 1 in the subsequent cycles, both were terminated earlier from the study due to similar complications. One developed CMV retinitis after fifth cycle of alemtuzumab-hyperCVAD while the other developed recurrent CMV reactivation following fourth cycle of alemtuzumab-hyperCVAD and subsequently died of neutropenic sepsis after fifth cycle of hyperCVAD.

Discussion

The cohort study design rather than randomized controlled trial was utilized in this study because the incidence of mature T-cell and NK/T-cell neoplasms is very low in our center. Hence, we are unable to compare the effectiveness of alemtuzumab-hyperCVAD against hyperCVAD regimen alone. Besides, the final sample size in this study was small, resulting in inability to arrive at statistically significant conclusion. This has been attributed to premature termination of the recruitment due high death rate and CMV reactivation and/or disease among the study subjects during the treatment period. Nevertheless, the results of this study provide some insight into the level of toxicity of alemtuzumab-hyperCVAD regimen.

The ORR in this study was high, about 69% for all patients, and 82% if excluding aggressive NK-cell leukemia, and 100% if excluding aggressive NK-cell leukemia and relapse cases. Although our ORR seems similar to other studies using various regimen [15–20, 23–27, 42–44, 53], the result needs to be interpreted carefully because other similar studies using alemtuzumab-CHOP have the highest number of PTCL, NOS cases among their study subjects [42, 43], whereas no PTCL, NOS subject was recruited into our study during the study period.

All subjects in our study developed cytopenia during the chemotherapy. This could be attributed to two reasons, the addition of alemtuzumab to standard hyperCVAD regimen and non-incorporation of routine granulocyte colony-stimulating factor (GCSF) (lenograstim or filgrastim) in our hyperCVAD regimen. In Australia, Gill et al. [54] reported 6.4% of the 187 cycles with dose delay among patients treated with hyperCVAD regimen were due to cytopenia and the cytopenia persisted in 59% of the 77 evaluable patients at three months after the completion of therapy. Although there was a considerable rate of cytopenia among their study subjects, the incidence was very much lower as compare to our study. This could be attributed to the benefit of GCSF used in their hyperCVAD regimen as compared to ours.

The OS rate is very poor and treatment related toxicities were very high in our study. This might not be solely attributed to the addition of alemtuzumab to hyperCVAD regimen as the regimen itself carries significant morbidity and mortality. However we are not able to establish the extent of which alemtuzumab contributed to the overall toxicities among the study subjects in addition to the standard hyperCVAD regimen. The data from the hyperCVAD origin center [22] does not provide adequate information on the efficacy and toxicities of hyperCVAD in treating PTCL as there was no specific breakdown of hyperCVAD toxicity in the original paper. In contrast, we

have 23% incidence of neutropenic sepsis death and 100% rate of chemotherapy delay. This could be due to the higher intensity of the protocol, our patient population's intolerance to the chemotherapy regimen as compared to Caucasian patient, non-incorporation of filgrastim routinely in hyperCVAD A and the use of lower dose filgrastim 5 mcg/kg/day instead of 10 mcg/kg/day in the original protocol [55]. Despite the higher chemotherapy intensity, our two patients with aggressive NK-cell leukemia did not responded well to the regimen. Based on the characteristics of clinical response in these two patients, it is felt that the combination therapy using hyperCVAD with alemtuzumab is not suitable for the treatment of aggressive NK-cell leukemia.

There is no much data on CD52 expression in precursor T-ALL, PTCL and NK/T-cell neoplasms during the initiation of this study. With the recent data, the incidence is about 35–40% by immunohistochemical (IHC) staining [56–58] to 92.3% by flow cytometry (FC) [59] in PTCL, NOS. CD52 expression in other subtype is quoted as ATLL 94.1% [59] to 100% [56], cutaneous T-cell lymphoma 87.5% [59], ALCL (no information whether ALK + or –) 0% [56], 22% [57] to 50% [59], NK/T-cell lymphoma 25% [59], 33% [56] to 47% [57], and precursor T-ALL 7% [56]. There is no good data on CD52 expression in aggressive NK-cell leukaemia and T-prolymphocytic leukaemia. It would be useful to know whether the presence of CD52 expression in our study correlates with the response. However, our study did not require presence of CD52 expression as an entrance criterion because of various reasons. Firstly, there is no study to examine the level of CD52 expression with clinical response to alemtuzumab. The available studies were not able to show any relationship between CD52 expression and outcome [43, 58]. Secondly, the method of examining CD52 expression is not standardized and the result varies from IHC to FC [56, 57, 59]. Lastly, the test was not readily available in local setting. The high ORR in our study might minimize the importance of using CD52 expression in predicting tumor response.

The dosing frequency of alemtuzumab in relation to the hyperCVAD are deemed adequate with one dose per cycle of hyperCVAD as evidenced by the high CMV reactivation and disease rate with higher dosing frequency per cycle. The CMV reactivation and/or disease are well known as one of the very common infectious complications [60]. In one of the alemtuzumab-CHOP trials of using oral acyclovir 400 mg twice daily as CMV prophylaxis, 15 episodes of CMV reactivation were observed in the total of 24 subjects [43]. Another alemtuzumab-CHOP study which used oral acyclovir 600 mg twice daily had CMV reactivation in five out of the total of 20 patients [42]. Our study used lower dose of oral acyclovir, which was 200 mg thrice

daily. This had likely contributed to our high incidence of CMV reactivation (50%) and disease (25%), although it could also be attributed to the more intensive chemotherapy regime used, the hyperCVAD as compared to CHOP. The incidence might have been lower if a better alternative, oral valganclovir was used as CMV prophylaxis [47–50].

It is also not uncommon to get invasive aspergillosis, *Pneumocystis carinii* pneumonia, JC viral encephalitis, EBV-positive immunodeficiency lymphoma or other uncommon infectious complications during or after the treatment period [37, 43, 61, 62]. The same phenomenon was also observed in our subjects. There were one case of psoas abscess, endophthalmitis, and fungal sepsis each. The patient with psoas abscess developed the condition one year after the completion of chemotherapy. Although the patient did not have microbiological culture evidence of tuberculosis from the psoas abscess drainage, the sputum smear was positive for pulmonary tuberculosis. The patient was treated with antituberculosis drug and responded completely with the treatment.

In conclusion, this study shows that alemtuzumab in combination with hyperCVAD regimen is a feasible regimen but with high toxicity. The toxicity might be reduced with the incorporation of filgrastim and use of valganclovir as CMV prophylaxis.

Conflict of interest The supply of alemtuzumab used in this study was partially sponsored by Bayer Healthcare Pharmaceuticals. The company has no role in the design of the study, collection and analysis of data and decision to publish the result of the study. All the authors have no affiliation or financial interest with the sponsoring company in all works related to the study.

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