表2 G-CSFの減量・中止基準(米国の場合は白血球数はなし．PLTは12万未満)

検査所見			G-CSF投与量の減量・中止
白血球数	50,000/μl以上		50%減量
	75,000/μl以上		投与中止
血小板数	100,000/μl未満(120,000未満)		50%減量
	50,000/μl未満		投与中止
症状	程度	Grade(CTC)	G-CSF投与量の減量・中止
骨痛	自制不能	4	50%減量，24時間後改善されなければ投与中止
頭痛	自制不能	4	50%減量，24時間後改善されなければ投与中止
吐き気	経口摂取不可能	3以上	投与中止
嘔吐	24時間で2～5回嘔吐	2	50%減量
	24時間で6回以上嘔吐	3以上	投与中止
身体反応	痛みもしくは腫れを伴う炎症・静脈炎		50%減量，24時間後改善されなければ投与中止

CTC：Common Toxicity Criteria；共通毒性基準
(財団法人骨髄移植推進財団：非血縁者間末梢血幹細胞採取マニュアル暫定版2010より引用改変)

2．末梢血幹細胞採取

PBSCHは非血縁者間末梢血幹細胞採取マニュアル[25]に従って行われる．PBSCH開始時もしくは採取中にどうしても十分な脱血路が確保できない場合には，習熟した医師が大腿静脈を確保する．G-CSFの投与については，身体症状と血球数で規定される．連日の採血と診察により，減量規定[25]を参照しつつ，当日の投与量を決定する(表2)．採取当日も採血結果を確認して投与量を決定する．採取は，当該施設の通常の方法にしたがって，G-CSF投与開始4日目ないし5日目より開始し，最大2日間行う．1日血液処理量の上限が，ドナー体重あたり250 ml/kgと定められている．原則としてCD34陽性細胞が2×10^6/kg(患者体重)採取できていないときは翌日も採取となり，翌日採血結果を診ながら再度G-CSFを投与する．1日目に血小板数が8万/μl以下になったときには，採取された幹細胞浮遊液からplatelet rich plasma(PRP)を作製し，ドナーに返血する．1日目に採取が終了したときは翌日退院，2日にわたったときは，2日目当日の退院も認められている．移植施設は，2回もらい受けに行く，2日目の期日でもらい受けに行く，2日目に業者に依頼する等の方法で幹細胞浮遊液を受領する．

症例が50例に達するまでは，「本邦における非血縁者間末梢血幹細胞移植の安全性と有効性に関する観察研究」と呼ばれる多施設前向きコホート研究に登録し，わが国における，URPBSCTの傾向を調査することになっている．

3．わが国の現状

2010年10月にURPBSCTが実施可能となり，第1例目は翌年3月に行われた．その後2013年5月31日現在で，URPBSCT認定施設数は52施設，URPBSCT数は24例，実際にPBSCTを行った施設数は13施設，実際にPBSCHを行った施設数は14施設であった．2010年10月から2013年3月末までの2年6か月間に，JMDPを介して行われたURBMTとURPBSCTの総数は，3,244件であり[26]，同時期にさい帯血バンクネットワークを介して行われた非血縁者間臍帯血移植は，2,846件であった[27]．2年半の時点では，URPBSCTはURBMTの0.76%というきわめて低頻度にとどまっている．

また，PBSCHを行った14施設のうち，G-CSFを外来で投与している施設は，2施設のみであり，多くのドナーにとってはPBSCHのほうが，入院期間が延びる結果となっている．コーディネート期間については，当初の予想どおり，自己血貯血のない末梢血のほうが短縮されている．

米国の現状

1．米国骨髄バンクによる非血縁者間同種移植

NMDPを介しての年間移植数は6,600件(2011事業年度)，内訳はBM 18%，PB 60%，臍帯血23%である(図5)．Collection center(BM)，Apheresis center(PB)，Transplant center，Do-

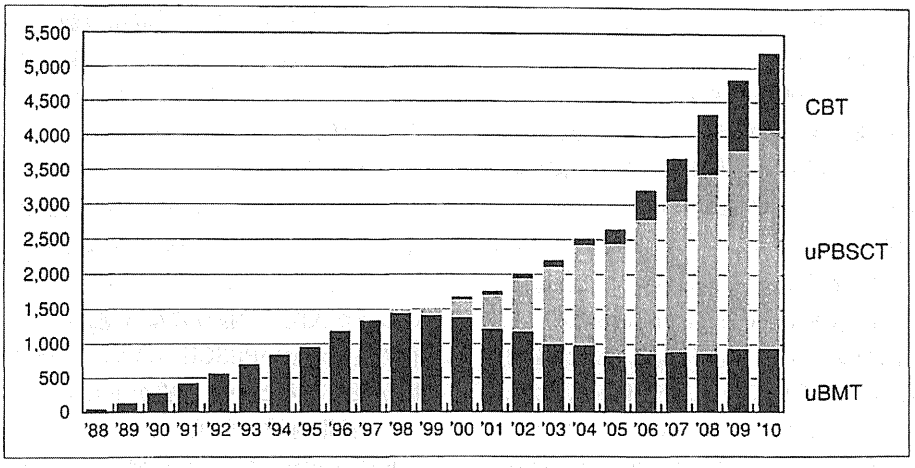

図5　NMDPにおける各種非血縁者間同種移植の年次別推移(1988～2010)
(National Marrow Donor Programホームページ，URL：http://bethematch.org/Home.aspxより抜粋)

表3　NMDPにおけるPBSCH時の血液処理量

患者体重(kg)	血液処理量(liters)
≦35	12
36～45	15
46～55	18
56～65	22
>65	24

アフェレシスにおける血液処理量は，ドナーではなく患者体重により規定される．
(文献[28]より引用改変)

nor center(coordination)に分かれている(兼務可)．Apheresis centerは95施設ある．採取施設にドナー居住地からの距離による制限はない．ドナー年齢は，18～60歳となっている[28]．

2．G-CSFの投与

G-CSFの投与は5日間行われ，採取は5日目と必要な場合6日目に行われる[28]．Filgrastimが投与されるが，2日間採取の場合でも6日目のG-CSF投与は行われない．初日は医療施設で投与が行われる．医療施設は，医師がいて，血球測定ができて，エピペンを備えていることが最低条件となり，投与後30分間，経過観察が行われる．2,3,4日目は，原則自宅で投与を受ける．Home health agencyから訪問看護師が往診して投与する．家族，隣人に医療関係者がいれば，その人でもよい．また場合によって自己注射も可能となっている．Filgrastimは，300 μgと480 μgのシリンジがあり，ドナー体重によって，必ず10 μl/kg以上となるシリンジの組み合わせを指示することで，誤りを回避している．自己注射を認めていない施設もある．たとえばEmory大学は自己注射を認めず，通院か看護師訪問で対応するとともに，24時間電話対応を行っている．WBC確認は初日と採取日と採取終了後であり，2～4日目は血液検査は行われない．身体症状による減量規定はあるが，血球数による変更はない[28](表2)．血小板数12万/μl以下で減量規定がある[28]．WBC増加では，大きな問題は生じないと考えている．脾臓破裂のリスクに関しては，腹痛や打診で痛みがあれば対応がとられる．同意文書では，脾臓破裂はpain & bleedingと説明されている[28]．

3．アフェレシス

血液センターがApheresis centerとなることもある．緊急時はエピペンと救急対応で，救急隊を待つ．血液センター採取のほうが，病院採取よりも安価である．Apheresis centerでの採取が困難であれば，他のNMDP施設(Collection center, Transplant center, Donor center)での採取が検討される．それも無理なら，非NMDP施設で条件の合う施設に依頼することもある．末梢ルートが確保困難であれば，医療設備の整った採取施設に搬送して，中心静脈を確保しその施設で採取する．中心静脈確保が必要な例は全体の9%，

男性の3%，女性の19%にあたる．

4．血液処理量

アフェレシスの血液処理量は，ドナーではなく患者の体重によって決まっている[28]（表3）．総血液処理量24 lが上限であるが，NIH（米国国立衛生研究所）など一部の施設では30 lのアフェレシスが認められている．ただし2日間採取でも総処理量が上限を超えることはない．各施設ともしだいにlarge volume leukapheresis（以下LVL）の方向に向かっており，以前は1/3，最近では2/3が1日採取で終了している．NIHでは，LVLにすると，バンクドナーでアフェレシスが2日にわたることはほぼないとのことであった．NMDPでは，採取CD34陽性細胞数の下限は決定されていない．ただし，移植施設と採取施設が処理量の範囲内で目標量を設定することはある．Ca^{2+}製剤の大量輸注を採取中に行うことをNMDPも推奨している．

5．血小板数

血小板数の低下は重要な問題との認識はあるが，PRPの返血はしない．NMDPのDr. Millerによると，汚染の問題を危惧するとのことであった．アフェレシス終了時8万/μl程度以上が望ましい．開始時12万/μl以下であれば，対応を検討することになっている（表2）．採取が2日にわたる場合は，2日目の血小板数が8万/μl以下なら採取しない．採取後の下限は5万/μl程度で，10万/μl以下なら1週間程度出血に注意を促すとのことであった．

今回の米国視察を通じて，PBとBMでは，採取終了までの苦痛はほぼ同じであるが，採取後の苦痛がまったく違う．ドナーのためにはPBを推進すべきである．また，LVLで1日での採取がほぼ可能であり推進すべきであるという意見が多くきかれた．

考　察

日本におけるURPBSCTは，解禁後2年6か月を経過しても，きわめて低頻度でしか行われていない．そこにはいくつかの事情が考えられる．

1．ドナーリクルート

PBSCT開始にあたって，PBドナーの要件として，症状出現時に採取施設を1時間以内に受診可能としたため，特に採取施設数が限られる今日の状況では，75%のドナー候補がドナー検索の前に不適格としてリストから外れることになり，移植施設がPBドナーを選ぶこと自体が困難な状況が続いている．現在認定施設数を増加させる努力とともに，PBSCTの認定施設以外にも，緊急時に対ドナー対応が可能な施設を増やすための調整が続けられている．ドナーにとっては，G-CSFが入院での投与になる施設が多いため，術後の負担が軽いPBSCHのほうがBMHより入院期間が長くなるという矛盾が生じている．

2．採取施設の負担

BMHの際には，全身管理は麻酔科医や病棟スタッフが多くの部分を担っている．PBSCHについては，血液内科医に負担がかかる部分が多い．欧米では，PBSCH以外の血漿交換療法をはじめとする治療的アフェレシスは，遠心式血液成分分離装置で輸血，血液部門が担っていることが多く，診療体制やスタッフも整備されている．一方わが国では治療的ヘムアフェレシスは大部分が膜を用いて透析関連部門で行われており，遠心式血液成分分離装置が用いられるのはPBSCHのときのみの施設が多い．2010年の造血細胞移植学会の統計から推測すると，1回のPBSCTに2回のアフェレシスが行われたとしても，PBSCTを経験した全施設を平均すると自家，同種移植用の採取を合わせても，アフェレシスの回数は1施設あたり1年に19.75回となる[29]．赤十字血液センターが採取に関与していない現状では，日常診療の中で習熟したアフェレシスを提供できる施設は限られている．PBSCTの認定施設が増えない理由として，血液内科医の数が少なく，ドナーの管理が負担になることに加えて，上記のような状況が背景にあると思われる．G-CSFについても外来投与可能な体制を病院全体で立ち上げる必要がある．また，CD34陽性細胞を院内で測定できない施設も多い．これについては，「移植に用いる造血幹細胞の適切な提供の推進に関する法律」の成立後，国庫からの補助が11施設に対して行われ，今年度も継続されることになっている．

3．治療成績

すでに述べたように，非血縁者間のBMとPBの幹細胞源の差による予後の解析は，NMDPを介

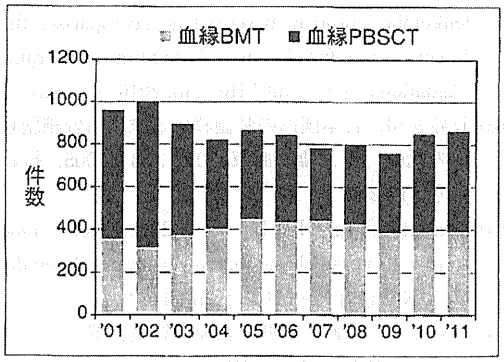

図6　わが国における各種血縁者間同種移植の年次別推移(2001～2011)
(文献[29]より一部抜粋しグラフ化)

した移植に関しての報告で，全生存率は変わらず，URPBSCTで慢性GVHDが多いとの報告がなされた[19]．一方国内では，RBMTに匹敵する数のRPBSCTが行われているものの(図6)，長藤らの造血細胞移植学会のデータを用いた血縁者間の解析で，RPBSCTのほうが，RBMTより有意に全生存率が低いという結果が出ている[17]．この点も，血縁者間よりもGVHDの発症が多いと予想されるURPBSCTを開始するにあたって，施設が躊躇する理由と思われる．

おわりに

図1に示したように，世界的にはURBMTに比べて，圧倒的にURPBSCTが多く行われている．わが国での，RPBSCTの後方視的移植成績の解析結果は否定的であるが，少なくともなんらかの工夫をすることで，URPBSCTの成績は，URBMTと遜色ない成績になることが予想される．ドナーの採取後の負担は明らかにPBで少ない．移植施設がPBを希望し，ドナーの了解が得られた場合に，迅速にコーディネートが進行する体制が望まれる．米国の現状を考えると，donationに関する考え方が基本的に異なっていることを感じる．訴訟社会といわれる米国で，わが国と比べるとかなり簡略化された方法で，NMDPを通じて年間4,000件のボランティアドナーからのPBSCHが行われていることに注目する必要がある．ドナーにとって負担の少ないPBSCHは，少なくとも，血縁者間移植と同様に，BMTと同程度以上に行われても不思議ではない(図6)．欧米に比べて困難な状況を克服するためには，各認定施設が輸血部門を充実させ，無理なく対応可能な状態を目指すとともに，G-CSFの外来投与が可能な体制を病院全体で立ち上げる必要がある．BMTのみの認定施設でも，ドナーに健康上の問題が生じた場合には対応することで，ドナー選択の際の距離制限を撤廃することが可能である．赤十字血液センターが，センター内での採取もしくは採取に関して技術協力を行うことで，URPBSCTの普及に貢献できる部分は大きい．また採取についての体制が整っている施設をセンター化することも考慮される．このほかにも，施設間でのCD34$^+$細胞数測定を標準化するため，国際的な測定法である，single platform法での測定に統一していくことや，LVLを導入してできるだけ1日で採取を終了するなどの改善策を探ることが望まれる．ドナーの負担軽減のためにも，これらの対策によって，インフラストラクチャーの脆弱性を克服していく必要がある．

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《シンポジウム3》採血副作用の原因とその防止対策（日本血液事業学会共催）

3-3　ドナーアフェレシス，治療的ヘムアフェレシスにおける留意点

公益財団法人大原記念倉敷中央医療機構
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ヘムアフェレシスは，体外循環を用いて血液成分を採取，除去，置換する手法である．世界的には，アフェレシスは遠心式血液成分分離装置を用いることが標準的であるが，我が国では，治療的ヘムアフェレシスの大半は，膜や吸着材を用いて行われており，特化された機器を用いる献血用の成分採血を除いて，輸血や血液疾患診療にたずさわるスタッフが，遠心式アフェレシスにかかわる機会は非常に少ない．非血縁者間末梢血幹細胞移植の日本での普及が遅れている理由の根本に，このような事情が推測される．体外循環を問題なく行うために最も大事な点は，十分なバスキュラーアクセスの確保である．安定した体外循環，所要時間の短縮，回路内凝固の回避をもたらす．必要時に安全に大腿静脈路を確保できる体制は重要である．抗凝固剤の適切な選択も重要である．ACD-A液は，理想的な局所抗凝固剤ではあるが，特にFFPを用いた血漿交換においては，クエン酸による低Ca血症を惹起しやすいので，病状によりヘパリンやメシル酸ナファモスタット等も考慮される．低Ca血症に対しては，初期徴候の早期発見と流量の下方修正，Ca^{2+}製剤の輸注を必要に応じて行う．連続式血液成分分離では，VVRを生じることは少ないが，初期徴候に気づき対応するとともに，重篤な場合は輸液路が確保されていることを認識し，落ち着いて体外循環を終了する．治療的アフェレシスにおいては，血漿分画膜や吸着により予想以上に浸透圧の変化が生じ，VVR様の症状を発現することがある．ACE阻害薬服用中の体外循環は，ブラディキニンの蓄積をもたらし重篤な血圧低下を生じる場合があるので，原則禁忌となる．病歴聴取は重要である．補体活性化や発熱物質による悪寒戦慄等も時に経験する．ヘムアフェレシスは，輸液路が確保され，体外循環量も献血量以下という基本的に安全な手技であることを理解し，十分な準備と細心の注意のもとに行うことが必要である．

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ORIGINAL ARTICLE

Autologous peripheral blood stem cell transplantation with granulocyte colony-stimulating factor combined conditioning regimen as a postremission therapy for acute myelogenous leukemia in first complete remission

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Abstract We retrospectively analyzed the outcomes of 81 patients with non-M3 acute myelogenous leukemia (AML) in first complete remission (CR1) who were treated with high-dose chemotherapy (HDCT) and autologous peripheral blood stem cell transplantation (Auto-PBSCT) by the Fukuoka Blood and Marrow Transplantation Group between 1989 and 2005. Cytogenetically, 16 patients were defined as good risk, 56 as intermediate risk, and nine as poor risk, following the Southwest Oncology Group criteria. The pre-transplant conditioning regimen consisted of high-dose busulfan, etoposide, and cytarabine (BEA regimen), combined with priming by granulocyte colony-stimulating factor (G-CSF). Disease-free survival (DFS) and overall survival at 5 years were 64.0 % (95 % CI 52.5–73.4) and 66.4 % (95 % CI 54.9–75.6) after Auto-PBSCT at a median follow-up time of 103 months (range 3–240 months), respectively. Two patients died of transplant-related pulmonary complications 6 months after Auto-PBSCT without relapse. The 5-year DFS rates of patients in the genetically good-, intermediate-, and poor-risk groups were 80.8, 64.3, and 33.3 %, respectively, but there was no significant difference statistically among the risk groups (log-rank $p = 0.0579$). These observations suggest that HDCT supported by Auto-PBSCT with the BEA regimen combined with G-CSF priming is a therapeutic option for postremission therapy of AML in CR1.

Keywords Acute myelogenous leukemia · First complete remission · Postremission therapy · Autologous peripheral blood stem cell transplantation · G-CSF combined conditioning regimen

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Introduction

Despite complete remission (CR) rates as high as 60–80 % with anthracycline/cytarabine-based induction therapy in de novo acute myelogenous leukemia (AML), relapse of leukemia is frequent and an obstacle to long-term survival. Therefore, postremission therapy is very important in preventing relapse and improving treatment outcome [1]. AML has various clinical features and is usually classified into three risk groups based on cytogenetic abnormality [2]. The good-risk group is characterized by t(15;17) translocation or core-binding factor (CBF) abnormalities, and the poor-risk group is characterized by complex cytogenetic abnormalities or deletion of chromosome 5 or 7. Although the intermediate-risk group is defined by normal karyotype, it is very heterogeneous in terms of its genetic abnormalities, and 50–60 % of AML patients belong to this group. For patients with CBF abnormality AML in first CR (CR1), postremission therapy with repetitive cycles of high-dose cytarabine (HiDAC) is considered to be a standard treatment option. Recently, repetitive cycles (usually 3–4 cycles) of HiDAC have been widely used as consolidation of CR for patients with intermediate-risk AML, but the treatment outcome is not completely satisfactory. For patients with poor-risk AML, the results of conventional consolidation therapy are poor [3]. Accordingly, allogeneic stem cell transplantation is clinically indicated for patients with high-risk AML if they have a human leukocyte antigen (HLA)-identical sibling, but only about one-third of patients can find a suitable donor [4].

In an attempt to improve the treatment outcome of AML, the Fukuoka Blood and Marrow Transplantation Group (FBMTG) started high-dose chemotherapy (HDCT) supported by autologous peripheral blood stem cell transplantation (Auto-PBSCT) as postremission therapy for patients with AML in CR1 in 1989. Since that time, we have employed a unique conditioning regimen, including granulocyte colony-stimulating factor (G-CSF) priming followed by conventional dose cytarabine to induce dormant residual leukemic cells into the cell cycle to increase their chemosensitivity [5]. In this study, we retrospectively analyzed the long-term outcomes of 81 patients with AML in CR1 who received HDCT combined with G-CSF priming followed by Auto-PBSCT to evaluate the safety and efficacy of this treatment modality as postremission therapy.

Patients and methods

Patients

We retrospectively analyzed 81 patients with de novo AML who received HDCT with Auto-PBSCT as postremission therapy between January 1989 and June 2005 at four institutions of the FBMTG in Japan. During that time, we employed this treatment in clinical practice on most AML patients in CR1; patients who were candidates for allogeneic stem cell transplantation with a suitable donor and patients who were of advanced age or with poor performance status because of organ dysfunction or severe infections were excluded. The 81 patients consisted of 48 males and 33 females with a median age of 37 years (range 16–75 years). The French–American–British classification of AML in these patients was as follows: M1 in 25, M2 in 35, M4 in 16, and M5 in 5 patients, and M3 patients were excluded. Seventy-six patients achieved CR after one course of remission induction chemotherapy, while 5 patients required two courses of the therapy to obtain CR. The median interval from CR1 to Auto-PBSCT was 4.1 months, with a range of 2.9–19.2 months. Patient characteristics are summarized in Table 1, in which patients are divided into three groups based on cytogenetic abnormalities according to the Southwest Oncology Group criteria [2]: 16 patients (20 %) were classified as good risk, 56 (69 %) as intermediate risk, and 9 (11 %) as poor risk. There were no significant differences in the distribution of FAB classification, numbers of induction chemotherapy to obtain CR, interval from CR1 to Auto-PBSCT, and numbers of CD34+ cells infused among these three groups. No significant differences in white blood cell (WBC) counts, lactate dehydrogenase (LDH) values, and CD56 positivity at diagnosis were observed among the three groups.

Chemotherapy and peripheral blood stem cell (PBSC) collection

Induction chemotherapy consisted of daunorubicin (45 mg/m^2 intravenously) or idarubicin (12 mg/m^2 intravenously) for 3 days combined with cytarabine (100 mg/m^2 by continuous infusion) for 7 days. Bone marrow aspiration was performed on day 7, and if the marrow was not severely hypoplastic, daunorubicin was added on days 8 and 9, and cytarabine administration was continued for 3 more days (days 8, 9, and 10) [5].

Consolidation chemotherapy consisting of an intermediate dose of cytarabine (500 mg/m^2 intravenously) every 12 h for 6 days combined with mitoxantrone (7 mg/m^2 intravenously) for 3 days in the first course and etoposide (100 mg/m^2 intravenously) for 5 days in the second course was started after achieving CR. Collection of PBSCs was performed with a continuous blood-cell separator during hemopoietic recovery after the second consolidation chemotherapy with ($n = 67$) or without ($n = 14$) G-CSF (filgrastim, 200 μg/m^2 intravenously). Unpurged PBSC harvests were cryopreserved using a simplified method without rate-controlled freezing until

Table 1 Patient characteristics

	Cytogenetics				
	All ($n = 81$)	Good ($n = 16$)	Intermediate ($n = 56$)	Poor ($n = 9$)	p value
Median age, years (range)	37 (16–75)	32 (19–59)	39 (16–75)	48 (20–68)	0.258
Male/female	48/33	11/5	29/27	8/1	0.075†
FAB subtype					
M1	25	2	20	3	0.284†
M2	35	11	20	4	
M4	16	3	12	1	
M5	5	0	4	1	
WBC counts at diagnosis					
Median (range)	9800 (800–198900)	6150 (1600–187000)	11450 (800–180000)	79640 (1800–198900)	0.331
<10,000	36	10	23	3	0.170†
≧10,000	36	4	27	5	
Unknown	9	2	6	1	
LDH values at diagnosis					
WNL	23	4	15	4	0.596†
High LDH	55	12	38	5	
Unknown	3	0	3	0	
CD56 expression at diagnosis					
Negative	53	11	37	5	0.609†
Positive	16	3	10	3	
Unknown	12	2	9	1	
No. of induction courses to remission					
1 course	76	14	53	9	0.466†
2 courses	5	2	3	0	
G-CSF for PBSC mobilization					
Yes	67	13	46	8	>0.99†
No	14	3	10	1	
No. of CD34+ cells infused					
Median, $\times 10^6$/kg (range)	4.3 (0.9–20.8)	6.2 (1.3–16.3)	4.2 (0.9–20.8)	3.9 (2.1–11.3)	0.230
<4	31	3	23	5	0.321†
≧4	40	9	27	4	
Unknown	10	4	6	0	
Interval from diagnosis to Auto-PBSCT					
Median, months (range)	5.4 (3.9–20.4)	5.5 (4.5–15.0)	5.2 (3.9–20.4)	6.3 (4.7–7.4)	0.493
Interval from CR1 to Auto-PBSCT					
Median, months (range)	4.1 (2.9–19.2)	4.0 (3.4–13.7)	4.1 (2.9–19.2)	5.5 (3.6–6.7)	0.315
<4 month	35	8	25	2	0.389†
≧4 months	46	8	31	7	

Kruskal–Wallis test

G-CSF granulocyte colony-stimulating factor, *PBSC* peripheral blood stem cell, *BEA* busulfan, etoposide, cytarabine, *PBSCT* peripheral blood stem cell transplantation, *CR1* first complete remission

† Fisher exact test

use for transplantation [6]. The target cell dose was greater than 2×10^6 CD34+ cells/kg in PBSC harvests.

Autologous peripheral blood stem cell transplantation

The pretransplant conditioning regimen consisted of high-dose busulfan (4 mg/kg orally) on days −8 to −5 (total dose 16 mg/kg), etoposide (20 mg/kg intravenously) on days −4 and −3 (total dose 40 mg/kg), and cytarabine (3 g/m^2 intravenously) every 12 h on days −3 and −2 (total 4 doses) (BEA regimen). Priming with human G-CSF was used prior to the BEA regimen to increase chemosensitivity of residual leukemic cells to cytarabine, an S-phase-specific anti-leukemic agent. G-CSF was

administered intravenously over 6 h at a dose of 5 μg/kg on days −14 to −8, 10 μg/kg on days −7 and −6, and 20 μg/kg on days −5 and −4 in combination with continuous infusion of conventional dose cytarabine (100 mg/m^2) on days −12 to −6 in addition to the above BEA regimen (G-CSF combined BEA regimen). On day 0, cryopreserved PBSCs were rapidly thawed in a 37 °C waterbath and infused through a central venous catheter without washing. Complications related to the pretransplant conditioning were classified according to the criteria proposed by Bearman et al. [7]. Engraftment was confirmed by recovery of granulocyte counts >0.5 × 10^9/l and platelet counts >20 × 10^9/l without dependence on platelet transfusion on three consecutive days.

Data management and statistical considerations

For overall survival (OS), death from any cause was used as the event. For disease-free survival (DFS), leukemic relapse or death from any cause was used as the event. Early death was defined as death within 100 days after Auto-PBSCT. Frequencies or median (range) were provided for age, sex, FAB subtype, WBC count at diagnosis, LDH value at diagnosis, CD56 expression at diagnosis, number of induction courses to remission, G-CSF for PBSC mobilization, number of CD34+ cells infused, interval from diagnosis to Auto-PBSCT, and interval from CR1 to Auto-PBSCT. Differences in those factors between groups according to cytogenetics were tested using the Fisher's exact test for frequencies and the Kruskal–Wallis test for medians. Survival curves were estimated using the product-limit method of Kaplan–Meier. The log-rank test was used for comparison of survival curves. Relapse mortality was calculated by the Kaplan–Meier method as cumulative incidence of deaths caused by leukemic relapse after Auto-PBSCT. For OS and DFS, the multivariate Cox proportional hazard models were constructed by applying the backward elimination method for covariates with p value less than 0.2 using the univariate Cox proportional hazards model. A two-sided p value <0.05 was considered to be statistically significant. All statistical analyses were conducted using the SAS Ver 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Engraftment, transplantation-related complications, and secondary malignancy

The median number of infused CD34+ cells was 4.3 × 10^6 cells/kg (range 0.9–20.8 × 10^6 cells/kg). There was no significant difference in the number of infused CD34+ cells among the risk groups (Table 1). G-CSF was given to 58 of 81 patients after transplantation. Engraftment failure was not observed, and granulocytic engraftment was rapid in all patients who were transplanted. The median number of days to granulocyte (>0.5 × 10^9/l) and platelet (>20 × 10^9/l) engraftment was 12 days (range 8–35 days) and 22 days (range 7 days, not reached), respectively. Five of 81 patients died without platelet recovery because of leukemic relapse (4, 6, 10, and 27 months after transplant) or cytomegalovirus pneumonitis (6 months after transplant).

Major regimen-related toxicities were mucositis and gastrointestinal toxicity, but most toxicities were less than grade 3 by Bearman's criteria and tolerable (Table 2). All patients suffered from fever during the post-transplant neutropenic periods. Three patients were diagnosed with pneumonia, 2 with sepsis, and the other 76 patients were diagnosed with febrile neutropenia. All patients showed improvement, which was due to effective courses of antibiotics or anti-fungal agents and rapid granulocytic recovery. None of the patients' courses were complicated by probable or proven fungal infections and viral infections, except for one case of cytomegalovirus pneumonitis (Table 3). Two patients died from pulmonary diseases 6 months after Auto-PBSCT (cytomegalovirus pneumonitis and interstitial pneumonitis) without relapse, namely transplant-related death. Two patients developed secondary malignancies; both were myelodysplastic syndrome. One patient progressed to transfusion-dependent refractory

Table 2 Regimen-related toxicities

	Grade				
	0	1	2	3	4
Regimen-related toxicities (Bearman's criteria) (n = 81)					
Cardiac	79	0	2	0	0
Pulmonary	81	0	0	0	0
Stomatitis	3	47	30	1	0
Gastrointestinal	9	66	6	0	0
Hepatic	59	19	3	0	0
Renal	73	6	2	0	0

Table 3 Transplant-related adverse events (infections and secondary malignancies)

Transplant-related adverse events ($n = 81$)	
Febrile neutropenia	76
Bacterial infection	
Pneumonia	3
Sepsis	2
Fungal infection	0
Viral infection	1
Secondary malignancies	
Myelodysplastic syndrome	2

anemia 11 years after Auto-PBSCT; that patient received allogeneic PBSCT from sibling and is now alive. The other patient progressed to refractory anemia 11 months after Auto-PBSCT and finally died of leukemic progression 4 years after Auto-PBSCT.

Disease-free survival and overall survival

The DFS and OS of 81 patients are shown in Fig. 1a, b. The 5-year DFS was 64.0 % [95 % confidence interval (CI) 52.5–73.4], and the 5-year OS was 66.4 % (95 % CI 54.9–75.6) after Auto-PBSCT at a median follow-up time of 103 months (range 3–240 months). The median follow-up time in surviving patients was 131 months (range 14–240 months). As shown in Fig. 2, the 5-year cumulative relapse mortality (RM) was 31.9 % (95 % CI 22.8–43.4). Two patients died of transplant-related pulmonary complications 6 months after Auto-PBSCT without relapse.

The 5-year DFS was also stratified by age. The 5-year DFS was significantly higher in the younger group of patients aged 60 years or less (68.7 %, 95 % CI 56.8–78.0, $n = 74$) than in that of the older group (14.3 %, 95 % CI 0.7–46.5, $n = 7$) (log-rank $p = 0.0013$) (Fig. 3a).

According to the cytogenetic risk groups, the 5-year DFS was 80.8 % (95 % CI 51.4–93.4, $n = 16$) for the good-risk group, 64.3 % (95 % CI 50.3–75.3, $n = 56$) for the intermediate-group, and 33.3 % (95 % CI 7.8–62.3, $n = 9$) for the poor-risk group, but there was no significant difference statistically among these three groups (log-rank $p = 0.0579$) (Fig. 3b).

There was no significant difference in the 5-year DFS between 1 (65.6 %, 95 % CI 53.7–75.1, $n = 76$) and 2 courses (40.0 %, 95 % CI 5.2–75.3, $n = 5$) of the induction chemotherapy required to achieve remission ($p = 0.150$). The usage of G-CSF at the PBSC collection did not affect the 5-year DFS: 63.9 % with G-CSF (95 % CI 51.2–74.2, $n = 67$) and 64.3 % without G-CSF (95 % CI 34.3–83.3, $n = 14$) ($p = 0.542$). We compared the 5-year DFS by the interval from CR1 to transplantation; the interval from CR1 to transplantation was less than 4 months in early transplant and 4 months or more in late transplant, because the median interval from CR1 to transplantation was 4.1 months in all 81 cases. There was no significant difference in the 5-year DFS between the early transplant (56.6 %, 95 % CI 38.7–71.2, $n = 35$) and the late transplant (69.5 %, 95 % CI 54.0–80.7, $n = 46$) ($p = 0.349$).

Twenty-nine of 81 patients relapsed after Auto-PBSCT. All those patients ultimately died. Fourteen patients received allogeneic stem cell transplantation (Allo-SCT), and 15 patients received only salvage chemotherapy. In the Allo-SCT patients, 5 patients died of leukemia progression, 6 of Allo-SCT related complications, and 3 of unknown causes. In the salvage chemotherapy patients, 12 patients died of leukemia progression, 1 of secondary myelodysplastic syndrome, 1 of complications from chemotherapy, and 1 patient died of unknown causes.

Prognostic factors affecting DFS and OS

The results of the univariate analyses using prognostic factors are listed in Table 4. Younger age was a significantly better prognostic factor for DFS [hazard ratio (HR) = 1.039; 95 % CI 1.011–1.067; $p = 0.005$] and OS (HR = 1.042; 95 % CI 1.014–1.072; $p = 0.003$). The poor risk by karyotype group had a significantly worse prognosis, as shown by DFS (HR = 2.856; 95 % CI 1.158–7.040; $p = 0.023$) and OS (HR = 2.672; 95 % CI 1.088–6.563; $p = 0.032$) compared to those in the good-/intermediate-risk group. Unusually, patients with WBC counts more than 10,000/μl at diagnosis had better DFS (HR = 0.422; 95 % CI 0.181–0.980; $p = 0.045$) and OS (HR = 0.417; 95 % CI 0.180–0.967; $p = 0.042$) compared to those with WBC counts less than 10,000/μl. Similarly, patients with high LDH values at diagnosis had better DFS (HR = 0.354; 95 % CI 0.172–0.726; $p = 0.005$) and OS (HR = 0.364; 95 % CI 0.177–0.746; $p = 0.006$) compared to those with normal LDH values. The numbers of induction chemotherapy courses to achieve remission, use of G-CSF for PBSC collection, cell number of CD34+ cells infused, interval from CR1 to transplantation, and CD56 positivity at diagnosis did not affect the outcomes of Auto-PBSCT for AML.

The details of the multivariable Cox proportional hazards regression analyses are shown in Table 5. Covariates with p value less than 0.2 using the univariate Cox proportional hazards model, such as age, karyotype, numbers of induction courses to remission, and WBC counts at diagnosis, were selected for the multivariate Cox proportional hazard models. As a result, only WBC counts at diagnosis was the significant prognostic factor affecting

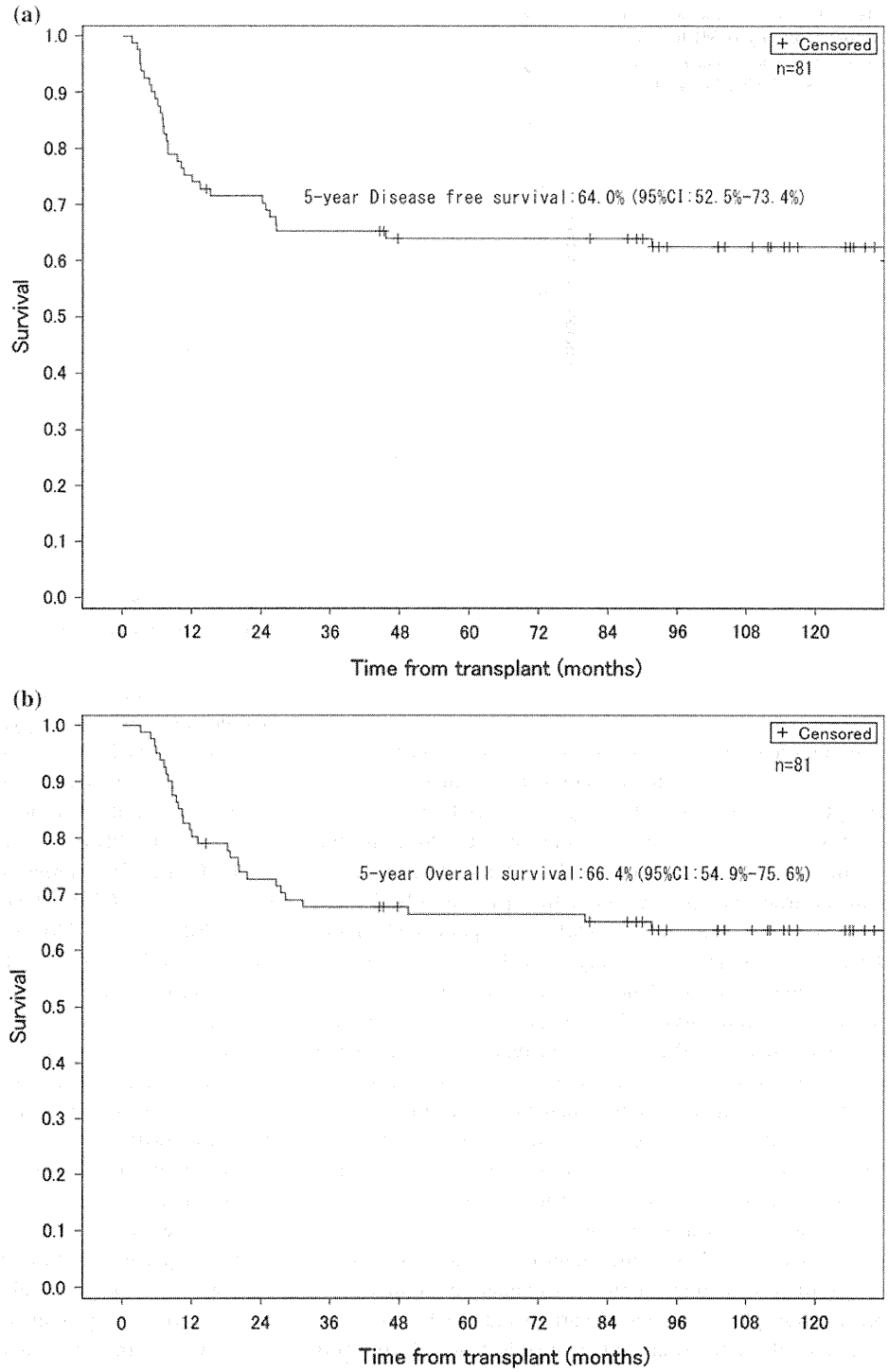

Fig. 1 **a** Disease-free survival (DFS) and **b** overall survival (OS) for all 81 patients. The 5-year DFS was 64.0 % (95 % confidence interval (CI) 52.5–73.4), and the 5-year OS was 66.4 % (95 % CI 54.9–75.6) after Auto-PBSCT at a median follow-up time of 103 months (range 3–240 months)

DFS and OS. Curiously, patients with WBC counts that were more than 10,000/μl at diagnosis had better DFS (HR = 0.282; 95 % CI 0.103–0.774; $p = 0.014$) and OS (HR = 0.273; 95 % CI 0.100–0.746; $p = 0.011$) compared to those with WBC counts less than 10,000/μl. The age, karyotype, and numbers of induction courses to achieve remission did not affect the outcomes of Auto-PBSCT for AML in this multivariate analysis.

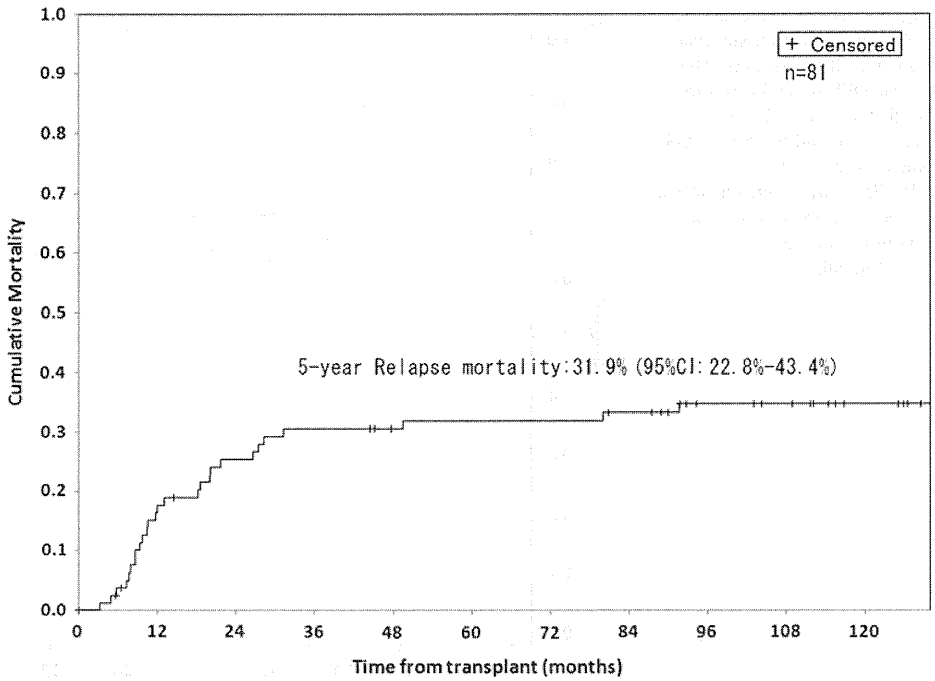

Fig. 2 Cumulative incidence of relapse mortality (RM) for all 81 patients. The 5-year RM was 31.9 % (95 % CI 22.8–43.4)

Discussion

Although autologous SCT (Auto-SCT) has improved survival in some hematologic malignancies, including non-Hodgkin's lymphoma [8], Hodgkin's lymphoma [9], and multiple myeloma [10], the role of Auto-SCT in the treatment of AML remains unclear. Several randomized clinical trials comparing chemotherapy, Auto-SCT, and Allo-SCT for treatment of AML were published in the 1990s [11–13]. The meta-analysis of these results did not show superiority of Auto-SCT to chemotherapy with regard to overall survival [14]. In those Auto-SCT protocols, bone marrow (BM) was exclusively used as a source of hematopoietic stem cells, and the rate of non-relapse mortality was slightly higher than that of chemotherapy. Moreover, there were some difficulties in salvage therapy for leukemia relapse after Auto-SCT. There were also some problems in the randomized control trials. In some reports, only about one-third of the patients were allocated to randomization, and, despite the intent-to-treat analysis, about one-third of the patients in the transplantation group did not receive transplantation due to various reasons [11, 12, 15].

In the 1990s, the source of stem cells changed from BM to PBSC in Auto-SCT to treat various types of malignancy. A more rapid hematopoietic recovery after Auto-PBSCT and subsequent decreases in infectious and hemorrhagic complications are advantages of Auto-PBSCT over autologous bone marrow transplantation (Auto-BMT). However, there are only a few reports of clinical trials of Auto-PBSCT for AML. Tsimberidou et al. [16] reported a prospective randomized trial comparing Auto-PBSCT and maintenance chemotherapy, but only 19 out of 120 patients in total received Auto-PBSCT. In a more extensive prospective clinical trial of the European Intergroup reported by Büchner et al. [17], only 24 % of patients received the assigned Auto-PBSCT. Recently, a retrospective analysis of the Center for International Blood and Marrow Transplant Research (CIBMTR) data of AML in CR1 comparing Auto-PBSCT ($n = 230$) and HLA-matched sibling BMT ($n = 475$)/PBSCT ($n = 428$) has been published [18]. The 5-year survival rates after Auto-PBSCT, allogeneic BMT (Allo-BMT), and allogeneic PBSCT (Allo-PBSCT) were 54, 64, and 59 %, respectively, but there were no significant differences among them. Although the relapse rate after Auto-PBSCT was significantly higher than those of Allo-BMT/PBSCT, the significantly lower transplant-related mortality (TRM) after Auto-PBSCT compensated for the survival disadvantage due to the high relapse rate. Since these data were not obtained through a prospective study, the place of Auto-PBSCT as a postremission therapy for AML has not been well established in the PBSCT era.

High-dose or myeloablative chemotherapy supported by Auto-SCT is the most intensive treatment modality for leukemia when the safety of the treatment is assured in postremission therapy. Factors increasing anti-leukemic effects include the more effective pretransplant conditioning and less contamination of the autograft with leukemic stem/progenitor cells. The combination of busulfan/cyclophosphamide and total body irradiation/cyclophosphamide are major preparative conditioning regimens frequently

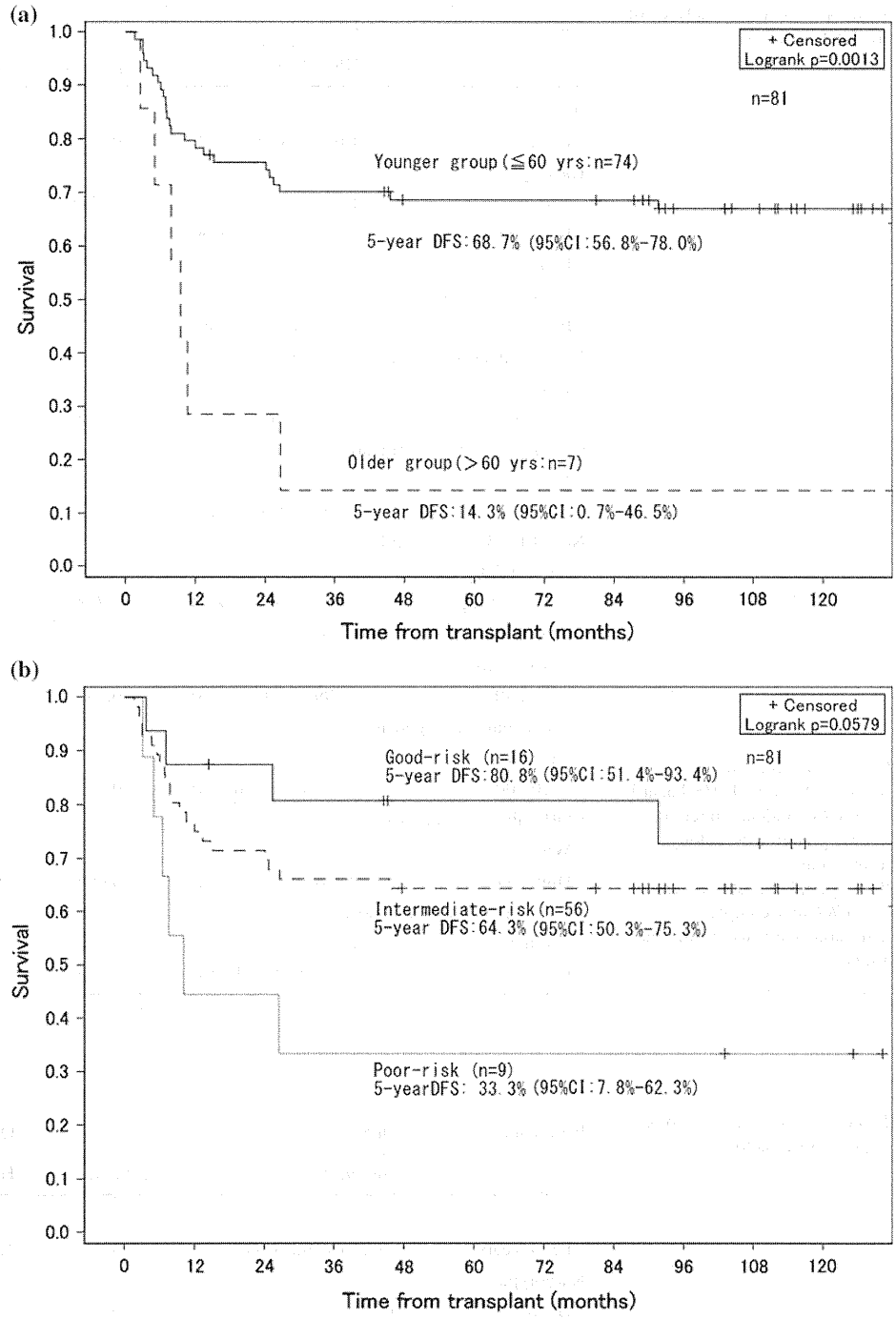

Fig. 3 The 5-year DFS was stratified by age and cytogenetic risk. **a** There was a significant difference in the 5-year DFS between the younger group of patients aged 60 years or less (68.7 %, 95 % CI 56.8–78.0, $n = 74$) and the older group (14.3 %, 95 % CI 0.7–46.5, $n = 7$) (log-rank $p = 0.0013$). **b** According to the cytogenetic risk groups, the 5-year DFS was 80.8 % (95 % CI 51.4–93.4, $n = 16$) for the good-risk group, 64.3 % (95 % CI 50.3–75.3, $n = 56$) for the intermediate-group, and 33.3 % (95 % CI 7.8–62.3, $n = 9$) for the poor-risk group. The differences among these three groups were not statistically significant (log-rank $p = 0.0579$)

used for Allo-SCT, but the most effective conditioning regimen for Auto-SCT remains controversial [19]. The priming effect of G-CSF to increase the chemosensitivity of myeloid leukemic cells to cytarabine, an S-phase-specific anti-leukemic agent, was already reported in vitro [20] and applied to chemotherapy and Allo-SCT [21, 22]. Moreover, it is possible that G-CSF enhances the chemosensitivity of leukemic stem/progenitor cells by mobilizing them into circulation from the bone marrow niche [23]. For these reasons, our pretransplant conditioning adopted G-CSF priming, and the toxicities of this conditioning regimen were tolerable.

On the other hand, focus must be directed at preventing contamination of leukemic stem/progenitor cells into the graft. A recent report has pointed out the higher incidence of relapse in patients transplanted with PBSC, especially

Table 4 Univariate analyses of prognostic factors

Parameter	DFS		OS	
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Age				
Per 1 years	1.039 (1.011–1.067)	0.005	1.042 (1.014–1.072)	0.003
Karyotype				
Good	1	0.074*	1	0.056*
Intermediate	1.620 (0.556–4.719)		1.591 (0.546–4.637)	
Poor	3.939 (1.105–14.038)		4.151 (1.162–14.828)	
Good/intermediate	1		1	
Poor	2.856 (1.158–7.040)	0.023	2.672 (1.088–6.563)	0.032
No. of induction courses to remission				
1 course	1		1	
2 courses	2.339 (0.709–7.722)	0.163	2.219 (0.671–7.336)	0.191
G-CSF for PBSC mobilization				
Yes	1		1	
No	1.299 (0.560–3.016)	0.543	1.351 (0.581–3.138)	0.485
No. of CD34 + cells infused				
$<4 \times 10^6$/kg	1		1	
$\geqq 4 \times 10^6$/kg	0.867 (0.388–1.937)	0.728	0.852 (0.382–1.904)	0.697
Interval from CR1 to transplant				
≧4 months	1		1	
<4 months	1.399 (0.691–2.833)	0.351	1.399 (0.691–2.833)	0.351
WBC counts at diagnosis				
<10,000	1		1	
≧10,000	0.422 (0.181–0.980)	0.045	0.417 (0.180–0.967)	0.042
LDH values at diagnosis				
WNL	1		1	
High LDH	0.354 (0.172–0.726)	0.005	0.364 (0.177–0.746)	0.006
CD56 expression at diagnosis				
Normal	1		1	0.636
High	0.603 (0.208–1.753)	0.353	0.580 (0.200–1.684)	0.316

DFS disease-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval, *G-CSF* granulocyte colony-stimulating factor, *PBSC* peripheral blood stem cell, *CR1* first complete remission, *WNL* within normal limits

* Overall *p* value

Table 5 Multivariate analyses of prognostic factors

Parameter	DFS		OS	
	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Age				
Per 1 years	1.025 (0.992–1.059)	0.143	1.027 (0.993–1.061)	0.117
Karyotype				
Good	1	0.107*	1	0.081*
Intermediate	2.365 (0.579–9.659)		2.039 (0.533–8.585)	
Poor	6.242 (1.087–35.851)		6.523 (1.150–36.999)	
Numbers of induction courses to remission				
1 course	1		1	
2 courses	3.563 (0.701–18.105)	0.126	3.135 (0.622–15.788)	0.166
WBC counts at diagnosis				
<10,000	1		1	
≧10,000	0.282 (0.103–0.774)	0.014	0.273 (0.100–0.746)	0.011

Backward elimination method was used to select the final multivariate models with characteristics whose *p* value in the univariate analysis were less than 0.2

DFS disease-free survival, *OS* overall survival, *HR* hazard ratio, *CI* confidence interval

* Overall *p* value

those harvested during the earlier phase of treatment [24]. They reported that relapse rates were 56 % in patients receiving Auto-PBSCT within 80 days after CR1, 46 % in those receiving Auto-PBSCT more than 80 days after CR1, and 39 % in those receiving Auto-BMT, and the differences among these three groups of patients were significant. However, they did not mention the induction and consolidation chemotherapy regimens, timing of PBSC harvest, and transplantation procedures in detail. Previously, we reported the efficacy of repetitive consolidation with an intermediate dose of cytarabine to minimize minimal residual disease in PBSC harvests from AML patients with t(8;21) chromosomal abnormality [25]. In this study, we used PBSC autografts obtained after the second round of consolidation chemotherapy with an intermediate dose of cytarabine to reduce the risk of leukemia contamination in the PBSC autografts through so-called in vivo purging [5]. Consequently, as shown in Fig. 2, the 5-year RM was 31.9 %.

We presented the long-term outcomes of AML patients in CR1 treated with HDCT followed by Auto-PBSCT; 5-year OS and DFS were 66.4 and 64.0 %, respectively. As shown in Fig. 1a, b, their survival curves are at a plateau. These data bear comparison to those of Allo-SCT reported by CIBMTR cited above [18]. Recently, low incidences of TRM after Auto-PBSCT (less than 5 %) have been reported and appear to contribute to improved outcomes [26]. In our data, only two 2 of 81 patients died of transplant-related complications. These data suggest that Auto-PBSCT may be an acceptable alternative to HLA-matched sibling donor transplantation for patients with AML in CR1, but close attention should be paid to secondary malignancies.

Although patients with CBF-AML are generally considered to have a good prognosis and may be cured with standard chemotherapy, a subgroup of them with c-kit mutation and a CD56 positive phenotype showed poor prognosis with a high relapse rate [27]. The European Group for Blood and Marrow Transplantation published a retrospective analysis of patients with CBF-AML who received Auto-SCT or Allo-SCT in CR1 [28]. Transplant outcomes were similar after Auto-SCT and Allo-SCT; the 5-year DFS rates of AML patients with inv 16 were 66 and 59 %, respectively, and those of AML patients with t(8;21) were 66 and 60 %, respectively. Meanwhile, an intermediate-risk group of AML with normal karyotype shows various clinical features, and the flt-3 mutation is considered to be one of the risk factors for poor prognosis [29]. Several reports suggest that high-dose chemotherapy with Auto-SCT may overcome poor prognostic factors, such as c-kit and flt-3 mutations, in subgroups of AML [30]. In our study, 5-year DFS rates of good-risk and intermediate-risk patients were 80.8 and 64.3 %, respectively (Fig. 3b). Although our results regarding Auto-PBSCT for good-risk and intermediate-risk AML are also promising, risk factors such as high-risk chromosomal abnormalities and advanced age are difficult to overcome, even with HDCT followed by Auto-PBSCT, as previously reported [31].

HDCT supported by Auto-PBSCT is a modified type of chemotherapy, since it lacks graft-versus-leukemia effects. Concerning the safety of transplantation procedures, Auto-PBSCT is characterized by rapid hematopoietic recovery, which can reduce treatment-related toxicities and infectious complications. Intravenous busulfan is expected to minimize serious adverse effects without loss of anti-leukemic activity [32]. These refinements will be beneficial to increase the safety of Auto-PBSCT and expand the clinical indication of Auto-PBSCT.

In conclusion, our observations have demonstrated the safety and efficacy of Auto-PBSCT for treatment of good-risk and intermediate-risk AML in CR1 and suggest that HDCT with Auto-PBSCT may be used as a postremission therapy. A prospective study will be necessary to know whether HDCT with Auto-PBSCT will influence outcomes in the treatment of AML. Currently, we are conducting a prospective randomized clinical trial comparing HDCT with Auto-PBSCT with high-dose cytarabine as postremission therapy for patients with AML in CR1.

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Conflict of interest The authors declare no conflict of interest.

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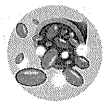

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RESEARCH Open Access

Monitoring of minimal residual disease (MRD) is useful to predict prognosis of adult patients with Ph-negative ALL: results of a prospective study (ALL MRD2002 Study)

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Abstract

Background: Allogeneic hematopoietic stem cell transplantation (HSCT) for patients with Philadelphia chromosome (Ph)-negative acute lymphoblastic leukemia (ALL) in first complete remission (CR1) is much more intensive than multi-agent combined chemotherapy, although allogeneic HSCT is associated with increased morbidity and mortality when compared with such chemotherapy. Minimal residual disease (MRD) status has been proven to be a strong prognostic factor for adult patients with Ph-negative ALL.

Methods: We investigated whether MRD status in adult patients with ALL is useful to decide clinical indications for allogeneic HSCT. We prospectively monitored MRD after induction and consolidation therapy in adult patients with Ph-negative ALL.

Results: Of 110 adult ALL patients enrolled between July 2002 and August 2008, 101 were eligible, including 59 Ph-negative patients. MRD status was assessed in 43 patients by the detection of major rearrangements in *TCR* and *Ig* and the presence of chimeric mRNA. Thirty-nine patients achieved CR1, and their probabilities of 3-year overall survival and disease-free survival (DFS) were 74% and 56%, respectively. Patients who were MRD-negative after induction therapy (n = 26) had a significantly better 3-year DFS compared with those who were MRD-positive (n = 13; 69% vs. 31%, p = 0.004). All of 3 patients who were MRD-positive following consolidation chemotherapy and did not undergo allogeneic HSCT, relapsed and died within 3 years after CR.

Conclusions: These results indicate that MRD monitoring is useful for determining the clinical indications for allogeneic HSCT in the treatment of ALL in CR1.

Keywords: Acute lymphoblastic leukemia, Minimal residual disease, Hematopoietic stem cell transplantation, Adult

Background

Although more than 80% of adult patients with Philadelphia chromosome (Ph)-negative acute lymphoblastic leukemia (ALL) achieve complete remission (CR) with conventional induction therapy, their 5-year survival is only 30%–40%. Leukemia relapse is the most common cause of treatment failure in ALL [1-6]. Therefore, post-remission therapy is necessary and should be optimized in the treatment of adult ALL patients. If prognosis of patients with ALL in CR1 is estimated to be favorable, chemotherapy is usually continued to prevent leukemia relapse. However, patients with less favorable prognosis should be treated more aggressively [7]. Although allogeneic hematopoietic stem cell transplantation (HSCT) for patients with ALL in CR1 is much more intensive than multi-agent combined chemotherapy, it is associated with increased morbidity and mortality when compared with such chemotherapy.

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Minimal residual disease (MRD) status has been proven to be a strong prognostic factor for adult patients with Ph-negative ALL [8-14]. In this study, we prospectively monitored the MRD status after CR induction and consolidation chemotherapies in adult patients with Ph-negative ALL to determine the clinical indications for allogeneic HSCT.

Patients & methods

Patient eligibility criteria

A total of 110 adult ALL patients were enrolled in this study between July 2002 and August 2008 on the basis of the following eligibility criteria: non-L3 ALL, 16–65 years of age, an Eastern Cooperative Oncology Group performance status of 0–2, and adequate liver and kidney function (serum bilirubin, ≤2.0 mg/dl and serum creatinine, ≤2.0 mg/dl, respectively). Cytogenetic studies were performed on pretreated bone marrow or unstimulated blood samples using the standard banding technique. The treatment protocol was approved by the institutional review board of each participating hospital. Written informed consent was obtained from all patients in accordance with the Declaration of Helsinki. Of the 110 patients enrolled, 42 were excluded from the study because of Ph-positivity, 5 because of misdiagnosis, 2 because of infectious complications, and 1 each because of liver damage and protocol violation. The remaining 59 patients were Ph-negative.

Treatment

We used a modified CALGB 19802 [15,16] treatment protocol that comprised 6 courses of chemotherapy administered in the order of A-B-C-A-B-C regimens, followed by a maintenance phase. Induction chemotherapy (course A) was a 21-day course consisting of cyclophosphamide (CPM; 1200 mg/m^2 on day 1), daunorubicin (DNR; 60 mg/m^2 on days 1, 2, and 3), vincristine (VCR;1.3 mg/m^2 [maximum 2 mg] on days 1, 8, 15, and 22), L-asparaginase (3000 U/m^2 on days 9, 11, 13, 16, 18, and 20), and prednisolone (PSL; 60 mg/m^2 [max 100 mg]). Granulocyte-colony stimulating factor (nartograstim) was administered starting from day 4 and continued until neutrophil recovery. For patients aged 55 years or older, the doses of CPM and DNR were reduced to 500 mg/m^2 and 50 mg/m^2, respectively. Furthermore, PSL therapy was shortened to 7 days in these patients. The first consolidation therapy (course B) consisted of mitoxantrone (MIT; 10 mg/m^2 on days 2 and 3), cytarabine (AraC; 2000 mg/m^2/day on days 1, 2, 3, and 4) and intrathecal administration of methotrexate (MTX; 15 mg/body on day 1). For patients aged 55 years or older, the doses of MIT and AraC were reduced to 7 mg/m^2 and 1500 mg/m^2/day, respectively. The second consolidation therapy (course C) consisted of VCR (1.3 mg/m^2 [max 2 mg] on days 1, 8, and 15) and MTX (1500 mg/m^2 on days 1, 8, and 15) with leucovorin rescue and intrathecal MTX on days 1, 8, and 15. The patients received the following maintenance chemotherapy on a monthly basis: PSL, 60 mg/m^2 on days 1–5; VCR, 1.3 mg/m^2 (max 2 mg) on day 1; oral MTX, 20 mg/m^2 weekly; and oral 6-mercaptopurine, 60 mg/m^2 daily. MRD status was evaluated after the induction therapy (first course A) and after the second consolidation therapy (first course C). Patients with positive MRD following the second consolidation therapy were considered to be indicated for allogeneic HSCT as soon as possible. Eligible donors included HLA-identical related, HLA-identical unrelated donors from Japan Marrow Donation Program, and cord blood from Japan Cord Blood Bank Network. Conditioning before allogeneic HSCT and prophylaxis for graft-versus-host disease was performed according to each institutional standard.

MRD analysis

Real-time quantitative polymerase chain reaction (RQ-PCR) analysis of chimeric mRNA

mRNA from bone marrow cells were analyzed for the presence of major and minor *BCR/ABL*, *TEL/AML1*, *MLL/AF4*, *MLL/AF9*, *MLL/AF6*, *MLL/ENL*, *E2A/PBX1*, and *SIL/TAL1* chimeric genes. Samples were amplified by RQ-PCR and quantified by parallel amplification of serial dilutions of transcript-containing plasmids [17,18].

PCR analysis of TCR/Ig rearrangement

High-molecular weight DNA from marrow cells was initially screened for major rearrangement patterns of *TCRγ*, *TCRδ*, and *Igκ*, and secondarily screened for rearrangements in *Ig heavy chain* (*IgH*), using previously described primers [19-21]. Two-step (nested) PCR for MRD quantification was performed using allele-specific oligonucleotide (ASO)-primers based on the sequence of PCR screening products, which had clonal recombinations by heteroduplex analyses. Prior to PCR analysis, DNA samples from post-treatment bone marrow samples and DNA from the samples obtained at diagnosis were serially diluted (between 10^{-2} and 10^{-5}) with buffy coat DNA from eight healthy volunteers. Buffy coat DNA was also used as a control for nonspecific amplification of comparable *Ig/TCR* arrangements present in normal cells. All PCR reactions were performed simultaneously and analyzed using ethidium staining and agarose gel electrophoresis. MRD was quantified by comparing the intensities of band signals on an agarose gel stained with ethidium bromide without amplification of the background. MRD quantifications were performed using ASO-primers with a sensitivity of $\leq 1 \times 10^{-4}$, and MRD positivity was defined as a lower limit of detection of $\geq 1 \times 10^{-3}$.

Statistical analysis

Statistical analyses of the data accumulated throughout October 2011 were performed. Overall survival (OS) was

defined as the time between diagnosis and the end of the trial or death, and disease-free survival (DFS) was defined as the time from CR to relapse or death while still in CR. Survival curves were estimated using the Kaplan–Meier method, and the statistical significance of differences in survival was determined using the log-rank test.

The influence of prognostic factors including age, white blood cell (WBC) count, and MRD status on DFS was estimated with multivariate Cox regression analysis. The level of statistical significance was set at 0.05.

Results

Treatment outcome

The median follow-up time was 1134 days (range, 14–3248 days). A total of 59 patients were Ph-negative (29 males and 30 females), and their median age was 35 years ranging from 16 to 63. The median white blood cell count at presentation was 11.0×10^3/L (range 0.9–409). CR was achieved in 47 patients (80%). Six patients died during induction; their causes of death included sepsis (n = 3), pneumonia (n = 2), and other (n = 1). There were 29 survivors after the median follow-up period. The probability of 3-year OS and DFS in these patients with Ph-negative ALL was 59% and 52%, respectively (Table 1).

Relationship between MRD status and treatment outcomes

Among the 59 Ph-negative ALL patients, 43 patients (73%) could be monitored for MRD status, and the remaining 16 patients were not because 10 had no clonal *TCR/Ig* targets or chimeric mRNA and 6 did not provide sufficient DNA or RNA from their samples. The MRD status of 43 patients (21 males and 22 females; median age: 31 years, ranging from 17 to 63; median WBC count at presentation: 10.6×10^3/L ranging 1–409) was determined by PCR analysis of major gene rearrangements and/or chimeric mRNAs (15 were positive for *TCRγ*, 6 for *TCRδ*, 6 for *Igκ*, 11 for *IgH*, 1 for *TCRγ* and *TCRδ*, 1 for *TCRδ* and *IgH*, 1 for *E2A-PBX*, 1 for *MLL-AF4*, and 1 for *MLL-ENL*). CR was achieved in 39 of these 43 patients with known MRD status (91%). The median follow-up time was 1421 days (range, 162–3248 days). The probability of 3-year OS and DFS in the Ph-negative patients with known MRD status was 74% and 56%, respectively (Table 1). In terms of CR1 status, MRD-negative patients after induction chemotherapy A in the first course (n = 26) showed a better 3-year DFS (69%) compared with MRD-positive patients (n = 13; 31%), as shown in Figure 1. The difference was statistically significant (p = 0.004). MRD-negative patients also showed a significantly lower 3-year relapse rate compared with MRD-positive patients (28% vs. 58%, p = 0.031).

Table 1 Patient characteristics and clinical outcome

	Ph negative	Ph negative & known MRD status
Total No. patients	59	43
Sex, No. (%)		
Male	29 (49)	21 (49)
Female	30 (51)	22 (51)
Median Age, (range)	35 (16-63)	31 (17-63)
Median WBC count, $\times 10^9$/L, (range)	11.0 (0.9-409)	10.6 (1-409)
Immunophenotype, No. (%)		
B-lineage	45 (76)	36 (84)
T-lineage	14 (24)	7 (16)
CR rate, No. (%)	47 (80)	39 (91)
3-years OS (%)	59	74
3-years DFS (%)	52	56

MRD, minimal residual disease; *Ph*, Philadelphia chromosome; *CR*, complete remission; *OS*, overall survival; *DFS*, disease-free survival.

There was no patient who proceeded to allogeneic HSCT among 26 MRD-negative patients after induction therapy in CR. In contrast, patients who were MRD-positive after induction but became MRD-negative after consolidation chemotherapy C in the first course (n = 7) showed a significantly worse 3-year DFS compared with patients who were MRD-negative after induction chemotherapy A in the first course (29% vs. 69%, p = 0.004), as shown in Figure 2. Among 7 late-attained MRD-negative patients, three patients proceeded to allogeneic HSCT when MRD status became positive again under maintenance therapy. Six patients were MRD-positive after consolidation chemotherapy C in the first course, and 3 patients among them proceeded to allogeneic HSCT, while other 3 patients did not because of lack of a suitable donor (n = 1) and of patients' refusal to allogeneic HSCT (n = 2). All of 3 MRD-positive patients who did not undergo allogeneic HSCT, relapsed and died within 3 years after CR, whereas 2 of 3 patients those who received allogeneic HSCT gave DFS at 3 years. Table 2 shows the results of multivariate Cox regression analysis for DFS in 43 MRD-evaluable patients. The analysis indicates that age (≥35 years vs. <35 years: Hazard ratio (HR) 5.067, and p = 0.005) and MRD status after induction therapy (positive vs. negative: HR 8.769, and p < 0.001) were significant prognostic factors, whereas WBC count ($\geq 30 \times 10^9$/L vs. $<30 \times 10^9$/L: HR 1.496, and p = 0.505) or MRD status after consolidation therapy (positive vs. negative: HR 0.675, and p = 0.556) was not.

Discussion

Compared with treatments for childhood ALL, those for adult ALL are far less effective [22], and allogeneic HSCT is frequently recommended as the most potent post-remission therapy for ALL patients in CR1 [23].