Table 4 Comparison of acute toxicity according to treatment arm with Japanese registration

Reported toxicity	International study[11]			Japanese registration		
	MTX1 arm 1,025 courses	MTX3 arm 1,025 courses	P	MTX1 arm 129 courses	MTX3 arm 129 courses	P
	% of courses with toxicity	% of courses with toxicity		% of courses with toxicity	% of courses with toxicity	
All types, all grades	97	92	.002	100	95	.014
Severe toxicity	83	68	<.0001	96	78	<.0001
Hematologic toxicity, grade 3–4	79	64	<.0001	95	74	<.0001
Infection						
Infection, grade 3–4	6	5	.32	2	2	.645
Infection, all grade	50	32	<.0001	53	29	<.0001
Other toxicity, grade 3–4	32	16	<.0001	28	21	.181
Stomatitis, grade 3–4	21	6	<.0001	5	0	.007
Liver toxicity, grade 3–4	13	10	.06	21	17	.41
Miscellaneous, grade 3–4	7	5	.13	6	4	.39

選択した場合の転帰，治療毒性とも，国際臨床試験結果と同様の結果であることが示された。本集計の結果は，今後，小児 ALCL に対する新たな国際臨床試験を計画する上で，有用な情報と考えられる。

国内登録例の集計と国際臨床試験報告の比較において，試験治療の有効性と安全性が同様であった結果には，試験に使用された薬剤の効果・毒性に関する人種差のみならず，腫瘍細胞の生物学的特性の差，生活・医療環境の違いなど，さまざまな要素が反映されている可能性が推測される。治療開発に際し，薬剤の効果・毒性に関する人種差の影響の検証は課題のひとつであるが，今回の集計において，これらの要素の影響の検討が可能な情報収集には至らなかった。

小児 ALCL は約 30％に再発を生じる。ALCL99-R1 においても，無作為割り付け対象 352 例中，84 例に再発が報告された。再発 ALCL に対する治療は未整備であり，造血細胞移植を含むさまざまな治療が選択されている[13~17]。共通のファーストライン治療が採用された国際臨床試験体制は，再発 ALCL に対する至適治療の整備にも貢献することが可能と考えられる。

ALCL99-R1 は小児 ALCL の約 70％に無イベント生存が期待される治療を示したものの，入院を要する 4〜6 か月の治療期間，晩期合併症のリスクが懸念されるアルキル化剤，アントラサイクリン製剤の使用などの課題が残されている。ファーストライン治療のさらなる改善のためには，進行，再発に関連するリスク因子の検出，新規薬剤の導入が期待される。ドイツ，イタリアのグループにより，ALCL の診断時の，定量 PCR 法により評価される骨髄，末梢血中の ALK コピー数[18, 19]，および血清抗 ALK 抗体価[20]が，進行，再発に関連することが報告されている。EICNHL，および JPLSG は，小児 ALCL に対する次期国際臨床試験の準備として，定量 PCR 法による骨髄，末梢血中の ALK コピー数，および血清抗 ALK 抗体価の測定の標準化の取り組みを続けている。

国際臨床試験は ALCL など頻度の低い小児リンパ腫に対する至適治療の整備に貢献する方法と考えられる。国際臨床試験において検証された事象について，少数の国内登録例において同様の傾向を確認することは，頻度の低い疾患に対する標準治療を提示する方法のひとつと考えられる。一方で，稀少な疾患を対象とした新規薬剤の導入に際しては，国際臨床試験参加各国における新規薬剤開発に関わる法律，指針，臨床研究支援体制，医療体制の違いなど，解決が求められる課題は少なくないと推測される。

謝　辞

ALCL99 研究に参加された患者様，およびそのご家族，試験治療を担当，および試験データを提示いただいた医療機関の皆様，および同研究に協力いただいたすべての関係者に深謝致します。

著者の COI (conflicts of interest) 開示：本論文発表内容に関連して特に申告なし

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Analysis of Japanese Registration from the Randomized International Trial for Childhood Anaplastic Large Cell Lymphoma (ALCL99-R1)

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Key words : ALCL, Childhood, International clinical trial, Collaboration

The randomized international trial for childhood anaplastic large cell lymphoma, (ALCL99-R1) involving European study groups and a Japanese group, compared six courses of methotrexate 1 g/m^2 over 24 hours with an intrathecal injection (IT) (MTX1 arm) with six courses of methotrexate 3 g/m^2 over 3 hours without IT (MTX3 arm). In this report, data from the Japanese portion of the trial are compared with the results of the international study. Overall, 352 patients were recruited for the international study, and 44 of these patients were from Japan. Median follow-up times were 3.8 and 3.5 years, respectively, in the international and Japanese studies. The two-year event-free and 2-year overall survival rates of the international study were 74% and 93%. The corresponding figures for those registered in Japan were 81% and 96%, respectively. Clinical characteristics and outcomes of patients were similar in the two groups. Incidences of grade 4 hematologic toxicity, infection, and grade 3 to 4 stomatitis, which were reported to be statistically significantly higher after the MTX1 arm in the international study, were also statistically significantly higher after the MTX1 arm for those registered in Japan. Results of ALCL99-R1 treatment in Japan were essentially the same as in the international study. The international study is anticipated to contribute to establishing an optimal treatment for ALCL, a rare childhood lymphoma.

小 児 科　第55巻　第11号（平成26年10月1日発行）別冊

Pediatrics of Japan
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金 原 出 版 株 式 会 社

特集 小児血液疾患—よくわかる最新知見— 38
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Key words
非ホジキンリンパ腫
化学療法
標準治療
分子標的治療

非ホジキンリンパ腫

森 鉄也*

要旨

小児非ホジキンリンパ腫の主要な病型に対する標準的な治療は概ね整備されている．標準的な治療により80%以上の小児リンパ腫患者は少なくとも5年以上生存する．さらに最近では，抗体，チロシンキナーゼ阻害薬など分子標的治療薬剤の開発が進められている．小児非ホジキンリンパ腫の診療に際しては，正確な診断，長期生存の可能性が高いことを前提とした治療選択・実践が求められる．また，晩期合併症の評価・対応，および軽減への取り組みは重要な課題である．

Ⅰ 疫学

リンパ腫は小児がんの約7%，1/100,000人の発生頻度のリンパ系細胞由来の悪性腫瘍である．病理組織像からホジキンリンパ腫（Hodgkin lymphoma：HL），非ホジキンリンパ腫（non-Hodgkin lymphoma：NHL）に大別される．大部分の小児NHLはさらにバーキットリンパ腫（Burkitt lymphoma：BL），びまん性大細胞型B細胞性リンパ腫（diffuse large B-cell lymphoma：DLBCL），リンパ芽球性リンパ腫（lymphoblastic lymphoma：LBL），未分化大細胞型リンパ腫（anaplastic large cell lymphoma：ALCL）に分類される．NHLは10歳以降の発症が多く，3歳未満は低頻度である．原発性縦隔B細胞性リンパ腫（primary mediastinal B-cell lymphoma）を除き，男児に高頻度である．NHLは小児後天性免疫不全症候群（AIDS）患者が発症する悪性腫瘍としてもっとも頻度が高く，低年齢で発症することも少なくない．**表1**に日本小児血液・がん学会疾患登録におけるリンパ腫の病型別登録数を示す[1]．

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Ⅱ 病因・病態

1．病因

小児リンパ腫の正確な発症機序は不明であるが，リンパ球が分化過程において抗原受容体や細胞増殖関連因子に異常を生じ，生理的な免疫監視機構や増殖制御機構を逸脱して増殖した結果と推測されている．骨髄移植後，臓器移植後などの細胞性免疫が低下した状況では，Epstein-Barr virus（EBV）感染により移植後リンパ増殖性疾患（post-transplant lymphoproliferative disorder：PTLD）を発症することがある．

2．病態

NHLの発症臓器や細胞増殖は病理組織型により異なり，病態，臨床像は多彩である．頻度の高い病型の病態，臨床像の特徴を以下に記す．

a．バーキットリンパ腫（BL）

高度に進行性で，しばしば急性白血病を含む

表1　日本小児血液・がん学会疾患登録におけるリンパ腫の病型別登録数（2008～2010年）

疾患	2008年	2009年	2010年
急性リンパ性白血病	532	504	478
急性骨髄性白血病	184	193	182
ホジキンリンパ腫	24	14	31
非ホジキンリンパ腫	138	137	108
T細胞性リンパ芽球性リンパ腫	32	29	21
B前駆細胞性リンパ芽球性リンパ腫	22	10	16
バーキットリンパ腫	33	37	29
びまん性大細胞型B細胞性リンパ腫	23	27	21
未分化大細胞型リンパ腫	16	24	15
その他の非ホジキンリンパ腫	12	10	6

リンパ節外病変を示す．腹部腫瘤で発症することが多く，他の病変としてワルダイエル咽頭輪，副鼻腔，骨，末梢リンパ節，皮膚，精巣，骨，骨髄，中枢神経がある．腸管リンパ節由来の腹部腫瘤は腸重積として発症することもある．

b．びまん性大細胞型B細胞性リンパ腫（DLBCL）

臨床像はBLに類似することが多いものの，DLBCLでは縦隔病変を認めることがあり，骨髄，中枢神経浸潤の頻度は低い．

c．リンパ芽球性リンパ腫（LBL）

縦隔腫瘤を有するT細胞性LBL例では，胸水，呼吸困難，上大静脈症候群などを伴うこともあり，骨髄，中枢神経に病変を認めることもある．限局病変のみのLBLはリンパ節，骨，皮下組織などにみられる．骨髄浸潤例では急性リンパ性白血病（acute lymphoblastic leukemia：ALL）との鑑別が問題になる．

d．未分化大細胞型リンパ腫（ALCL）

大部分の症例は進行病期であり，しばしば発熱を伴う．リンパ節外病変を認めることが多く，皮膚，骨，軟部組織，肺，肝病変の頻度が高い．消化管や中枢神経浸潤はまれである．血球貪食性リンパ組織球症（hemophagocytic lymphohistiocytosis：HLH）の病態を合併することもある．

Ⅲ 診断

1．生検と病理診断

生検などにより採取された腫瘍組織を用いて病理組織学的に診断する．末梢血，骨髄，胸水，あるいは腹水中に評価可能な割合のリンパ腫細胞を認める場合には，これらを診断材料とすることが可能である．生検では，もっとも採取しやすい病変から組織を入手することが原則である．リンパ腫の診断に必要な免疫組織染色，染色体・遺伝子検査などの解析を行うためには，摘出された腫瘍検体を適切に処理，保存しなければならない．

病理診断のための生検は最低限の侵襲による方法を選択すべきである．巨大縦隔腫瘤を伴う例では全身麻酔や深い鎮静に伴い心肺停止を生じる危険を有する．診断に要する手術手技の危険性が著しく高い場合は，放射線局所照射などによる診断前治療を考慮する．

2．病期診断

小児NHLの病期診断にはSt. Jude staging system（Murphy分類）が汎用されている[2]．治療の根拠となる臨床試験プロトコール（あるいは報告）における病期診断方法に従うことが原則である．

※成人リンパ腫例においてfluorodeoxyglucos(FDG)-PET scanによる評価は，診断時のより多くの病巣の検出，治療後の残存腫瘤中の腫瘍細胞の検出においてガリウムシンチグラフィーと比較して有用であることが示されている．本稿執筆時（2014年7月）まで，小児NHLにおけるFDG-PET scanによる評価の経験は十分でなく，結果の解釈には慎重な姿勢が求められる．

3．合併症

小児NHLの発症・診断・治療開始時には，気道圧迫・狭窄，上大静脈症候群，脊髄圧迫，腫瘍崩壊症候群など緊急対応を要する病態（oncologic emergency）が少なくない．診断確定までの期間，あるいは治療開始早期にこれらの合併症が急速に進行する可能性を十分に想定し，適切な対応の準備が求められる．

Ⅳ 治療

小児NHLに対する代表的な臨床試験におけるリスク分類，治療期間，成績を**表2**に示す．本項では，各病型に対する最近の取り組み，話題を紹介する．

1．成熟B細胞性非ホジキンリンパ腫（B-NHL）

BL，DLBCLをB-NHLとして同一の治療を適用する．ドイツなどにおいて行われた臨床試験であるNHL-BFM95[3]，フランス，英国，米国などにおいて行われたFAB/LMB96[4)~6)]，日本において行われたJPLSG B-NHL03[7]などが標準的な治療に位置づけられる．

リツキシマブはCD20に対する抗体製剤であり，補体依存性細胞傷害作用，および抗体依存性細胞介在性細胞傷害作用により効果を発現する．成人DLBCLに対する複数の第Ⅲ相試験において，CHOP（シクロホスファミド，ドキソルビシン，ビンクリスチン，プレドニゾロン）あるいはCHOP類似化学療法にリツキシマブを追加する有効性が示されている[8)9)]．小児DLBCLおよびBLはいずれもCD20を発現する．小児にB-NHLに対するリツキシマブの使用経験は限られている．主な知見を以下に示す．

① Children's Oncology Group（COG）は再発B-NHLを対象としたICE療法（イホスファミド，カルボプラチン，エトポシド）にリツキシマブを追加する（R-ICE）臨床試験を行った[10]．DLBCLの6例中3例，BLの14例中4例が完全寛解に到達し，6例が大量化学療法，造血幹細胞移植に進んだ．

② COGはリツキシマブ追加LMB化学療法の毒性の評価を目的としたパイロット臨床試験ANHL01P1を行った[11)12)]．リツキシマブは，2コースの寛解導入療法開始時に48時間間隔で2回，その後の各強化療法開始時に1回追加された．LMB化学療法単独と比較してリツキシマブ追加による毒性の増強は明らかでなく，小児におけるリツキシマブの薬物動態は成人と類似していることが示された．3年無イベント生存率は，グループB（40例）で92%，グループC（37例）で86%であった．

③ Berlin-Frankfurt-Münster Group（BFM）は標準化学療法の開始5日前にリツキシマブを投与する第Ⅱ相ウィンドウ試験を行った（BFM04 trial）[13]．評価可能な87例のうち，36例に効果（少なくとも1カ所の病変，あるいは骨髄，あるいは末梢血における25%以上の減少）を認めた．病理組織型による治療反応の差は明らかでなかった．骨髄病変における治療反応は腫瘤病変と比較して良好であった．

現在，6カ月以上，18歳未満の高リスクB-NHLに対する標準LMB化学療法とリツキシマブ追加LMB化学療法の比較により，リツキシマブ追加による3年無イベント生存率の改善の検証を目的とした欧州，米国など12カ国による国際共同臨床試験が行われている（EudraCT N°：2010-019224-31）．

表2　小児非リンパ腫に対する主な臨床試験におけるリスク分類，治療期間，予後

病型	研究 対象症例数	リスク群	治療期間	無イベント 生存率 （観察期間）	文献
成熟B細胞性非ホジキンリンパ腫	NHL-BFM95 R1＝48 R2＝233 R3＝82 R4＝142	R1：病変が摘出された病期Ⅰ＋Ⅱ	2 courses	94％（3年）	3
		R2：病変が摘出されていない病期Ⅰ＋Ⅱ 病期Ⅲで LDH＜500	Pre-phase ＋4 courses	94％（3年）	
		R3：病期Ⅲで 500≦LDH＜1,000 病期Ⅳで LDH＜1,000 かつ中枢神経浸潤なし	Pre-phase ＋5 courses	85％（3年）	
		R4：病期Ⅲ＋Ⅳで LDH≧1,000 かつ/または中枢神経浸潤あり	Pre-phase ＋6 courses	81％（3年）	
	FAB/LMB96 Group A＝132 Group B＝762 Group C＝190	A：病変が摘出された病期Ⅰ 腹部病変が摘出された病期Ⅱ	2 courses	98％（3年）	4
		B：病変が摘出されていない病期Ⅰ 腹部病変が摘出された例以外の病期Ⅱ 病期Ⅲ＋Ⅳで骨髄中の芽球≦25％かつ中枢神経浸潤なし	Pre-phase ＋4 courses	90％（4年）	5
		C：病期Ⅲ＋Ⅳで骨髄中の芽球＞25％かつ/または中枢神経浸潤あり	Pre-phase ＋8 courses	79％（4年）	6
	JPLSG B-NHL03 Group 1＝17 Group 2＝103 Group 3＝111 Group 4＝90	G1：病変が摘出された病期Ⅰ＋Ⅱ	2 courses	97％（4年）	7
		G2：病変が摘出されていない病期Ⅰ＋Ⅱ	Pre-phase ＋4 courses	97％（4年）	
		G3：病期Ⅲ 病期Ⅳで骨髄中の芽球≦25％かつ中枢神経浸潤なし	Pre-phase ＋6 courses	82％（4年）	
		G4：病期Ⅳで骨髄中の芽球＞25％かつ/または中枢神経浸潤あり	Pre-phase ＋6 courses	71％（4年）	
リンパ芽球性リンパ腫	NHL-BFM95 n＝156	病期Ⅲ＋Ⅳ	2 years	82％（5年）	14
未分化大細胞リンパ腫	ALCL99-R1 n＝352	標準リスク：皮膚，縦隔，肝臓，脾臓のいずれにも病変なし	Pre-phase ＋6 courses	74％（2年）	17
		高リスク：皮膚，縦隔，肝臓，脾臓のいずれかに病変あり			

2．リンパ芽球性リンパ腫（LBL）

ドイツなどで行われた臨床試験である NHL-BFM95[14]が標準的な治療に位置づけられる．

日本小児白血病リンパ腫研究グループ（Japanese Pediatric Leukemia/Lymphoma Study Group：JPLSG）は進行病期 LBL を対象とした NHL-BFM95 類似化学療法の維持療法を強化した臨床試験（JPLSG ALB-NHL03, UMIN000002212）を行い，2010 年 10 月に 154 例の登録を終了した．登録例の染色体解析の集計結果が発表されている[15]．今後，治療成績などが発表される予定である．

Pediatric Oncology Group は T 細胞性 ALL，および T 細胞性 LBL に対する化学療法に大量メトトレキサート療法（high-dose methotrexate：HD-MTX）追加の有効性を検証する臨床

試験を行った[16]．少数（66例）の集計ではあるが，HD-MTXを追加しない化学療法で治療されたT細胞性LBLの5年無イベント生存率は88%と報告された．ただし，この対象例には中枢神経予防治療として全脳全脊髄照射が行われていることに留意しなければならない．

3．未分化大細胞リンパ腫（ALCL）

欧州の国際共同研究グループ（European Intergroup for Childhood non-Hodgkin lymphoma：EICNHL）とJPLSGにより行われた国際臨床試験ALCL99が標準的な治療に位置づけられている[17)~19)]．

クリゾチニブは，anaplastic lymphoma kinase（ALK）の受容体チロシンキナーゼとその発がん性変異体に対するチロシンキナーゼ阻害薬であり，国内外でALK陽性の切除不能な進行・再発の非小細胞肺癌に対する製造販売が承認されている．ALCLに対するクリゾチニブの開発は途上であり[20)21)]，本稿執筆時（2014年7月），欧米，成人においても未承認である．米国で行われた小児固形腫瘍を対象とした第Ⅰ相臨床試験（ADVL0912）によりクリゾチニブ280 mg/m^2 1日2回投与の忍容性が認められ，9例の再発・増悪ALCL中8例に奏功を認めたことが報告された[21)]．

ブレンツキシマブベドチンは，CD30に対する抗体薬物複合体であり，再発成人ALCLに対する第Ⅱ相試験において，55～60%の完全寛解率が報告された[22)23)]．国内においても，再発ALCL，および再発HLに対する製造販売が2014年1月に承認された．小児におけるブレンツキシマブベドチンの開発は，本稿執筆時点（2014年7月）で，欧米において第Ⅰ/Ⅱ相臨床試験が行われている段階である．

4．治療抵抗・再発リンパ腫

小児NHLに対するfirst line治療成績は良好であり，治療抵抗・再発リンパ腫に対する情報は乏しく，治療は未整備である．一部のALCLを除き，治療抵抗・再発NHLの予後は著しく不良である．

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Pediatr Blood Cancer 2015;62:674–679

Hematopoietic Stem Cell Transplantation Following Unsuccessful Salvage Treatment for Relapsed Acute Lymphoblastic Leukemia in Children

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Background. For children who experience a re-induction failure or multiple recurrences following the first relapse of acute lymphoblastic leukemia (ALL), it is uncertain whether additional intensive chemotherapy aimed at hematopoietic stem cell transplantation (SCT) in complete remission (CR) or immediate SCT even in non-CR should be performed. This study aimed to investigate the impact of disease status at SCT on the outcomes of SCT for these children, whose prognosis is considered unquestionably poor even with SCT. ***Procedure.*** The medical records of 55 children with ALL who underwent SCT following the experience of re-induction failure (n = 25) or multiple relapses (n = 30) were retrospectively analyzed. ***Results.*** Twenty-one patients underwent SCT in CR (delayed CR2, CR3, and CR4) and 34 in non-CR (first or subsequent relapse). The probability of overall survival of patients with CR and with non-CR at SCT was 42.9% and 23.5% ($P=0.15$), leukemia-free survival was 38.1% and 20.6% ($P=0.18$), and the cumulative incidence of relapse at 2 years was 23.8% and 50%, respectively ($P=0.05$). In multivariate analysis, non-CR at SCT was a significant risk factor for higher relapse incidence and male sex was a significant risk factor for lower survival. ***Conclusions.*** Our results indicated that in case of tolerable patient condition, further re-induction chemotherapy might be reasonable so that SCT could be performed in CR, which might result in a low incidence of relapse after SCT. Novel approaches are required to induce CR for the treatment of children with relapsed/refractory ALL. Pediatr Blood Cancer 2015;62:674–679.

Key words: pediatric ALL; multiple relapse; re-induction failure; disease status at transplantation; stem cell transplantation

INTRODUCTION

The prognosis of children with acute lymphoblastic leukemia (ALL) who fail salvage treatment following the first relapse is reportedly very poor [1–4]. Further treatment with curative intent can only induce a second complete remission (CR2) in a small subset of these patients while associating with high treatment-related mortality. In a recent retrospective analysis of patients with first relapse in the Berlin/Frankfurt/Muenster (ALL-REZ BFM) study group, 51 patients who did not respond to the initial re-induction regimen continued to receive multi-agent chemotherapy with 22 of them also undergoing stem cell transplantation (SCT) [1]. Less than one third (16/51; 31%) of these patients achieved CR2 and 13 did only after SCT. Of the 16 patients with CR2, only 2 remained in continuous remission at the time of analysis. In a retrospective multi-institutional review, the re-induction rates after second and third relapse in children with ALL were 44% and 27%, respectively and the 5-year disease-free survival was 27% and 15%, respectively [2]. The chance of remission and cure is reduced with every subsequent relapse and the risk of sequelae and toxicities associated with intensive therapy increases in patients with multiple relapses.

Generally, the prognosis of patients who experience a re-induction failure or a second or subsequent relapse following the first relapse of ALL is considered very poor even if they undergo SCT. Previous reports suggest extremely poor SCT outcomes for patients in relapse [5–7] or third and further CR [6,8,9] at SCT. However, it remains uncertain whether these patients should definitely be transplanted in CR or transplantation performed in non-CR would lead to similar clinical outcomes. As a result, a decision between immediate SCT to avoid toxicities associated with further intensive chemotherapy and additional intensive chemotherapy to hopefully achieve CR before SCT is often difficult.

This study investigated whether disease status at SCT (CR or non-CR) affected the outcomes of SCT for children experiencing re-induction failure or multiple recurrences after the first relapse of ALL.

METHODS

Patient Characteristics

Between 1990 and 2013, a total of 101 children with B-cell precursor or T-cell ALL who relapsed following the front-line chemotherapy underwent SCT. Of these, the medical records of 55 who did not achieve CR2 after the first re-induction chemotherapy (re-induction failure, n = 25), or achieved CR2 after the first re-induction chemotherapy but later suffered a subsequent relapse (multiple relapses, n = 30), were retrospectively analyzed in this study. No patients with primary induction failure or previous transplantation were included. Additionally, those with repeated isolated central nervous system relapse were also excluded as they received extended period of post-transplant intrathecal chemotherapy [10]. CR was defined as a bone marrow (BM) morphological blast count of less than 5% with the absence of blast in peripheral blood (PB) and extramedullary (EM) disease as well as the sufficient recovery of nuclear cell count. All patients or their parents provided written informed consent to hematopoietic SCT. This study and its use of patient data were approved by the institutional review board of the National Kyushu Cancer Center.

After the first relapse, all 55 patients received various intensive re-induction chemotherapy mainly consisted of four to five drugs already used in their first-line therapy, such as anthracycline, vinca alkaloid, L-asparaginase, alkylating agent, or corticosteroid. For most patients included in this study, there were no standardized relapse study in Japan at the time of their treatment. Therefore, the

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Conflict of interest: Nothing to declare.

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Received 9 June 2014; Accepted 22 October 2014

DOI 10.1002/pbc.25353
Published online 24 December 2014 in Wiley Online Library (wileyonlinelibrary.com).

TABLE I. Patient Characteristics

	All patients (n = 55)	CR at SCT (n = 21)	Non-CR at SCT (n = 34)	*P*-value
Age at SCT (median) years	9 (2–19)	10 (4–19)	9 (2–16)	0.7
Sex				
Male	32	10	22	0.21
Female	23	11	12	
Immunophenotype				
BCP (Ph)	48 (4)	19 (2)	29 (2)	0.48
T	7	2	5	
Time of the first relapse				
Very early[a]	20	5	15	0.24
Early[b]	24	10	14	
Late[c]	11	6	5	
Site of the first relapse				
BM	44	16	28	0.08
BM + EM	6	1	5	
EM	5	4	1	
Response to the first re-induction chemotherapy				
CR2	30	11	19	0.8
Re-induction failure	25	10	15	
Number of drugs used in the first re-induction chemotherapy				
3–4	31	10	21	0.44
5–6	21	9	12	
Missing data	3	2	1	
Number of re-induction chemotherapy (times)				
1–2	36	13	23	0.66
3≤	19	8	11	
Interval from the first relapse to SCT (months)				
≤7	31	8	23	0.03
8≤	24	13	11	
Year of SCT				
1990–2000	24	10	14	0.64
2001–2013	31	11	20	
Donor				
HLA-matched related	18	7	11	0.81
HLA-mismatched related	13	4	9	
HLA-matched unrelated	17	7	10	
HLA-mismatched unrelated	7	3	4	
Stem cell source				
BM	47	19	28	0.66
CB	5	1	4	
PB	3	1	2	
Conditioning regimen				
TBI/BU/Mel	19	8	11	0.76
TBI/Mel	19	6	13	
Others	17	7	10	
GVHD prophylaxis				
MTX	4	1	3	0.43
CsA ± sMTX	28	13	15	
Tac ± sMTX	23	7	16	

SCT, stem cell transplantation; CR, complete remission; BCP, B-cell precursor; BM, bone marrow; EM, extra medullary; HLA, human leukocyte antigen; CB, cord blood; PB, peripheral blood; TBI, total body irradiation; BU, busulfan; Mel., melphalan; GVHD, graft-versus-host disease; MTX, methotrexate; CsA, cyclosporine A; Tac, tacrolimus; sMTX, short-term methotrexate. [a]Relapse within 18 months from diagnosis of ALL. [b]Relapse after 18 months from initial diagnosis and up to 6 months after cessation of front-line treatment. [c]Relapse later than 6 months after cessation of front-line treatment.

composition of chemotherapies was decided at the discretion of their attending physician. In recent years, 8 patients received multi-drug chemotherapy according to the ALL-relapse protocol of the ALL-REZ BFM study group (ALL-REZ BFM 95/96).

The median number of intensive chemotherapy trials aimed at CR (re-induction chemotherapy) before SCT was two (range, 1–6) times and the median interval from the first relapse to SCT was 7 (range, 2–83) months for all patients. Patient characteristics are shown in Table I. The study included 32 male and 23 female patients, with a median age of 9 (range, 2–19) years at the time of SCT. Twenty patients (36%) relapsed within 18 months from diagnosis of ALL (very early relapse), 24 (44%) did more

than 18 months after the initial diagnosis and up to 6 months after cessation of front-line treatment (early relapse), 11 (20%) relapsed later than 6 months after cessation of front-line treatment (late relapse). Site of the first relapse was isolated BM in 44 patients (80%), isolated EM in 5 (9%), and combined BM and EM in 6 (11%). All five patients who had isolated EM relapse later suffered a subsequent relapse in BM.

Transplantation Procedures

Characteristics of patients and transplantation factors are shown in Table I. Stem cell sources were BM for 47 patients, cord blood (CB) for five, and PB in three. Eighteen patients underwent SCT from human leukocyte antigen (HLA)-matched related donors, 17 from matched unrelated donors, 13 from mismatched related donors, and seven from mismatched unrelated donors. All of the 55 patients received myeloablative conditioning regimens including total body irradiation (TBI) of 12.0 or 13.2 Gy. In combination with TBI, 19 patients received melphalan (Mel) at 180–210mg/m^2; another 19 received Mel and busulfan at 8 mg/kg or 280 mg/m^2; six received Mel and cytarabine (CA) at 12 g/m^2; five received CA and cyclophosphamide at 120 mg/kg, and five received other agents. Cyclosporine A was used with or without short-term methotrexate (sMTX) as prophylaxis for acute graft-versus-host disease (GVHD) in 28 patients, whereas tacrolimus with or without sMTX was administered in 23. The remaining four patients received weekly MTX only.

Statistical Analysis

Differences in the proportions of clinical characteristics of the two treatment groups were compared by the Chi-square test, Fisher exact tests, and Mann-Whitney test as appropriate. The probability of overall survival (OS) and leukemia-free survival (LFS) was estimated by the Kaplan–Meier method. Univariate analysis of OS, LFS was performed using the log-rank test. Cumulative incidence of relapse and non-relapse mortality (NRM) was estimated on the basis of cumulative incidence method treating each of relapse and NRM as a competing risk for the other. Groups were compared using Gray's test. Multivariate analysis was performed using the Cox proportional hazard regression model for OS and LFS, and the method of Fine and Gray for relapse and NRM. Variables considered were patient's age, sex, time and site of the first relapse, response to re-induction chemotherapy, number of re-induction chemotherapy, duration from the first relapse to SCT, status at SCT, donor type, HLA disparity, and conditioning regimen. Statistical analyses were performed using SPSS version 19 and EZR (Saitama Medical Center, Jichi Medical University) and a P value of less than 0.05 was considered to be statistically significant.

RESULTS

Patients

The clinical course after the first re-induction chemotherapy of all 55 patients is shown in Figure 1. Of 30 patients who suffered a

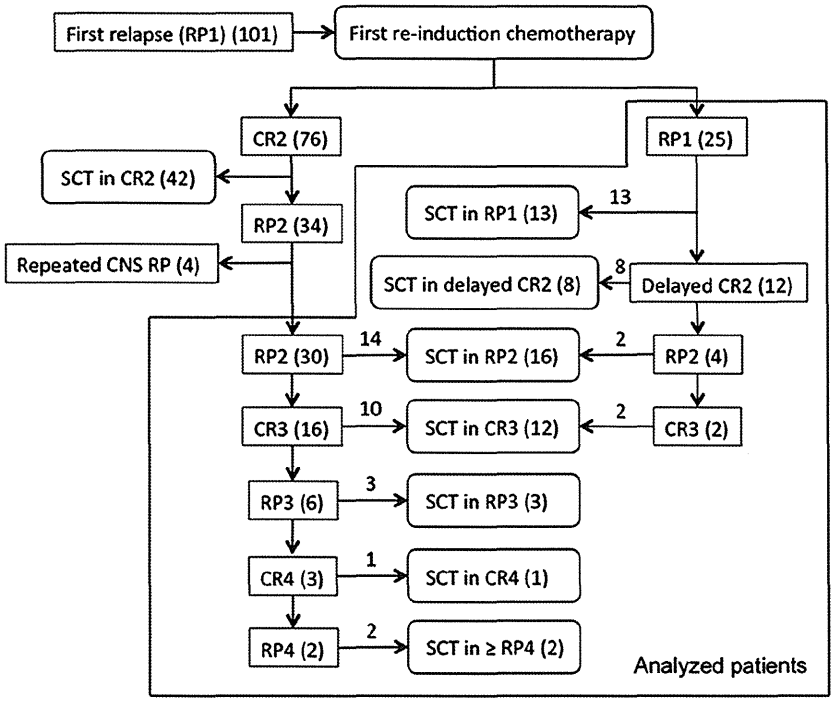

Fig. 1. Clinical course of patients with relapsed ALL. Of 101 patients who suffered the first relapse, 42 receiving SCT in CR2 and 4 with repeated CNS relapse were excluded from this study. As a result, 21 patients received SCT in CR (delayed CR2 in 8 patients, CR3 in 12, and CR4 in 1) and 34 received SCT in non-CR (RP1 in 13 patients, RP2 in 16, RP3 in 3, and beyond RP3 in 2).

second relapse (RP2), 14 underwent SCT in RP2, whereas 16 achieved CR3 after additional re-induction treatment. Ten of these 16 patients who achieved CR3 subsequently underwent SCT, but the other six suffered subsequent relapse. On the other hand, of the 25 patients who did not achieve CR2 after the first re-induction chemotherapy, 13 underwent SCT in RP1 and 12 achieved delayed CR2 after additional re-induction chemotherapy. Eight of the 12 patients who achieved delayed CR2 subsequently underwent SCT, but the other four suffered subsequent relapse. As a result, 21 patients underwent SCT in CR (CR at SCT group; delayed CR2 in 8 patients, CR3 in 12 and CR4 in 1) and 34 in non-CR (non-CR at SCT group; RP1 in 13 patients, RP2 in 16, RP3 in 3 and beyond RP3 in 2).

There were no statistically significant differences associated with age, sex, immuno-phenotype, time and site of the first relapse and the number of re-induction chemotherapy trials between the two groups except for the duration from the first relapse to SCT (Table I). Patient responses to the first re-induction chemotherapy (achieving CR2 or re-induction failure) were similar ($P = 0.8$) between the two groups. Transplantation characteristics were well balanced and not statistically different between the groups (Table I).

SCT Outcomes

The median follow-up period was 81 months (range, 22–241 months) after SCT in all surviving patients. The estimated probabilities of OS and LFS at 3 years after SCT were 30.7% (95% Confidence interval [CI], 19.1–43.1) and 27% (95% CI, 16.1–39.2), respectively, whereas the cumulative incidence of relapse and NRM at 2 years were 40.2% (95% CI, 27.1–53.0) and 32.7% (95% CI, 20.7–45.3), respectively.

The 3-year OS of patients with CR (n = 21) and with non-CR at SCT (n = 34) was 42.9% (95% CI, 21.9–62.3) and 23.5% (95% CI, 11.1–38.6), respectively ($P = 0.15$, Fig. 2A), whereas LFS was 38.1% (95% CI, 18.3–57.8) and 20.6% (95% CI, 9.1–35.5), respectively, ($P = 0.18$, Fig. 2B). The CR at SCT group showed a tendency toward higher OS and LFS probability compared with the non-CR group, but the difference was not statistically significant.

Of 21 patients with CR at SCT, five patients relapsed at a median duration of 13 months (range, 3–20 months) after SCT, whereas 17 of 34 patients with non-CR at SCT relapsed at a median duration of 4 months (range, 1–24 months) after SCT. The cumulative incidence of relapse at 2 years in patients with CR and non-CR at SCT was 23.8% (95% CI, 8.2–43.8) and 50% (95% CI, 32.1–65.6), respectively ($P = 0.05$, Fig. 2C).

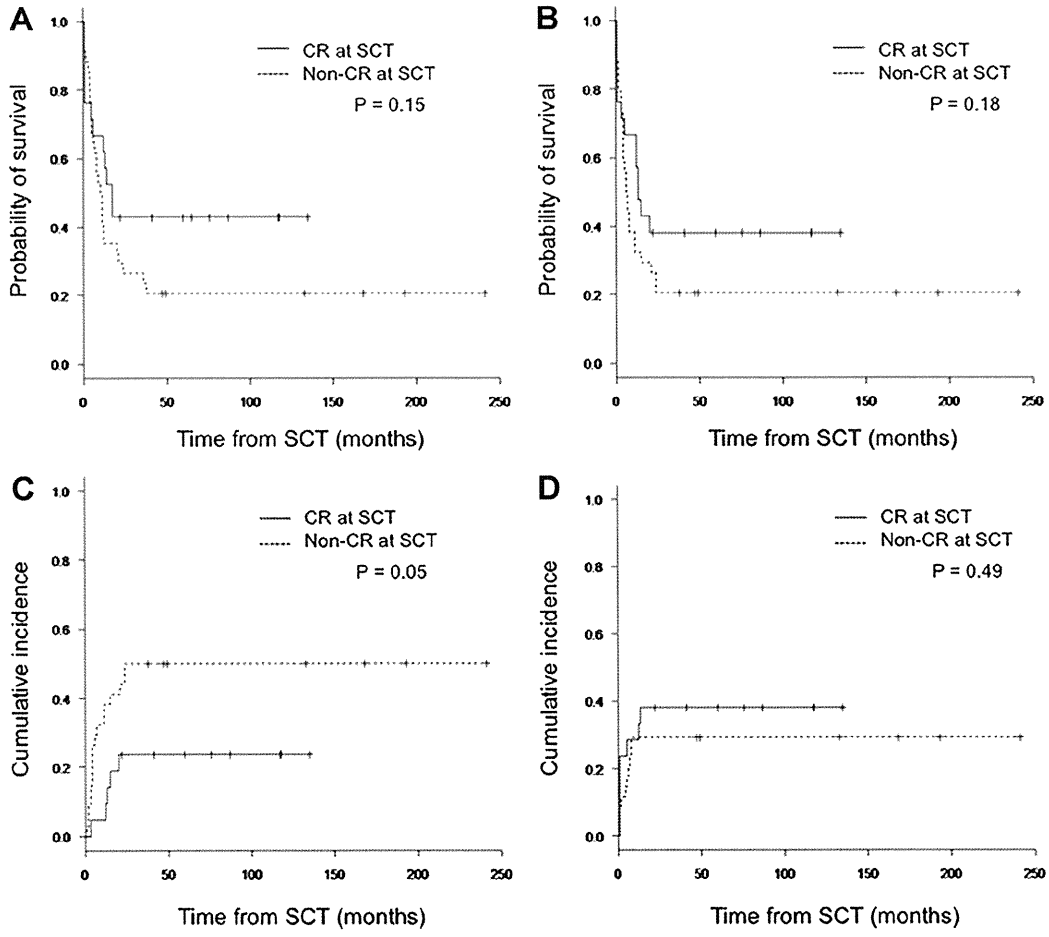

Fig. 2. Overall survival (A), leukemia-free survival (B), and cumulative incidence of relapse (C) or non-relapse mortality (D) according to status at SCT (CR or non-CR) in 55 children with ALL who failed the treatments provided after the first relapse.

Treatment-related death was observed in 8 of 21 patients with CR at SCT at a median duration of 34 days (range, 16–385 days) after SCT and 10 of 34 non-CR patients at a median duration of 146 days (range, 16–238 days). Death before engraftment occurred in five patients (three in the CR group and two in the non-CR group) at a median duration of 17 days (range, 16–28 days). The incidence of acute GVHD (grade III–IV) at day 100 was 27.8% and 22% in patients with CR and non-CR at SCT, respectively ($P = 0.6$). The cumulative incidence of NRM at 100 days after SCT for the two patient groups was 23.8% (95% CI, 8.4–43.6) and 11.8% (95% CI, 3.6–25.1), respectively, whereas that at 2 years after SCT was 38.1% (95% CI, 17.8–58.3) and 29.4% (95% CI, 15.2–45.2) ($P = 0.49$, Fig. 2D), respectively. The causes of death were infectious complications (n = 10), bronchiolitis obliterans (n = 1), diffuse alveolar damage (n = 1), acute GVHD of grade III (n = 1), sinusoidal obstructive syndrome (n = 2), necrotic encephalopathy (n = 1) and cardiac failure (n = 2). The incidence of NRM was significantly higher in patients who relapsed within 18 months from diagnosis (very early relapse) and who received conditioning regimens other than TBI and Mel regimen by Gray's test.

Multivariate analysis revealed that disease status at SCT was not statistically associated with OS, LFS and NRM. However, a significantly higher incidence of relapse was observed in patients who underwent SCT in non-CR (hazard ratio [HR] = 2.59; $P = 0.049$)(Table II). Male sex was a significant risk factor for lower OS (HR = 2.3; $P = 0.018$) and LFS (HR = 2.2; $P = 0.019$), respectively. Time of the first relapse and conditioning regimen were significant risk factors for NRM (Table II). Both acute GVHD grade and severity of chronic GVHD were analyzed as time-dependent covariate and were not associated OS and LFS.

Patient response to the first trial of re-induction chemotherapy (achieving CR2 or re-induction failure) was not associated with OS, LFS, relapse, and NRM in univariate and multivariate analyses. Of 25 patients who experienced re-induction failure, 10 achieved CR thereafter, underwent SCT in CR, and had a significantly lower incidence of relapse at 2 years compared to the 15 who underwent SCT in non-CR (10.0% (95% CI, 0.4–39.2%) and 53.3% (95% CI, 23.5–76.1%), respectively, $P = 0.034$).

TABLE II. Multivariate Analysis

	Hazard ratio	95% CI	*P*-value
Overall survival			
Sex			
Female	1		
Male	2.3	1.15–4.57	0.018
Leukemia-free survival			
Sex			
Female	1		
Male	2.2	1.14–4.35	0.019
Relapse			
Status at SCT			
CR	1		
Non-CR	2.6	1.01–6.7	0.049
Non-relapse mortality			
Time of the first relapse			
Late or Early	1		
Very early	3.8	1.53–9.5	0.004
Conditioning regimen			
TBI/Mel	1		
Others	5	1.54–16.3	0.007

CI, confidence interval; SCT, stem cell transplantation; CR, complete remission; TBI, total body irradiation; Mel, melphalan.

DISCUSSION

When the first relapse of childhood ALL occurs, a patient usually receives re-induction chemotherapy to achieve CR2. Previous reports have suggested that CR2 can be achieved in >70% of early relapse and in up to 96% of late BM relapse cases [11–13]. Recently, SCT has frequently been indicated for children with ALL in CR2 and many studies have reported the outcome of SCT in such cases [13–17] however, limited data are available on the outcome of SCT for children who do not achieve or maintain CR2 [1,6–9]. When a patient does not respond to re-induction chemotherapy or relapses after achieving CR2, it is often difficult for the attending physician to decide on additional intensive chemotherapy for the purpose of achieving CR and subsequent SCT in CR versus immediate SCT even in non-CR to avoid toxicities associated with further intensive treatment. In this study, although the difference was not statistically significant, the CR at SCT group showed a tendency toward higher probability of OS and LFS compared with the non-CR group. In addition, non-CR at SCT was a significant risk factor for higher incidence of relapse in multivariate analysis. Therefore, in case of tolerable patient condition, further re-induction treatment aimed at CR might be reasonable, and ideally, SCT should be offered as soon as CR is achieved. However, for patients who never achieve CR despite various aggressive chemotherapeutic regimens, how long and how many trials of chemotherapy are necessary, and when SCT is performed remain uncertain. The number of intensive chemotherapy trials aimed at CR and the duration from the first relapse to SCT were not associated with SCT outcomes in this study. However, since the patient cohort is small and the treatment plans were heterogeneous and customized in this study, it was difficult to draw any definitive conclusion. Further studies with a large number of patients who are treated with an identical treatment strategy are required.

The results in the current study did not demonstrate the impact of donor type and stem cell source on SCT outcomes in children with treatment-resistant ALL. The timing of SCT is in part, concerned with donor availability. If matched related donor or unrelated cord blood is available, SCT can be performed at the expected time according to a patient's condition. Otherwise, in cases of unrelated BM or PB donor, the timing depends heavily on the donor's situations and it might take several months until SCT can be arranged. Some CR patients might suffer a subsequent relapse while waiting for SCT. In this regard, for patients without an HLA-matched related donor and restored CR after re-induction chemotherapy, immediate unrelated cord blood transplantation (CBT) or SCT from HLA-haploidentical related donor might be of choice. Unrelated CBT or haplo-identical SCT has been developed as a suitable treatment option for children with acute leukemia lacking an HLA-matched related donor [18,19], although the feasibility of these transplantation modalities is not established for very high-risk ALL cases like those analyzed in this study.

In this study, comparable or even higher NRM was observed in CR at SCT group than in non-CR at SCT group. The reason for this finding is possibly that the proportion of both death before engraftment and acute GVHD of grade III–IV were slightly higher in CR at SCT group. This could relate to the fact that the proportion

of patients who received conditioning regimens other than TBI and Mel regimen, which is the risk factor for higher NRM, was also slightly higher in CR at SCT group.

Male sex was a significant risk factor for lower survival by multivariate analysis. As there were no statistically significant differences in clinical and transplantation characteristics between males and female patients, the reasons for worse outcome in male patients were unclear. Male sex has long been recognized as an adverse prognostic factor for the treatment of childhood ALL, partially explained by differences between males and females in the distribution of immune-phenotype and DNA index [20,21]. However, the association between male sex and poor prognosis after SCT in high-risk childhood ALL has not been previously reported. Further evaluation will be of interest in the future.

Because many children with ALL who experience re-induction failure or multiple relapses generally do not respond to chemotherapy consisted of multiple drugs used during front-line treatment, they possibly become refractory to these drugs. Therefore, novel experimental approaches should be considered for this subset of patients. In the last decade, novel pharmacologic agents including nucleoside analogues and monoclonal antibodies have been developed. Clofarabine or nelarabine has shown significant activity in children with refractory/relapsed BCP- or T-ALL in phase 1 or 2 clinical trial as a single agent or in combination with cyclophosphamide and etoposide [22–28]. In addition to nucleoside analogues, monoclonal antibodies (epratuzumab and blinatumomab) or proteasome inhibitor (bortezomib) have shown promising efficacy for pediatric refractory/relapsed ALL as a single agent or in combination with other drugs [29–31]. These novel agents may be helpful to reduce disease burden and achieve CR before SCT, which may leads to a decreased incidence of relapse after SCT in very high-risk patients such those included in this study. It is expected that the most successful of these treatment strategy will be incorporated into the first-line treatment of children with refractory/relapsed ALL. Furthermore, more effective treatments for pre-SCT refractory disease are needed.

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Hematopoietic Stem Cell Transplantation in Children with Refractory Cytopenia of Childhood: Single-Center Experience Using High-Dose Cytarabine Containing Myeloablative and Aplastic Anemia Oriented Reduced-Intensity Conditioning Regimens

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Article history:
Received 17 October 2014
Accepted 1 December 2014

Key Words:
Refractory cytopenia of childhood
Reduced-intensity conditioning
Hematopoietic stem cell transplantation

A B S T R A C T

Refractory cytopenia of childhood (RCC) is the most common subtype of myelodysplastic syndrome in children, and the clinical course of RCC is heterogeneous. A certain proportion of RCC patients need allogeneic hematopoietic stem cell transplantation (HSCT); however, data on HSCT outcomes are not abundant, and the optimal intensity of a preparative conditioning regimen remains uncertain. In this study, we evaluated the outcomes of HSCT in 24 patients with RCC. Eleven patients received myeloablative conditioning (MAC) consisting of high-dose cytarabine, cyclophosphamide, and either total body irradiation (TBI) or busulfan. Nine patients (38%) received a reduced-intensity conditioning (RIC) regimen; of these, 7 received low-dose TBI and cyclophosphamide (200 mg/kg) with or without antithymocyte globulin or fludarabine, and 2 patients received low-dose TBI, fludarabine, and melphalan (140 mg/m^2). The remaining 4 patients had disease progression before HSCT and received the MAC regimen. With a median follow-up of 91 months (range, 6 to 263), the probability of overall survival at 5 years was 81.1% (95% CI, 57.0 to 92.5). The 5-year overall survival for the 15 patients who received MAC was 73.3% (95% CI, 43.6 to 89.1), and all 9 patients with RIC are alive without any events. Further study is needed to evaluate the efficacy of RIC for children with RCC with an expectation for reduction of late effects such as growth retardation and infertility.

INTRODUCTION

Refractory cytopenia of childhood (RCC) is the most common subtype of myelodysplastic syndrome (MDS) in children, characterized by persistent cytopenia with <5% blasts in the bone marrow (BM), <2% blasts in the peripheral blood, and dysplastic changes in 2 or 3 lineages or exceeding 10% in 1 single cell line [1-4]. Some patients with RCC without cytogenetic abnormalities may experience a long, stable clinical course of the disease. For patients without a transfusion requirement, severe neutropenia, or infections, careful observation (watch and wait) is the reasonable treatment strategy. In contrast, hematopoietic stem cell transplantation (HSCT) is the treatment of choice for patients with monosomy 7 or complex karyotypes because of the risk of progression to advanced MDS [3,4]. HSCT can also be recommended for patients with other karyotypes if a suitable donor is available [2,4]. Practically, some RCC patients need HSCT for the reason of transfusion dependency or severe neutropenia and/or infection irrespective of the presence of cytogenetic abnormalities.

Reported disease-free survival rates after HSCT in patients with MDS range between 30% and 75% in previous reports, most of which included patients with various subtypes of MDS, ranging from RCC to advanced MDS, juvenile myelomonocytic leukemia, and therapy-related MDS [5-10]. However, limited data are available on the outcome of HSCT focusing on patients with RCC [3,11]. Therefore, little is known regarding treatment-related factors associated with transplant outcome, such as the optimal intensity of conditioning regimen for patients with RCC. In 2007, Strahm et al. [11] reported that a reduced-intensity conditioning (RIC) regimen consisting of fludarabine (Flu), thiotepa, and antithymocyte globulin achieved favorable outcomes of HSCT in children with hypocellular refractory cytopenia without chromosomal abnormality. In this study, we report the outcomes of HSCT in 24 pediatric patients with RCC after conditioning regimens of myeloablative conditioning (MAC) with high-dose cytarabine, cyclophosphamide (CY), and total body irradiation (TBI) or busulfan (BU) and RIC with low-dose TBI and CY or melphalan (Mel).

METHODS

Patients

Twenty-four patients with RCC aged < 19 years at diagnosis received HSCT at the Department of Pediatrics, National Kyushu Cancer Center between April 1991 and May 2014. Fifteen patients were diagnosed with refractory anemia or refractory cytopenia with multilineage dysplasia based on the French-American-British classification or World Health Organization 1999 criteria [12,13]. All 24 patients were compatible with the classification of RCC according to the World Health Organization 2008 criteria [4,14].

BM biopsy to evaluate marrow cellularity was performed in 19 patients (79%). Patients who had received previous HSCT, those with inherited BM failure disorder, such as Fanconi anemia or dyskeratosis congenita, or those with treatment-related MDS were excluded. The most frequent reasons for recommendation of HSCT were transfusion dependency in 14 patients, followed by monosomy 7 in 4, and disease progression to advanced MDS in 3.

All patients or their parents provided written informed consent for HSCT. This retrospective study and patient data use was approved by the institutional review board of the National Kyushu Cancer Center. Patient characteristics are shown in Table 1.

Financial disclosure: See Acknowledgments on page 568.

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1083-8791/© 2015 American Society for Blood and Marrow Transplantation.
http://dx.doi.org/10.1016/j.bbmt.2014.12.003

Table 1
Patient Characteristics

Number of patients	24
Median age at HSCT, yr (range)	10 (3-21)
Median time from diagnosis to HSCT, mo (range)	13.5 (3-95)
Sex	
Male	14
Female	10
Cytogenetics at diagnosis	
Normal	14
Monosomy 7	5
Trisomy 8	2
Structural complex	1
Other abnormalities	1
Unknown	1
Disease progression before HSCT	
Yes	4
No	20
Year of HSCT	
1991-2000	8
2001-2014	16
Donor	
MSD-BM	8
MUD-BM	12
UCB	4
Conditioning regimen	
BU/CA/CY	8
TBI/CA/CY	5
TBI/Others	2
RIC	9
GVHD prophylaxis	
CsA based	11
TaC based	13

MSD indicates matched sibling donor; MUD, matched unrelated donor; UCB, unrelated cord blood; CA, cytarabine; CsA, cyclosporine; Tac, tacrolimus.

The study included 14 male and 10 female patients, with a median age of 10 years (range, 3 to 21) at the time of HSCT. Before HSCT, 18 patients had neutropenia (absolute neutrophil count $< 1.0 \times 10^9$/L), and 12 patients had ANC $< .5 \times 10^9$/L. The number of transfusions for RBCs and/or platelets before HSCT was > 10 times in 20 patients and >20 times in 17 patients. Four patients experienced disease progression to advanced MDS (refractory anemia with excess blast in 3 patients and acute myeloid leukemia in 1 patient) before HSCT at a median of 11 months (range, 3 to 17) from diagnosis. Cytogenetic analysis at diagnosis was available in 23 patients, including normal karyotype (n = 14), monosomy 7 with or without additional aberrations (n = 5), structurally complex karyotype (defined as more than 3 chromosomal aberrations in the presence of at least 1 structural aberration, n = 1), or other abnormalities such as trisomy 8 (n = 3). Sixteen patients received immunosuppressive therapy, including steroid therapy in 4 patients, cyclosporine A in 2 patients, and antithymocyte globulin in combination with cyclosporine A in 10 patients as the initial treatment for RCC. Two patients with disease progression received acute myeloid leukemia–oriented chemotherapy.

Transplantation Procedures

Until 2006, 11 patients received the MAC regimen consisting of high-dose cytarabine (12 g/m^2), CY (120 mg/kg), and either TBI (≥12 Gy) or BU (Figure 1). Another 4 patients with disease progression before HSCT received the MAC regimen, which was determined at the treating physician's discretion. Since 2007, the RIC regimen has been applied to patients with RCC, and a total of 9 patients (38%) received RIC. Six patients with hypocellular marrow without monosomy 7 or a structural complex karyotype received low-dose TBI (3 Gy) and CY (200 mg/kg) with antithymocyte globulin or Flu. Two patients with normocellular marrow received low-dose TBI (3 or 4 Gy), Flu (125 mg/m^2), and Mel (140 mg/m^2). One patient with hypocellular marrow who underwent HSCT in 2004 received RIC with TBI (3 Gy) and CY (200 mg/kg), because he was initially diagnosed with aplastic anemia. Transplantation characteristics of these 9 patients who received the RIC regimen are shown in Table 2.

Cyclosporine A with or without short-term methotrexate was used as prophylaxis for acute graft-versus-host disease (GVHD) in 11 patients; tacrolimus was used with or without short-term methotrexate in 13 patients. Stem cell sources were BM for 20 patients and cord blood for 4 patients. Eight patients received BM transplantation from an HLA-matched sibling donor, 12 patients received BM transplantation from an HLA-matched unrelated donor, and 4 patients received unrelated cord blood stem cell transplantation.

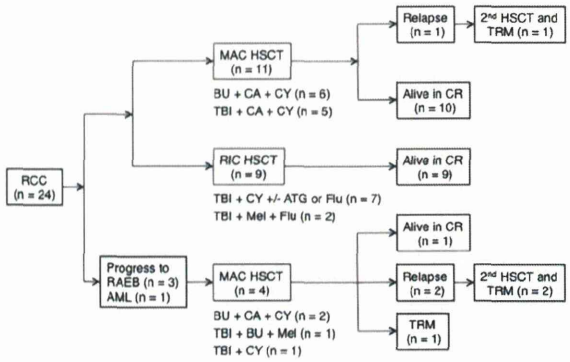

Figure 1. Outcomes of HSCT in 24 patients with RCC. After the first HSCT, treatment-related death was observed in 1 patient who had disease progression before HSCT. No patient who received RIC relapsed, and 1 of 11 patients who received MAC without disease progression relapsed after HSCT. One of 4 patients with disease progression before HSCT is alive in remission.

Definitions and Statistical Analysis

Engraftment was defined as the first day when the absolute neutrophil count reached .5 × 10^9/L for 3 consecutive days. Both the platelets and reticulocytes were considered to be recovered when they reached counts >20 × 10^9/L and >1%, respectively, without transfusion support. Graft failure was defined as a lack of engraftment irrespective of donor chimerism. A chimerism analysis was performed using BM samples when engraftment was observed. Fluorescence in situ hybridization with sex chromosomes for sex-mismatched HSCT and short tandem repeat PCR for sex-matched HSCT was carried out. Acute and chronic GVHD were diagnosed and classified according to previously reported criteria [15,16]. Overall survival (OS) was calculated as the time from HSCT to death or last follow-up. Probability of survival was estimated by the Kaplan-Meier method with the corresponding 95% confidence intervals (CI). Univariate analysis of OS was performed using the log-rank test. All statistical analyses were performed using SPSS version 19 (SPSS Co., Tokyo, Japan).

RESULTS

Engraftment, GVHD, and Complications

Twenty-three of 24 patients (96%) were engrafted on a median of day 17 (range, 11 to 49). Graft failure occurred in 1 patient who had disease progression to refractory anemia with excess blast before unrelated cord blood stem cell transplantation. Thereafter, he had autologous recovery and relapsed. Platelet and reticulocyte recovery was achieved on a median of day 27 (range, 17 to 75) in 21 assessable patients and day 25 (range, 17 to 41) in 22 assessable patients, respectively. The data on 16 patients were available for chimerism analysis, and all 16 patients showed complete donor chimerism when engraftment was observed. All 9 patients who received the RIC regimen engrafted with complete donor chimerism.

Acute GVHD of grades II to III occurred in 12 of 23 assessable patients including 8 of 14 patients (57%) with MAC and 4 of 9 (44%) with RIC. No grade IV acute GVHD occurred. Chronic GVHD occurred in 5 of 21 assessable patients. In 9 patients with RIC, only 1 (11%) developed mild chronic GVHD limited to skin, whereas 4 of 12 patients (33%) with MAC developed chronic GVHD, including 3 with the severe type. Within 100 days after HSCT, viral infections were the most frequent complication. In patients with MAC, 9 patients (60%) developed cytomegalovirus infection, including 8 patients with cytomegalovirus reactivation in the blood and 1 with interstitial pneumonia. In patients with RIC,

Table 2
Characteristics and Outcomes of RCC Patients with RIC

Patient No.	Age (yr)/ Sex	Cellularity	Cytogenetics	Treatment before HSCT	Diagnosis to HSCT (mo)	Donor	Conditioning Regimen (Dose of TBI or chemotherapy)**	GVHD Prophylaxis	Acute GVHD	Chronic GVHD	Complication within 100 days	Prognosis (follow-up mo)
1	5/M	Hypocellular	Unknown	PSL	28	MSD-BM	TBI (3)/CY (200)	CsA	0	No		Alive in remission (122)
2	14/M	Hypocellular	Normal	ATG, CsA, mPSL	40	MUD-BM	TBI (3)/CY (200)/ATG (10)	Tac/sMTX	II	Mild	CMV antigenemia	Alive in remission (87)
3	10/F	Hypocellular	Normal	ATG, CsA, mPSL	10	MUD-BM	TBI (3)/CY (200)/ATG (10)	Tac/sMTX	0	No	CMV antigenemia HC (BK virus)	Alive in remission (48)
4	14/M	Hypocellular	Trisomy 8	ATG, CsA, mPSL	14	MUD-BM	TBI (3)/CY (200)/ATG (10)	Tac/sMTX	0	No		Alive in remission (37)
5	4/F	Hypocellular	Normal	CsA	22	MUD-BM	TBI (3)/CY (200)/ATG (10)	Tac/sMTX	I	No		Alive in remission (39)
6	11/F	Hypocellular	Normal	CsA	15	MUD-BM	TBI (3)/CY (200)/ATG (5)	Tac/sMTX	II	No		Alive in remission (11)
7	5/F	Hypocellular	Normal	ATG, CsA, mPSL	13	MUD-BM	TBI (3)/CY (200)/Flu (120)/ ATG (2.5)*	Tac/sMTX	III	No	CMV antigenemia HC (BK virus)	Alive in remission (13)
8	3/F	Normocellular	Structural complex	No treatment	3	MSD-BM	TBI (3)/Flu (125)/Mel (140)	CsA	I	No		Alive in remission (6)
9	13/M	Normocellular	Normal	ATG, CsA, mPSL	20	UCB	TBI (4)/Flu (125)/Mel (140)	Tac/sMTX/MMF	III	No	CMV entelitis	Alive in remission (12)

PSL indicates prednisone; ATG, antithymocyte globulin; sMTX, short-term methotrexate; CMV, cytomegalovirus; mPSL, methylprednisolone; MUD, matched unrelated donor; MMF, mycophenolate mofetil.
All patients received rabbit ATG (Zetbulin [Fresenius, Bad Homburg, Germany] for early 4 patients and Thymoglobulin [Genzyme, Lyon, France] for 2 patients).

* This patient was intolerant of ATG and received Flu instead of ATG.

** TBI, Gy; CY, mg/kg; ATG, mg/kg; Flu, mg/m^2; Mel, mg/m^2.

cytomegalovirus infection occurred in 4 patients (44%; reactivation in the blood in 3 patients and enteritis in 1). All were treated successfully with ganciclovir or foscarnet sodium hydrate. Six patients (4 patients with MAC and 2 with RIC) developed hemorrhagic cystitis due to BK virus infection in 3 patients, adenovirus in 1, and unknown cause in the remaining 2 patients. They were treated with hydration and diuretics and thereafter improved.

Treatment-Related Death, Relapse, and Survival

Outcomes of HSCT of all patients are shown in Figure 1. Treatment-related death was observed in 1 patient who had disease progression and underwent HSCT with MAC, resulting in death from interstitial pneumonia 1 month after HSCT. No patient who received RIC relapsed, whereas 3 of 15 patients who received MAC relapsed at 1, 2, and 20 months after HSCT. One of the 3 relapsed patients had monosomy 7, and the remaining 2 relapsed patients had disease progression before HSCT. All 3 patients underwent a second HSCT, resulting in treatment-related death (interstitial pneumonia, sinusoidal obstructive syndrome, and bronchiolitis obliterans organizing pneumonia).

With a median follow-up of 91 months (range, 6 to 263), 20 patients survived, and the probability of OS at 5 years was 81.1% (95% CI, 57.0 to 92.5). The 5-year OS for the 15 patients who received MAC was 73.3% (95% CI, 43.6 to 89.1), and all 9 patients who received RIC are alive in remission with a median follow-up of 37 months (range, 6 to 122) without relapse, treatment-related death, and chronic GVHD for which systemic treatment is required (Table 2).

DISCUSSION

In the recent report of the European Working Groups of MDS, of 115 hypocellular RCC children without a high-risk karyotype who were given immunosuppressive therapy as frontline treatment, 40 patients ultimately underwent HSCT because of failure in immunosuppressive therapy [17]. The report from the Japanese Society of Pediatric Hematology/Oncology shows that 10 of 25 patients who received immunosuppressive therapy and 6 of 27 patients who were treated with a watch and wait strategy were thereafter transplanted [18]. The results of these 2 reports indicate that a certain proportion of RCC patients need HSCT after first-line treatment, and the OS rate after HSCT was favorable (80% at 4 years in the former and 66% at 5 years in the latter report) with an extremely low relapse rate. In the current study, the probability of OS at 5 years for all 24 patients was 81%, which compares favorably with these reports and a previous report [3]. Although only a small number of patients were included, the present results suggest that both the MAC and RIC regimens used in our institution are feasible for patients with RCC without increasing the risk of treatment-related mortality, which remains the most common cause of death in HSCT for children with MDS [5-7]. As previously described, the prognoses of patients with disease progression before HSCT were dismal [3]. Early referral for HSCT might be preferred for patients with unfavorable cytogenetic abnormalities or transfusion dependency.

For patients with advanced MDS, the MAC regimen consisting of BU, CY, and Mel offered about 60% of event-free survival in the EWOG-MDS report [7]. In contrast, the optimal conditioning regimen for HSCT in patients with RCC is uncertain. At our institution, pediatric patients with MDS have been treated with MAC with high-dose cytarabine, CY, and either TBI (12 to 13.2 Gy) or BU irrespective of morphological classification or BM cellularity. A RIC regimen, which is the same as regimens for aplastic anemia, was applied to patients with hypocellular RCC, because of a suggested pathological overlap between aplastic anemia and hypoplastic RCC [19,20]. As shown in Table 2, all 7 patients who received the RIC regimen consisting of TBI (3 Gy) and CY (200 mg/kg) had hypocellular marrow without unfavorable cytogenetics such as monosomy 7 or a structural complex karyotype. In support of the report by Strahm et al. [11], the current results suggest that HSCT using RIC could be promising in children with hypocellular RCC, with an expected reduction in late effects such as growth retardation and infertility.

The feasibility of RIC for RCC patients with normocellular or hypercellular marrow and/or with unfavorable cytogenetics remains to be evaluated. In our study, HSCT using RIC consisting of low-dose TBI, Flu (125 mg/m^2), and Mel (140 mg/m^2) was performed for 2 patients with normocellular RCC, whose follow-up period is too short. Further large-sized studies with a long-term follow-up will better evaluate the efficacy of RIC for children with RCC.

ACKNOWLEDGMENTS

Financial disclosure: The authors have no relevant financial conflicts of interest.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: J.I. was the principal investigator for this study and wrote the manuscript. J.I. and J.O. had responsibility for study design, data analysis, and interpretation. R.F., K.K., M.N., S.T., and J.O. collected data and critically reviewed the manuscript. All authors approved the final version of the manuscript.

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