Key words
小児CML
イマチニブ
造血幹細胞移植
成長障害
JPLSG

特集

分子標的治療　4

分子標的治療薬を用いた治療法の現状と問題点

小児慢性骨髄性白血病における近年の治療成績の向上と今後の課題

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チロシンキナーゼ阻害薬であるイマチニブは多くの患者において，従来の治療薬では成し得なかった長期の慢性期維持を可能にした．重篤な有害事象はほとんどみられず，コンプライアンスも悪くない．小児のガイドラインでは，イマチニブに反応良好かつ耐容である慢性期の患者にはイマチニブ継続が推奨されている．しかし，現時点では中止基準がないため，小児ではイマチニブによる長期毒性が懸念される．一方，根治療法としての同種造血幹細胞移植の位置づけは変わらず，その成績も向上していることから，移植適応の判断に苦慮することも少なくない．本稿では，今後の治療戦略を考察するため，イマチニブ導入前後の小児慢性骨髄性白血病（CML）の治療成績を概説する．

はじめに

イマチニブ時代が訪れ，慢性骨髄性白血病（chronic myeloid leukemia：CML）の治療は一変した．成人では造血幹細胞移植の適応が限られようになり，ほとんどの患者はイマチニブの内服により，QOLの高い生活が送られるようになった．小児においても現在，成人の臨床試験のエビデンスをもとに，イマチニブの内服継続が標準的治療とされている．しかし，成長・発達期にイマチニブを内服するという小児特有の問題や治癒を目標としない治療が小児に適切かどうかという問題がある．一方，慢性期の患者に対し移植に踏み切るのは，その不確実さのため患者や担当医にとって決断のいる選択である．造血幹細胞移植が真に根治療法であるかということに関して否定的な意見もある．

本稿では，Japan Pediatric Leukemia/Lymphoma Study Group（JPLSG）CML委員会が最近行った調査結果に基づいて，小児CML治療の現状と問題点を解説する．

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Ⅰ 患者の年齢・性別分布

CMLは小児の全白血病の中で2～3%を占める比較的まれな疾患である．JPLSG CML委員会による全国調査で1996～2006年に診断された小児CML166例の年齢・性別分布を**図1**に示す．多くは思春期以降に発症するが，2～6歳の幼児期発症例もまれではない．15歳以上の患者の多くは内科で治療されている．

Ⅱ 治療成績

1．イマチニブ導入前後の治療成績

全国調査の対象となった1996～2006年に診断された小児CML166例の診断時年代別の全生存率を**図2**に示す[1]．2002年以降に診断され

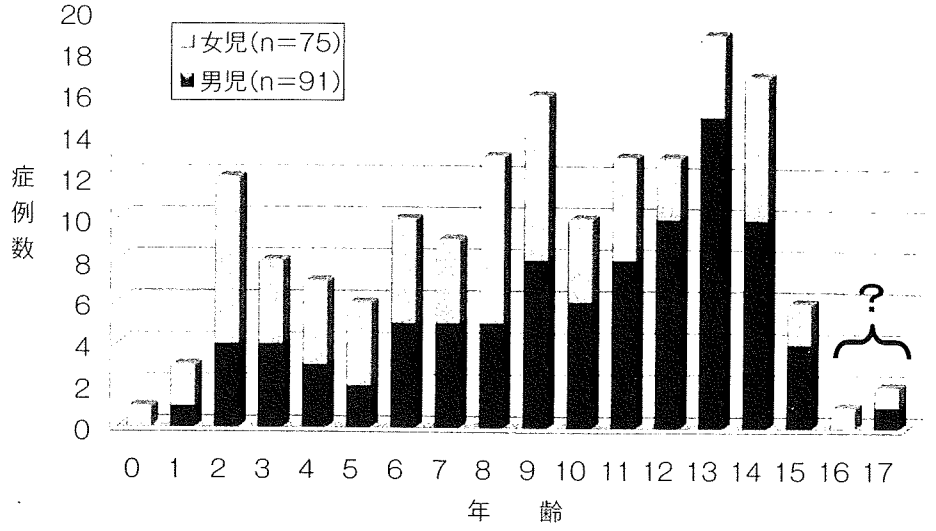

図1　小児 CML の年齢・性別分布（JPLSG CML 委員会による全国調査）

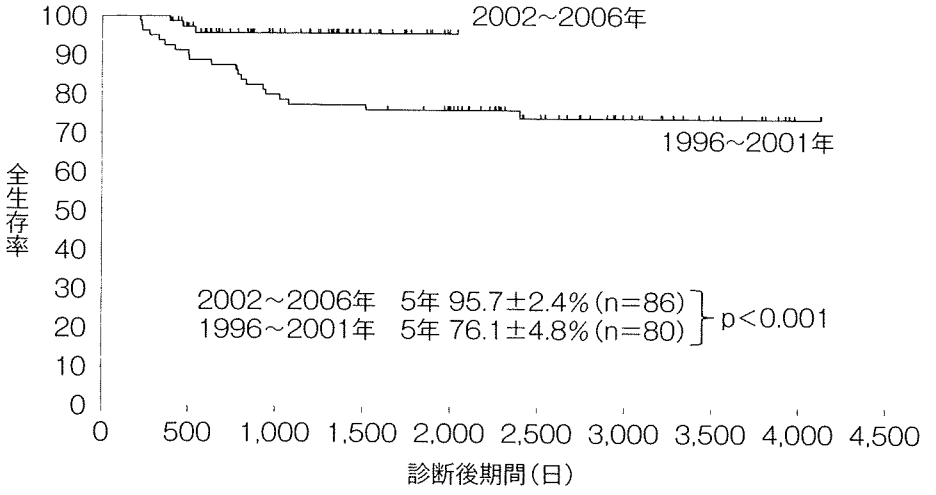

図2　診断時年代別の全生存率（病期進行例を含む）（JPLSG CML 委員会による全国調査）

た症例の5年全生存率の著しい向上がみられた（95.7±2.4% (n=86) vs. 76.1±4.8% (n=80), $p<0.001$）．発症時病期の内訳は，慢性期（CP）146例（88.0%），移行期（AP）9例（6.2%），急性転化期（BC）11例（6.6%）であり，年代別に有意差はない．成績向上の最大の要因は2001年12月のグリベック®発売に伴う治療選択の変化と考えられる．**表1**に示すとおり，2002年以降有意にイマチニブ内服歴のある症例および造血幹細胞移植歴のない症例が増加している．

2．イマチニブ導入後（2002年以降）の成績向上の要因

イマチニブ導入後の成績向上の要因として，CP維持率の向上および造血幹細胞移植成績の向上，の2つの要因が考えられる．

a．CP維持率の向上

イマチニブ導入前の時代では，インターフェロンαなどの化学療法のみでCPを維持するのは難しく，病期が進行する前に造血幹細胞移植を行うことが治療の目標であった．15歳以下の小児CMLを対象とした小児CML研究会に

表1　2001年12月グリベック®発売による治療選択への影響

		全体	～2001年	2002年～	p-value
症例数		166	80	86	
イマチニブ内服	有	102	18	84	p＜0.001
	無	64	62	2	
造血幹細胞移植	有	109	68	41	p＜0.001
	無	57	12	45	

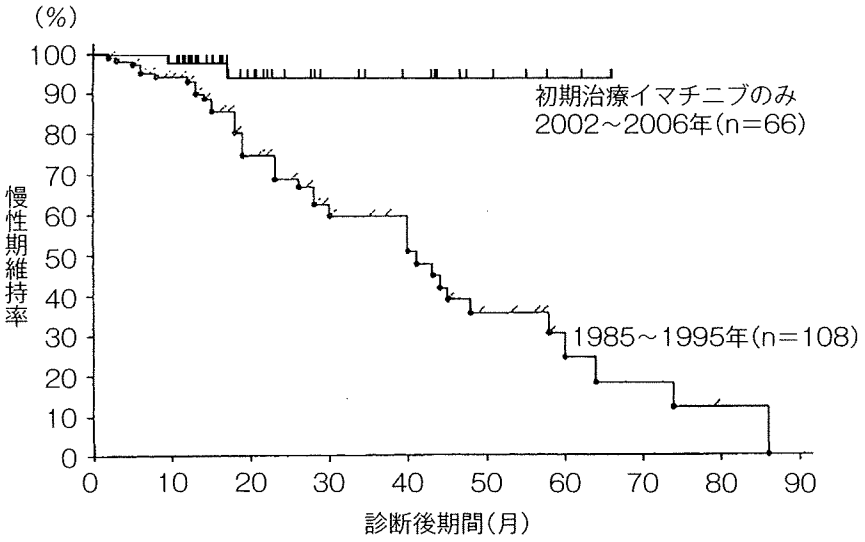

図3　イマチニブ導入前後の慢性期維持率の比較

よる1985～1995年の全国調査では，慢性期CML 108例のCP維持率は1年後には91%と高かったが，2年後には69%，5年後には39%と経時的に低下し，移植なしではほとんどの症例がCPを維持できなかった[2]．一方，JPLSG CML委員会による全国調査では，初期治療薬としてイマチニブのみが投与された慢性期CML 66例のCP維持率は，5年後も94.2±4.1%と高く，移植なしでも40例中38例がCPを維持した（**図3**）[3]．イマチニブによるCP維持率の向上はイマチニブを中止できないという制約があるものの，CMLが真の“慢性白血病”となり得たという点において治療成績の向上に直接関連する要因と考えられる．

b．造血幹細胞移植成績の向上

造血幹細胞移植を実施された病期進行例を含む109例の解析（JPLSG CML委員会の全国調査）では，**図4**に示すように，2002年以降診断例の移植成績の向上がみられた（5年全生存率

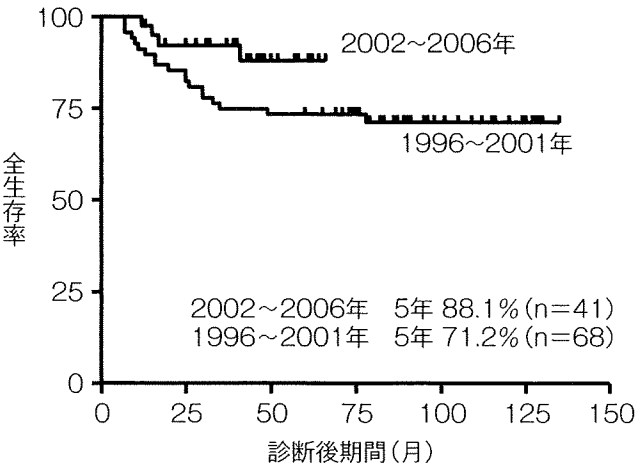

図4　造血幹細胞移植症例における診断時年代別の全生存率（病期進行例を含む）（JPLSG CML委員会による全国調査）

表2　小児CMLにおけるイマチニブ投与の成績

文　献	症例数（病期）	年齢（中央値）	開始投与量（mg/m²）（中央値）	成　績（%）	
				CHR	CCyR
Champagne et al[6]	14（CP）	3～20（14）	260～570	92	83
Millot et al[8]	22（CP） 5（AP） 3（BC）	1～17（13）	260～340（292）	80 80 67	60 40 0
谷澤ら[3]	66（CP）	0～15（9）	74～403（292）	97（3カ月）	83（12カ月）

CP：慢性期，AP：移行期，BC：急性転化期，CHR：血液学的完全寛解，CCyR：細胞遺伝学的完全寛解

88.1%（n＝41）vs. 71.2%（n＝68））[4]．症例数が少ないため有意差が出ないが，イマチニブ導入後の造血幹細胞移植成績の向上は明らかと考えられる．ドナー別の5年全生存率は2002年以降および2001年以前に分けて，HLA一致血縁100%（n＝14）vs. 82%（n＝22），HLA一致非血縁88%（n＝17）vs. 71%（n＝28），その他78%（n＝10）vs. 61%（n＝18）であった．

成人CMLの解析（International Blood and Marrow Transplant Research）では，移植時第1慢性期（CP1）の症例において移植前イマチニブ投与が有意に全生存率を改善することが報告されたが[5]，JPLSG CML委員会による109例の解析では，2002年以降診断例でもっとも顕著に5年全生存率が向上したのは移植時CP2以降の慢性期（higher CP）の症例であった（100%（n＝11）vs. 64.3%（n＝14）；p＝0.04）[4]．移植時CP1の症例では改善傾向を認めたが（89.1%（n＝28）vs. 80.1%（n＝47）），移植時AP/BCの成績は不良のままであった（0%（n＝2）vs. 28.6%（n＝7））．これらの結果から，2002年以降診断例の移植成績の向上は移植時higher CPの生存率の改善および移植時AP/BC症例数の減少によると考えられる．移植時higher CPの成績向上の原因については，病期進行時にイマチニブによって早期にかつ深い寛解レベルのhigher CPが得られたこと，移植後増悪時にイマチニブによって再移植を回避できたことなどが関与していると考えられる．

3．小児CML例におけるイマチニブ投与の経験

a．欧米の報告

小児CMLにイマチニブを用いた治療成績を表2に示す．Children's Oncology Group（COG）により慢性期CML 14例を含むPh陽性白血病（CML，AML，ALL）31例を対象とした第1相試験が行われた．260～570 mg/m²/日のイマチニブが3～20歳（年齢中央値14歳）の小児に安全に投与され，最大耐容量は示されなかった[6]．また，薬物動態の結果から，260 mg/m²，340 mg/m²がそれぞれ成人の400 mg，600 mgに相当することが明らかになった．日本小児血液学会のガイドラインではこの結果に基づいて，イマチニブの初期投与量を成人の標準開始投与量に相当する260 mg/m²/日としている[7]．

ヨーロッパ8カ国で行われた多施設共同第2相試験では，1～17.5歳（年齢中央値13歳）のCML 30例に260～340 mg/m²/日のイマチニブが安全に投与された．治療効果の評価が可能であった27例のうち，CP症例の60%（12/20例），AP/BC症例の29%（2/7例）で骨髄中のPh陽性細胞の消失を認め，12カ月時点での全生存率はCP症例95%，AP/BC症例は75%と成人と同等の効果が得られている[8]．

b．本邦の報告

JPLSG CML委員会による全国調査では，初期治療薬としてイマチニブのみが投与された小児慢性期CML 66例（年齢中央値9歳）の解析

が報告されている[3]．イマチニブ開始投与量の中央値（範囲）は292（74～403）mg/m^2，診断時からの観察期間の中央値（範囲）は873（176～1,999日）であった．血液学的完全寛解（CHR）の3カ月達成率は96.8%（61/63例），細胞遺伝学的完全寛解（CCyR）の12カ月達成率は83.3%（45/54例）であり，成人例と同等の臨床効果が確認された．治療に伴うグレード3以上の有害事象は，血液毒性（顆粒球減少15.2%，血小板減少9.1%，貧血4.5%）が主であり，非血液毒性はCPK上昇7.6%，筋肉痛6.1%，発熱1.5%，疲労1.5%，胸やけ1.5%，関節痛1.5%，けいれん1.5%であった．成人例と比較し，CPKの上昇と筋肉痛の頻度が高い傾向があった．

66例中26例に対して同種造血幹細胞移植が行われたが，うち3例が移植関連合併症で死亡した．移植後にイマチニブを内服している症例はなかったが，26%の症例が移植関連合併症に対する治療を続けており，移植によりイマチニブを中止できたとしても必ずしも全例が投薬自体を中止できるわけではないことが示された．一方，移植を選択せずイマチニブ内服を継続している40例は全例CP1を維持して生存していた．全体の5年全生存率は94.2±3.2%，無増悪生存率は90.6±4.0%であった．

移植前のイベントは4例に5件（細胞遺伝学的主要寛解（major cytogenetic response：MCyR）の消失2件，BC進行3件）発生した．初回のイベントは成人より早期（6カ月1件，9カ月1件，12カ月1件，18カ月1件）に集中して発生していたが，経過中に移植を選択された症例が成人に比し多かったからかもしれない．興味深いことに，3例（3件）のイベントがoptimal responseで経過中に突然発生し，この3例はイマチニブ開始後29日の時点ではCHRを達成していなかったことがわかった．29日は治療効果判定および治療介入の時期ではないが，29日反応不良の症例は29日時点でのイマチニブ増量の検討，可能であればイマチニブ血中濃度測定による用量調整，29日以降の頻回のモニタリングなどの対応が必要と考えられた．

表3　移植時病期別の転帰（JPLSG CML委員会による全国調査）

移植時病期	CP1 (n=75)	higher CP (n=25)	AP/BC (n=9)
生	63	20	3
死亡	12	5	6
移植関連死	12	5	4
原病死	0	0	2

4．移植成績を向上させる条件

小児CMLにおいても成人同様，イマチニブの内服によって移植なしでQOLの高い長期生存が得られるようになったが，イマチニブ不応例または不耐容例，あるいは発症時AP/BC例に対する根治療法としての同種造血幹細胞移植の位置づけは変わっていない．したがって，移植成績を向上させることがCML全体の治療成績の向上につながる．

JPLSG CML委員会による全国調査で同種造血幹細胞移植が実施された109例の移植時病期別の転帰を**表3**に示す[4]．注目すべきはCP1/higher CPの症例に原病死を認めないことである．死因が移植関連死のみというこの結果は，移植時CPの症例は造血器腫瘍よりむしろ再生不良性貧血などの良性疾患と同様の戦略が適切であることを示唆する．すなわち，いかに移植関連死を減少させるかが移植成績を向上させるための重要課題となる．移植時AP/BC症例に対しては，原病に対する治療も重視する必要があるが，CP症例と同様に移植関連死は注意すべき死因となる．

非血縁者間移植では小児CMLに限らず移植関連死が問題となる．JPLSG CML委員会では移植関連死を少なくする対策を検討するため，骨髄移植推進財団（JMDP）および日本さい帯血バンクネットワーク（JCBBN）を介して非血

表4　非血縁者間骨髄移植における移植関連死に関与する予後因子（多変量解析）

	RR（95% CI）	p-value
移植関連死		
輸注有核細胞数		
≥314×10^6/kg	(1)	
<314×10^6/kg	2.347	0.013
	(1.195～4.610)	
移植時細胞遺伝学的反応		
MCyR 達成　あり	(1)	
なし	9.055	0.003
	(2.151～38.127)	

MCyR：細胞遺伝学的主要寛解

縁者間移植を行った小児 CML の解析を行った．また，移植関連死の減少を目標に，小児 CML に対する骨髄非破壊的造血幹細胞移植（reduced-intensity stem cell transplantation：RIST）の適応を検討するため，全国で RIST が実施された 15 例の解析を行った．

a．非血縁者間同種骨髄移植の解析（JMDP）

1993～2003 年に JMDP を介して骨髄移植を行った 20 歳未満の CML125 例の解析を行った[9]．全体の 5 年全生存率は 59.3±4.5%，移植時病期 CP1 88 例の 5 年全生存率は 70.7±5.0%であった．全生存率に関連する独立した予後因子は多変量解析により，輸注有核細胞数，移植時病期，移植時細胞遺伝学的反応（MCyR 達成の有無）であることが示された．これらのうち，原病死ではなく移植関連死に関連するのは輸注有核細胞数と移植時細胞遺伝学的反応であった（**表4**）．

これらの結果から非血縁者間骨髄移植においては，骨髄採取施設に十分な有核細胞数の採取を求めること，また移植までに MCyR 以上の深い寛解を目標とした治療を行うことが移植関連死を減少させるための重要な条件と考えられた．移植時 CP1 の subgroup 解析においては，イマチニブを移植前に投与された 16 例のうち 15 例（94%）が移植時に MCyR を達成しており，その 5 年全生存率は非投与群と比較して有意差はないものの良好であった（87.1±8.6% vs 68.0±5.5%，p=0.195）．イマチニブによる移植時寛解状態の改善が移植関連死の減少につながると考えられた．

b．非血縁者間同種臍帯血移植の解析（JCBBN）

1998～2006 年に JCBBN を介して臍帯血移植を行った 20 歳未満の CML15 例の解析を行った[10]．全体の 5 年全生存率は 65.2±12.7%であったが，移植歴のある 2 例を除くと 72.0%±12.0%となり，小児 CML に対する alternative donor として選択しうると考えられた．死亡 5 例の死因内訳は再発が 1 例，移植関連死が 4 例であり，やはり移植関連死の回避が課題であった．生存に関する単変量解析では，性別不一致，輸注 CD34 陽性細胞数，移植時病期が有意な予後因子であり，5 年全生存率では，ドナー女性から患者男性（n=6）とそれ以外の組み合わせ（n=9）が 16.7±15.2% vs. 100±0.0%，輸注 CD34 陽性細胞数 1.01×10^5/kg 未満（n=7）と以上（n=8）が 28.6±17.1% vs. 100±0.0%，移植時 AP/BC（n=6）と移植時 CP（n=9）が 25.0±20.4% vs. 88.9±10.5%であった．性別と CD34 陽性細胞数を考慮したドナー選択が移植関連死の減少につながると考えられた．

c．骨髄非破壊的造血幹細胞移植（RIST）の解析（JPLSG）

JPLSG CML 委員会による全国調査で移植が実施された 109 例のうち 15 例に RIST が選択されたことがわかった．**表5** に示すように移植時 CP1（11 例）および higher CP（3 例）における RIST の 5 年全生存率はそれぞれ 90.9%，100%と良好な成績が得られていた．症例数が少ないため正確な評価は難しいが，移植時病期が CP であれば RIST は効果の面で十分であり，かつ移植関連死や晩期合併症の軽減を考慮するとより適切な選択であると考えられる．

表5 骨髄破壊的および骨髄非破壊的前処置による移植成績の比較（5年全生存率）（JPLSG CML委員会による全国調査）

移植時病期	CP1	higher CP	AP/BC
骨髄破壊的前処置	82.1% (n=64)	76.7% (n=22)	30.0% (n=8)
骨髄非破壊的前処置	90.9% (n=11)	100% (n=3)	0% (n=1)

前処置のレジメンはフルダラビン125 mg/m^2＋メルファラン180 mg/m^{2}がもっとも多く，移植時CP 6例（診断時BC 1例を含む）に適用され全例無病生存中であった[11]．これら6例のドナーはHLA一致血縁2例，HLA一致非血縁4例であり，非血縁ドナーでもHLA一致であればこのレジメンは適用可能と考えられた．HLA不一致ドナーからのRISTでは，6例中5例で抗胸腺細胞グロブリン（ATG）が追加され，移植時APの1例を除き生着が得られていた．成人CML186例（年齢中央値50歳）におけるRISTの報告では，3年全生存率は移植時CP1（118例）69.0%，CP2（26例）57.1%，AP（30例）24.4%，BC（12例）6%であり，フルダラビン＋ブスルファン＋ATGの前処置が他のレジメンと比較して良好な3年全生存率を示した（64.3% vs 46.9%，p=0.015）[12]．JPLSG CML委員会の解析では，15例中3例の前処置でブスルファン8 mg/kgが使用されていたが，そのうち2例がそれぞれ閉塞性細気管支炎と生着不全から再移植後の合併症で死亡しており，ブスルファンの選択には慎重であるべきと考えられた．10年以上の長期生存率や晩期合併症についての最終的な評価が今後の課題である．

Ⅲ イマチニブによる成長障害

JPLSG CML委員会によって行われた全国調査において，イマチニブが初期治療薬であり，かつ10カ月以上単独投与された48例の解析を行ったところ，イマチニブ開始時から調査時にかけて身長の平均SDスコアが-0.21±0.9から-0.85±0.98に有意に低下しており，イマチニブに成長障害を起こす有害作用があることが明らかになった（投稿中）．男女別に思春期開始前後の年齢に分け，1年ごとのイマチニブの平均投与量を計算して解析したところ，年齢および投与量がイマチニブによる成長障害に影響することが判明した．すなわち，女児9歳未満，男児10歳未満の症例は女児9歳以上，男児10歳以上の症例に比し，イマチニブによる成長障害の影響を受けやすく，かつ投与量による成長障害の程度の差が顕著に現れることわかった（**図5**）．経過観察の長い症例では，思春期開始年齢を境に成長のcatch-upを認める傾向があり，成長板へのエストロゲンの直接作用による成長促進（成長スパート）はイマチニブによって阻害されない可能性がある．

成長障害の機序として，PDGFR活性阻害による骨代謝回転の低下[13]などが考えられているが，成長板に強発現することが知られているABL自体に成長板の増殖・分化に関与する機能があり[14]，その阻害により成長障害が生じる可能性も考えられる．

今回の解析では最終身長まで経過観察できた症例がわずかなため，イマチニブによる最終的な成長障害の程度については数年後まで結論を待たなければならない．イマチニブが50 mg単位（半錠）での調整しかできないため年少児ほど過剰投与になる傾向がある．年少児に成長障害を認めた場合には標準量（260 mg/m^2/日）に近くなるよう内服方法を工夫すべきであり，可能であれば血中濃度により投与量を調整するのが望ましい．ニロチニブやダサチニブにイマチニブと同様の成長への影響があるかどうかは不明である．

おわりに

イマチニブによって小児CMLの成績は飛躍

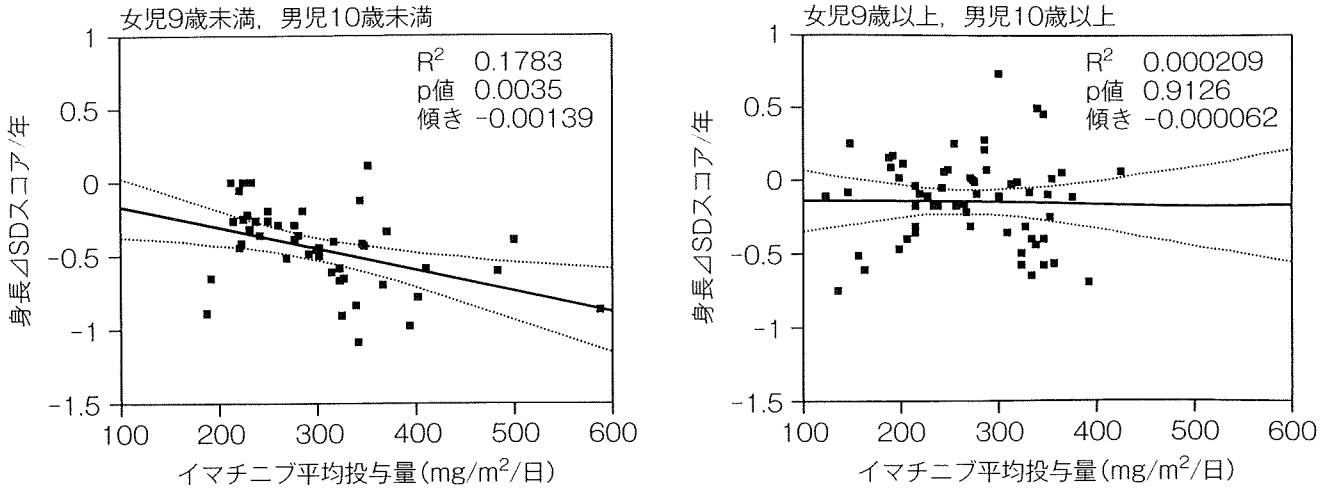

図5　イマチニブによる成長障害に対する年齢と投与量の影響

的に向上し，造血幹細胞移植を行わなくても長期生存が得られるようになった．小児にとって移植関連死や合併症を回避できるのは大きな意味がある．一方で，イマチニブは年少児に成長障害をきたすことが明らかになり，性腺機能異常や骨代謝異常などの合併症も起こす可能性がある．さらに，CML 幹細胞はイマチニブにより根絶されず，イマチニブ単独による CML の治癒は難しいと考えられている．

根治療法としての RIST は有望な手段であり，晩期合併症の軽減も期待できる．適合ドナーが存在すれば小児では早期の実施も許容されるであろう．しかし，10 年以上の長期生存率や晩期合併症についてはイマチニブと同様にいまだ不明である．ニロチニブ，ダサチニブの適応は現在イマチニブ不応・不耐容例に限られているが，数年後には初期治療薬としてイマチニブに取って代わる可能性が高い．また，HSP90 阻害薬，β カテニン阻害薬，ヘッジホッグ阻害薬など CML 幹細胞を標的にした分子標的薬の開発も進んでいる．

CML の治療は今後もしばらく進歩し変化していくだろう．JPLSG CML 委員会では 2009 年 10 月に多施設共同観察研究 CML-08 を開始した．稀少疾患である小児 CML の標準的治療を確立するためには多くの患者や医師の協力が必要である．

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お知らせ

第 43 回　太平洋小児外科学会

43rd Annual Meeting of Pacific Association of Pediatric Surgery（PAPS）

会　　期：平成 22 年 5 月 23 日（日）～27 日（木）
会　　場：神戸ポートピアホテル
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Pediatr Blood Cancer 2010;54:299–306

Hematopoietic Stem Cell Transplantation for Familial Hemophagocytic Lymphohistiocytosis and Epstein–Barr Virus-Associated Hemophagocytic Lymphohistiocytosis in Japan

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Background. Post-transplant outcomes of hemophagocytic lymphohistiocytosis (HLH) patients were analyzed in Japan where Epstein–Barr virus (EBV)-associated severe forms are problematic. ***Methods.*** Fifty-seven patients (43 familial HLH [12 FHL2, 11 FHL3, 20 undefined], 14 EBV-HLH) who underwent stem cell transplantation (SCT) between 1995 and 2005 were enrolled based on the nationwide registration. ***Results.*** Fifty-seven patients underwent 61 SCTs, including 4 consecutive SCTs. SCTs were employed using allogeneic donors in 93% of cases (allo 53, twin 1, auto 3). Unrelated donor cord blood transplantation (UCBT) was employed in half of cases (21 FHL, 7 EBV-HLH). Reduced intensity conditioning was used in 26% of cases. The 10-year overall survival rates (median ± SE%) were 65.0 ± 7.9% in FHL and 85.7 ± 9.4% in EBV-HLH patients, respectively. The survival of UCBT recipients was ≥65% in both FHL and EBV-HLH patients. Three out of four patients were alive with successful engraftment after second UCBT. FHL patients showed a poorer outcome due to early treatment-related deaths (≤100 days, seven patients) and a higher incidence of sequelae than EBV-HLH patients ($P = 0.02$). The risk of death for FHL patients having received an unrelated donor bone marrow transplant was marginally higher than that for a related donor SCT ($P = 0.05$) and that for UCBT ($P = 0.07$). ***Conclusions.*** EBV-HLH patients had a better prognosis after SCT than FHL patients. FHL patients showed either an equal or better outcome even after UCBT compared with the recent reports. UCB might therefore be acceptable as an alternate SCT source for HLH patients, although the optimal conditioning remains to be determined. Pediatr Blood Cancer 2010;54:299–306. © 2009 Wiley-Liss, Inc.

Key words: central nervous system disease; Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis; familial hemophagocytic lymphohistiocytosis; hematopoietic stem cell transplantation; reduced intensity conditioning; umbilical cord blood transplantation

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is an immunohematologic emergency, characterized by fever, cytopenias, hepatosplenomegaly, hyperferritinemia, and disseminated intravascular coagulopathy (DIC) [1,2]. HLH comprises primary form of familial hemophagocytic lymphohistiocytosis (FHL) and secondary form occurring in association with infections, malignancies, and rheumatic diseases. FHL has currently been classified into FHL1 linked to chromosome 9, FHL2 with *PRF1* mutation, FHL3 with *UNC13D* mutation, and FHL4 with *STX11* mutation, although more than half of patients have no mutations of these genes [1]. HLH could also be a presenting symptom in patients with the other inherited disorders including X-linked lymphoproliferative disease (XLP), Griscelli syndrome, Hermansky–Pudlak syndrome, Chediak–Higashi syndrome and primary immunodeficiency diseases. HLH accounts for the common basis of hypercytokinemia arising from excessive immune activation, in which activated lymphocytes and hemophagocytosing-macrophages without malignant morphology infiltrate into systemic organs, including the bone

Additional Supporting Information may be found in the online version of this article.

Abbreviations: BM, bone marrow; BMT, bone marrow transplantation; CB, cord blood; CBT, cord blood transplantation; CNS, central nervous system; CT, computed tomography; EBV-HLH, Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis; EEG, electroencephalography; FHL, familial hemophagocytic lymphohistiocytosis; HLH, hemophagocytic lymphohistiocytosis; PB, peripheral blood; SCT, hematopoietic stem cell transplantation; MRI, magnetic resonance imaging; OS, overall survival; SCT, hematopoietic stem cell transplantation; TRM, treatment-related mortality; RIC, reduced intensity conditioning; VOD, venoocclusive disease; XLP, X-linked lymphoproliferative disease/syndrome.

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marrow (BM), liver, spleen, lymph nodes, skin, and central nervous system (CNS) [3,4]. FHL is a fatal disease if allogeneic hematopoietic stem cell transplantation (SCT) has not been successfully performed.

Epstein–Barr virus (EBV)-associated HLH (EBV-HLH) is a severe form of secondary HLH more frequently occurring in Asian children [5–7]. Activated EBV-infected CD8$^+$ T cells account for the disease process of EBV-HLH [8], however no predisposing factors have yet been clarified. EBV-HLH patients mostly respond to immunochemotherapy, but a small fraction of patients experience a fatal course without SCT. Therefore, although numbers were still small, SCT has been included in the salvage for refractory EBV-HLH cases [9–11]. The optimal timing of SCT, the source of donor cells and the conditioning are critical, particularly for young HLH patients. In this setting, the appropriate SCT for HLH patients needs to be established.

This study analyzed the outcomes of patients with FHL or EBV-HLH who underwent SCT in Japan over the past 10 years, in order to address the issues in the transplant-related problems including engraftment, late sequelae as well as to find out if there are distinct transplant strategies for FHL and EBV-HLH patients.

PATIENTS AND METHODS

Data Collection

The HLH/LCH Committee in the Japanese Society of Pediatric Hematology (JSPH) sent the first questionnaires to the hospitals administered by JSPH members based on the SCT registry in JSPH, asking if SCT was performed for any HLH patients between 1995 and 2005. The second questionnaires were sent to 57 hospitals with SCT cases, asking the patients' characteristics, treatment prior to SCT, donor sources, conditioning regimens, complications, and outcome. Of the 47 responses (recover rate 82%), 61 definite SCT cases from 33 hospitals were eligible for the study (mean 1.7 case/hospital, Supplemental Table). Forty-three FHL patients underwent 46 SCT, while 14 EBV-HLH patients underwent a total of 15 SCT. The majority of SCT (EBV-HLH 87%, FHL 89%) were performed between 2000 and 2005.

Diagnosis and Classification

All 57 patients fulfilled the diagnostic criteria of HLH [12]. FHL was diagnosed when the patient had a genetic abnormality, positive family history, and/or other evidence such as impaired natural killer cell activity [13]. The genetic study of FHL 2, 3, and 4, approved by the ethics committee of Kyushu University, Japan (No. 45), was partly completed postmortem according to our methods [14–17]. FHL2 and FHL3 determined by *PRF1* or *UNC13D* mutations accounted for 28% (n = 12), and 26% (n = 11), respectively, in this group. In addition, a total of eight patients were found with siblings diagnosed as having HLH. EBV infection might be associated with the development of HLH in four FHL patients (one FHL2, one FHL3, and two familial). These cases were classified as FHL, not as EBV-HLH. Other types of primary HLH such as XLP were excluded in this study.

EBV-HLH was diagnosed when a non-FHL patient had a primary infection or reactivation of EBV at the onset of HLH. EBV infection was assessed by the detection of EBV DNA and/or the pattern of serum EBV-specific antibody titers [18]. Cases with secondary HLH occurring in a chronic active EBV infection [19], and/or a histologically confirmed EBV-related lymphoma were excluded in this study. CNS involvement was determined when patients showed neurological manifestations, clinically as well as with any evidence of abnormality in the cerebrospinal fluids (CSF), neuroimagings (CT/MRI), and/or electroencephalography (EEG).

Prior Treatment to SCT

Treatment was based on the HLH-94 protocol using a combination of corticosteroid, cyclosporine-A (CSA), and etoposide (VP16) for both groups [20,21]. As the multidrug chemotherapy, CHOP-VP16-based regimen (VP16, vincristine, cyclophosphamide [CY], doxorubicin, and prednisolone) was chiefly employed. SCT was performed for all FHL patients, but limited for EBV-HLH patients who were resistant to any other treatments.

SCT

Allogeneic SCT was performed in 53 of the 57 patients (93%). Autologous SCT and identical-twin donor SCT were performed in three and one sporadic patients, respectively, because the molecular diagnosis was not available at the time of SCT. Donor sources, infused cell doses, conditioning regimens, and other SCT-related data are summarized in Table I. Allogeneic donor sources for EBV-HLH were HLA-matched sibling peripheral blood (PB) 1, haploidentical parent BM/PB 2, HLA-matched unrelated BM 1, HLA-matched unrelated cord blood (UCB) 2, and HLA-mismatched UCB 5, and those for FHL were HLA-matched related BM 7 (sibling 6), haploidentical parent BM/PB 2, HLA-matched unrelated BM 12, HLA-matched UCB 9, and HLA-mismatched UCB 12. All CBs were obtained from unrelated donors registered in the Japanese Cord Blood Bank Network. All unrelated donor BMs were obtained from the Japanese Marrow Donor Program. Myeloablative conditioning for EBV-HLH included VP16/busulfan (BU)/CY in 8 patients (4 in UCB transplantation [UCBT]) and other regimens in 3 patients, while those for FHL were VP16/BU/CY plus or minus anti-thymocyte globulin (ATG) in 23 patients (10 in UCBT) and others in 8 patients. Reduced intensity conditioning (RIC) for EBV-HLH included melphalan (MEL)/fludarabine (FLU) plus or minus thoracoabdominal irradiation in three patients (two in UCBT), and those for FHL were MEL/FLU plus or minus low-dose total body irradiation plus or minus ATG in eight patients (four in UCBT) and others in three patients. Donor chimerism was assessed by using short tandem repeats or sex chromosome analyses.

Evaluation of Late Sequelae

Long-term survivors were further questioned concerning their physical growth, endocrinological status, and neurological deficits. Neurological development including cognitive functions was assessed by Karnofsky score, developmental quotient and/or school performance.

Statistical Analysis

The 10-year overall survival (OS) rate with 95% confidence intervals were estimated by the Kaplan–Meier method. The OS was calculated for the period from the day of SCT until the death of any cause or the final observation. All results were updated to May 31,

TABLE I. Profiles of Patients Who Underwent Hematopoietic Stem Cell Transplantation

	EBV-HLH	FHL	*P*-value
Number, male:female	14, 4:10	43, 23:20	0.37
Age at onset (median, range)	5.5y, 6m–18y	0.5y, 6d–12y	<0.0001
Age at SCT (median, range)	5.9y, 1.4–18y	1.2y, 0.4–15y	0.0002
Observation period (median, range)	5.5y, 0.3–16y	4.8y, 0.2–19y	0.94
Manifestation at diagnosis (%)			
Fever	100	95	>0.99
Hepatosplenomegaly	86	86	>0.99
Lymphadenopathy	36	21	0.30
Skin eruption	7	14	0.67
Respiratory failure	36	14	0.12
DIC	50	33	0.26
Treatment prior to SCT (%)			
HLH94 only	36 (5/14)	60 (25/42)	0.14
Multidrug chemotherapy	57 (8/14)	19 (8/42)	0.017
Diagnosis to SCT (median, range)	5.8m, 1.8–24m	7.5m, 1.6–84m	0.18
SCT (n)			
Allogeneic	11	42	
Auto/Identical twin	3	1	
Nucleated cell doses ($\times 10^8$/kg)	1.3 (0.2–6.6)	2.5 (0.1–12.7)	0.14
Donor			
UCB	7	21	0.94
Others	7	22	
HLA disparity no	4	28	0.09
HLA disparity yes (>1 locus[a])	7	14	
Conditioning			
Myeloablative[b]	11	31	>0.99
RIC[c]	3	11	
Irradiation yes	4	11	0.73
Irradiation no	9	31	
ATG yes	0	8	0.18
ATG no	14	34	
CNS abnormality (%)			
At diagnosis	29[d] (4/14)	21[d] (9/42)	0.72
Before SCT	57 (8/14)	67 (28/42)	0.52
CSF pleocytosis	25 (2/8)	32 (7/22)	>0.99
MRI abnormality	36 (5/14)	51 (20/39)	0.36
Convulsion	43 (6/14)	41 (17/41)	0.93
Disturbed consciousness	36 (5/14)	24 (10/41)	0.49
Post-transplant state (n)			
Early death (<100 days)	2	7	0.48
Alive	12	29	0.31
Neurological deficit (%)	8[d] (1/12)	29[d] (7/24)	0.22
Late sequelae[e] (%)	8 (1/12)	52 (11/21)	0.022

ATG, anti-thymocyte globulin; BU, busulfan; CNS, central nervous system; CSF, cerebrospinal fluid; CY, cyclophosphamide; DIC, disseminated intravascular coagulopathy; EBV, Epstein–Barr virus; FHL, familial hemophagocytic lymphohistiocytosis; FLU, fludarabine; HLH, hemophagocytic lymphohistiocytosis; MEL, melphalan; MRI, magnetic resonance imaging; SCT, hematopoietic stem cell transplantation; TAI, thoracoabdominal irradiation; TBI, total body irradiation; UCBT, unrelated donor cord blood transplantation; VP16, etoposide. Parenthesis means the positive number of patients per the evaluable number of patients. The observation period means the time from the onset to the last visit or death. [a]Human leukocyte antigen (HLA) disparity was assessed by the serotyping data of HLA-A, -B, and -DR; [b]Myeloablative conditionings for EBV-HLH were VP16/BU/CY 8 (4 in UCBT) and others 3, and those for FHL were VP16/BU/CY + ATG 23 (10 in UCBT) and others 8; [c]Reduced intensity conditionings (RIC) for EBV-HLH were MEL/FLU + TAI 3 (2 in UCBT), and those for FHL were MEL/FLU + low dose TBI + ATG 8 (4 in UCBT) and others 3; [d]The proportion of patients having neurological abnormality was lower in survived patients with EBV-HLH ($P = 0.0015$). Survived patients were neurodevelopmentally assessed at the last visit to the hospital; [e]Late sequela(e) in EBV-HLH was hemiparesis (n = 1), and those in FHL were short stature (n = 5), endocrinological abnormality (n = 1), psychomotor retardation with or without seizure (n = 5), brain atrophy (n = 1), and hearing difficulty (n = 1).

2008. An analysis of the risk factors for SCT outcome was possible for FHL, but not for EBV-HLH because of the small number of subjects. Age at onset of HLH or at the SCT, duration from the onset to SCT, CNS disease before SCT, donor sources, and the type of conditioning were tested using the log-rank method. Cox proportional-hazard model was employed to examine the association between selected clinical variables and the risk for death. A logistic regression model was used to investigate factors associated with neurological sequelae. Chi-square test or Fisher's exact test were employed in other comparisons. P values less than 0.05 were considered to be significant.

RESULTS

Profiles of EBV-HLH and FHL Patients

A comparison of the clinical profiles (Table I) revealed that the ages at disease onset and at the time of SCT were each higher in EBV-HLH than in FHL patients ($P < 0.0001$, $P = 0.0002$, respectively). No clinical manifestations differed between the two groups during the disease course, including respiratory failure as well as CNS abnormalities at diagnosis. The proportion of patients who failed VP16 and CSA therapy including HLH94 protocol and needed combination chemotherapy such as CHOP-VP16 before planning SCT was higher in EBV-HLH patients than FHL patients (57% vs. 19%, $P = 0.0168$).

Outcomes of SCT

Engraftment and survival. Post-transplant outcomes of 43 FHL patients and 14 EBV-HLH patients are summarized in Figures 1 and 2. The 10-year OS rates (median ± SE%) of FHL and EBV-HLH patients were 65.0 ± 7.9% and 85.7 ± 9.4%, respectively ($P = 0.24$; Fig. 3). In the allogeneic SCT cases with FHL (Fig. 1), 29 attained engraftment, 6 had rejection or graft failure, and 7 were undetermined. On the other hand, in EBV-HLH (Fig. 2), seven were engrafted, three were rejected, and one was undetermined. Of all 29 FHL patients engrafted after the first SCT, 26 were alive with no HLH relapse, but 3 died of treatment-related mortality (TRM). Seven engrafted patients with EBV-HLH were alive and well at the final follow-up. Among the nine rejection/graft failure patients (six FHL, three EBV-HLH), a second UCBT was successful in three of the four patients (three FHL, one EBV-HLH). Twelve of the UCBT recipients for FHL that received a graft with the first UCBT and two that received a second UCBT were alive at the last follow-up; while seven died; six were due to TRM and one was due to active HLH disease. Six of the seven UCBT recipients for EBV-HLH were alive and well at the last follow-up, while only one died of active HLH disease on day 18 post-transplant. A total of 29 FHL survivors after allogeneic SCT(s) had 17 complete donor chimera (2 patients after second UCBTs), 3 mixed chimera (1 had 42% donor chimera in remission 18 months after SCT, 2 attained >90% donor chimera until 6 months after SCT), 8 undefined, and 1 graft failure with CNS disease. Ten EBV-HLH survivors after allogeneic SCT attained eight complete donor chimera (seven patients after the first SCT and one patient after second SCT [UCBT]), and two with autologous recovery. Two of three EBV-HLH patients who rejected allogeneic cells were alive and disease free more than 6 years post-transplant. One of two EBV-HLH patients who underwent autologous SCT was alive and well 13 years post-transplant [22]. One EBV-HLH patient was alive and well 10 years after the identical twin donor BMT.

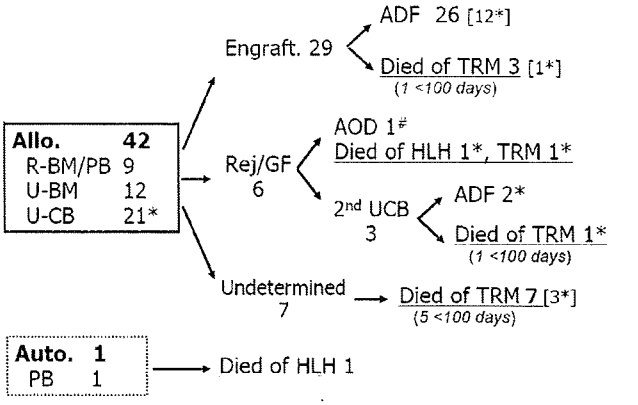

Fig. 1. Cohort diagram for the clinical outcome of 43 patients with familial hemophagocytic lymphohistiocytosis (FHL) who underwent stem cell transplantation (SCT). Of 42 patients after allogeneic SCT, 29 achieved engraftment (18 complete, 3 mixed) and 6 failed to engraft. One (#) with graft failure was alive with central nervous system disease 12 years after SCT. A total of 29 patients (67%) were alive after SCT. The underlined data indicate the number of deceased patients. Seven patients died within 100 days post-SCT (parenthesis). Asterisk (*) means UCB. R, related; U, unrelated; BM, bone marrow; PB, peripheral blood; CB, cord blood; ADF, alive with the disease free state; AOD, alive on disease; Rej/GF, rejection or graft failure; TRM, treatment-related mortality.

Causes of death. Of 14 deceased FHL patients, 12 died of TRM, including 3 chronic GVHD while 2 died of recurrent HLH. Seven patients experienced early death from TRM within 100 days after SCT (Fig. 1). One patient, later diagnosed with FHL2, died of CNS disease 5 years after autologous SCT [14]. Two EBV-HLH patients died of recurrent HLH within 50 days after SCT (Fig. 1). No TRM-related deaths were noted among the EBV-HLH patients.

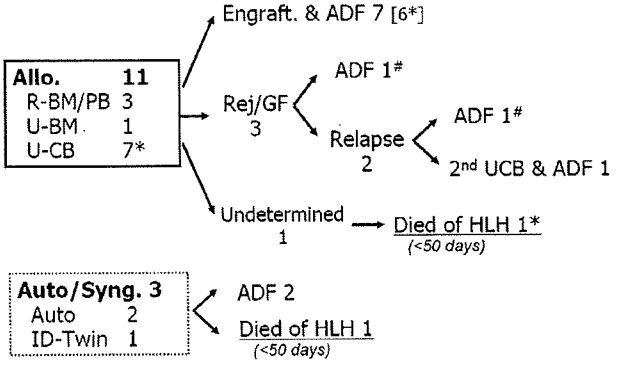

Fig. 2. Cohort diagram for the clinical outcome of 14 patients with Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) who underwent SCT. Among 11 patients after the first allogeneic SCT, 7 achieved successful engraftment and 3 failed to engraft. A total of 12 patients (86%) were alive after SCT. Two patients (#) were alive and well more than 6 years after SCT failure. The underlined data indicate the number of deceased patients. Two patients died within 50 days post-SCT (parenthesis). Asterisk (*) means UCB. Auto/Syng: autologous/syngeneic, ID: identical.

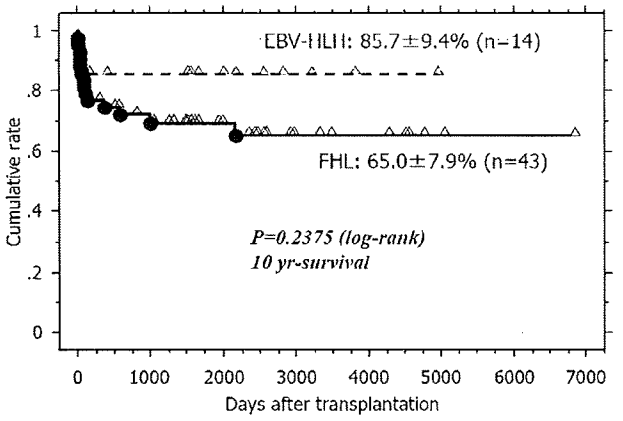

Fig. 3. Cumulative probability of post-transplant overall survival of FHL (*solid line*) and EBV-HLH patients (*dashed line*) who underwent SCT. Closed circle and open triangle represent deceased and alive patients, respectively. Each value indicates the 10-year overall survival rate plus or minus standard error assessed by the log-rank test.

Analysis of Prognostic Factors in FHL

A log-rank test on the OS rate did not show any significant difference in terms of age at SCT (<2 years vs. ≥2 years), time of SCT from HLH treatment (<6 months vs. ≥6 months), conditioning regimens (myeloablative vs. RIC) and various donor sources (R-PB/BM vs. UCBT vs. UBM; Table II). The Cox hazard model with adjustment for gender and age at engraftment indicated that the risk of death for UBM might be higher than that for R-PB/BM (adjusted hazard ratio = 0.07, 95% confidence interval [CI] = 0.01–1.02, $P = 0.05$) and that for UCB (0.27, 95% CI = 0.07–1.09, $P = 0.07$; Table II). No significant variables were found to predict the risk of early death within 100 days post-transplant, or the risk of neurological sequelae.

CNS Abnormalities and Late Sequelae

Table I shows that the frequency of CNS abnormalities at onset and the time of SCT did not differ between the EBV-HLH and FHL patients. Whereas, post-transplant CNS abnormalities were significantly higher in the FHL patients ($P = 0.0015$). Eleven FHL patients (52%) have had late sequelae including neurological as well as endocrinological problems, in comparison to only one EBV-HLH patient with left hemiparesis ($P = 0.022$). Late sequelae of FHL

TABLE II. Association Variables Influencing on the Risk of Mortality in FHL Patients

(A) Log-rank analysis Variables	No.	Survival (OS %)		*P*-value
Age				
<2 years	30	66.2 ± 8.7		0.56
>2 years	12	75.0 ± 12.5		
Time from HLH treatment				
<6 months	14	62.9 ± 13.3		0.65
≥6 months	28	71.4 ± 8.5		
Conditioning				
Myeloablative	31	71.0 ± 8.2		0.50
RIC	11	60.6 ± 15.7		
Donor sources				
R-PB/BM, a	9	88.9 ± 10.5	a vs. b	0.22
UCB, b	21	65.6 ± 10.6	a vs c	0.15
UBM, c	12	58.3 ± 14.2	b vs c	0.61

(B) Cox's model analysis Variables	No.	Adjusted hazard ratio	95% CI lower–upper limit	*P*-value
Stem cell source				
Unrelated BM	12	1.00	Reference	
Unrelated CB	21	0.27	0.07–1.09	0.07
Related PB/BM	9	0.07	0.01–1.02	0.05
Conditioning				
Reduced intensity	11	1.00	Reference	
Myeloablative	31	0.48	0.09–2.47	0.38
Radiation				
No	31	1.00	Reference	
Yes	11	0.52	0.11–2.52	0.41
Use of ATG				
No	34	1.00	Reference	
Yes	8	0.91	0.18–4.70	0.91
HLA disparity				
No	28	1.00	Reference	
Yes (>1 locus)	14	2.79	0.75–10.38	0.13

Both analyses (A, B) were performed for 42 FHL patients who underwent the first allogeneic SCT. The Cox model analysis was performed with adjustment for selected variables including sex and age at engraftment.

included psychomotor retardation with or without seizures (n = 5), brain atrophy (n = 1), hearing difficulty (n = 1), short stature (n = 5), and impaired sexual development (n = 1).

DISCUSSION

No underlying immunodeficiency has yet been identified for idiopathic EBV-HLH, which has been recognized to be distinct from familial or inherited disease-related HLH like FHL. However, EBV also acts as a trigger in the development of HLH episodes in FHL patients. Therefore, caution must be exercised in the differentiation of the two types of HLH disease. Strict use of the renewed diagnostic criteria for the registered cases in Japan enabled an analysis of the SCT results of 43 FHL and 14 EBV-HLH patients. The data first revealed a high survival rate in UCBT recipients in either type of HLH, indicating that CB could be preferable BM as the unrelated donor source in SCT for pediatric patients with refractory HLH. In addition, SCT in FHL patients was more problematic than that in EBV-HLH, where it was associated with a high incidence of post-transplant early death rate as well as late sequelae including neurological deficits. The EBV-HLH patients showed no apparent sequelae even if they had CNS involvement at diagnosis.

Information concerning SCT for HLH patients has been accumulated mostly in FHL, but little has been published in EBV-HLH except for sporadic case reports [10,11]. Previously published major studies on SCT in FHL patients are summarized in Table III. Because of the historical changes in the available genetic analyses, supportive care practices, donor sources and conditioning, the pre-2000 studies [23–27] might not be comparable to the current data. Henter et al. [21] showed the improved survival of patients treated with HLH-94 followed by BMT, in which the 3-year post-BMT survival was 62%. Horne et al. [28] noted significant TRM due to venoocclusive disease (VOD) after myeloablative conditioning, and that an active disease status at SCT was associated with a poor prognosis. Ouachee-Chardin et al. [29] reported 59% of OS in a series of 48 patients including 60% of haploidentical SCT, and indicated a high TRM due to VOD associated with young age. Recently, Baker et al. [30] reported that BU/CY/VP16 plus or minus ATG-conditioning provided a cure in 53% of patients after unrelated donor BMT, but a high mortality rate at day 100 (32 of 50 [64%] deceased patients). The present study showed a comparably high OS rate (69%) and similarly high incidence of early death until day 100 (7 of 13 [54%] deaths after allogeneic SCT) in Japan. Probably, the major distinction of the current study from the other reports is a higher usage of UCBT (50%) and RIC (26%). Unfortunately, the combined usage of RIC-UCBT was applied only in eight cases (14%) in this study, which was insufficient to fully evaluate its effectiveness. With regard to RIC-SCT with or without UCBT for FHL, Cooper et al. [31] reported a high disease free survival (75%) in 12 HLH patients (including 5 FHL) who underwent RIC-SCT from matched family/unrelated or haploidentical donor, in which 3 of 9 survivors had mixed chimerism but remain free of disease. The most recent report by Cesaro et al. [32] analyzed 61 cases including an appreciable number of RIC (18%) and UCBT (10%), but did not document the superiority of RIC-UCBT. In the present study, UCBT had a tendency to yield a more favorable outcome than UBMT, although the difference was not statistically significant. FHL infants received SCT early; however the fact that survival of FHL patients who underwent SCT at <2 years of age was not better than later SCT might reflect the difficulty in determining the optimal timing of SCT

TABLE III. Reports on the Clinical Outcome of Patients With HLH Who Underwent Allogeneic Hematopoietic Stem Cell Transplantation

No. pts	Median age at SCT (months)	FH (%)	Major conditioning regimen	Donor	Source	OS (%)	Engraft. (%)	Causes of death	Refs.
9	13	45	Myeloab	VP16/BU/CY ± anti-LFA1	MRD/MMRD/haplo	BM	44.0	100	TR, HLH	[24]
29	NR	48	Myeloab	NR	MRD/MUD/haplo	BM	66.0	72	TR, HLH	[25]
20	9	30	Myeloab	VP16/BU/CY ± ATG	MSD/URD (80%)	BM	45.0	90	TR, HLH	[26]
14	14	36	Myeloab	VP16/BU/CY, ATG/BU/CY	MMRD/MUD	BM (T cell depleted)	64.3	65	TR, HLH	[27]
12	18	42	Myeloab	VP16/BU/CY	MSD/URD (67%)	BM	100	100	No	[33]
17	NR	NR	Myeloab	VP16/BU/CY ± ATG, TBI	MRD/URD/haplo	BM, CB (2), PB, CD34	58.0	94	TR, HLH, lymphoma	[8]
65[a]	13	31	Myeloab	VP16/BU/CY ± ATG	MRD/URD/haplo	BM, CB (5), PB, CD34	62.0	89	TR, HLH, AML	[21]
86[a]	13	34	Myeloab	VP16/BU/CY ± ATG, TBI	MRD/URD/haplo	BM, CB (7)	64.0	90	TR, HLH, 2nd AML	[28]
48	6	35	Myeloab	VP16/BU/CY, ATG/BU/CY	MSD/URD/haplo	BM, PB	58.5	78	HLH	[29]
12	14	17	RIC	FLU/MEL ± BUS, FLU/2GyTBI	MRD/URD/haplo	BM, CD34	75.0	100	TR	[31]
91	12	NR	Myeloab	VP16/BU/CY ± ATG	URD	BM, PB, CB (9)	45.0	83	TR, HLH	[30]
61	13	20	RIC (18%)	VP16 or MEL/BU/CY ± ATG	MRD/MMRD/URD	BM, PB, CB (6)	63.9	78	TR (68%), HLH (27%)	[32]
42	17	55	RIC (26%)	VP16/BU/CY ± ATG, TBI	MRD/MMRD/URD	BM, PB, CB (21)	69.0	78	TR (79%), HLH (21%)	Ours

AML, acute myelogeneous leukemia; BM, bone marrow; BU, busulfan; CB, cord blood; CY, cyclophosphamide; FHL, familial hemophagocytic lymphohistiocytosis; FH, family history; FLU, fludarabine; MEL, melphalan; MMRD, HLA-mismatched related donor; MRD, HLA-matched related donor; MSD, HLA-matched sibling donor; MUD, HLA-matched unrelated donor; NR, not recorded; PB, peripheral blood; RIC, reduced intensity conditioning; TBI, total body irradiation; TR, transplantation-related events; URD, unrelated donor; VP16, etoposide. [a]Sixty four of 65 patients studied by Henter et al. [21] were included in 86 patients by Horne et al. [28].

or introducing appropriate RIC regimens in young infants. In UCBT, a major obstacle was thought to be early graft failure, but once engrafted no late graft failure could not be seen [29]. We confirmed this finding in our UCBT cases.

Dürken et al. [33] reported that six HLH patients with CNS disease underwent allogeneic BMT and three of them had no persistent neurological problems after transplant. More recently, SCT is thought to be preferable for FHL patients at the early stage of CNS disease with variable presentation [34,35]. Fludarabine-based RIC has been preferred in SCT for FHL patients in order to reduce late sequelae [36,37]. Since CNS disease itself had no impact on the OS in the current study, but nearly half of the long-term survivors of FHL had late sequelae associated with growth and development, further prospective studies should be focused on how to reduce late sequelae in SCT for FHL patients.

In the treatment of refractory EBV-HLH, no consensus has yet been reached concerning the treatment of patients who fail to respond to the HLH-2004 protocol type immunochemotherapy. Several reports documented that SCT led to a complete remission in such cases [8,10,11,28,38,39]. The present study revealed that use of pre-SCT combination chemotherapy might be associated with a better therapeutic impact on subsequent SCT in patients with EBV-HLH. Furthermore, long-term survival, that is, a probable cure, could be obtained even after autologous SCT [22] or identical twin donor BMT, suggesting that a reconstitution of allogeneic hematopoietic stem cells was not essential in the successful SCT for EBV-HLH patients as described in the autologous PBSCT success for lymphoma-associated HLH [40]. In addition, long-term survival even after graft failure or post-transplant relapse in EBV-HLH patients might suggest the possibility of resetting the adaptive immune response to the virus as postulated in autologous SCT for the treatment of autoimmune diseases [41,42]. Moreover, successful syngeneic SCT may imply that EBV-HLH is not a monogenic disease, since Chen et al. [43] observed that a primary infection of EBV incited HLH in a pair of the twins, but not in the identical twin counterpart. These observations implied that the genetic influence in patients with EBV-HLH might be distinct from that in patients with FHL on precipitating the excessive immune activation. Further prospective studies should therefore be directed toward not only the optimization of UCBT-RIC to improve survival of FHL patients, but to better understanding of the pathological interaction between cytotoxic granule disorders and EBV.

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原　著

小児再発急性リンパ性白血病における分子生物学的微小残存病変定量の臨床的意義

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Clinical Implication of the Molecular Biological MRD Quantification on the Children with Relapsed Acute Lymphoblastic Leukemia

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Abstract Here, we studied the correlation between the molecular minimal residual disease (MRD) levels and clinical outcome after the first bone marrow relapse in childhood acute lymphoblastic leukemia (ALL). The age of onset of the 4 children ranged from 4 to 9 years old. All of them were boys and stratified into the standard risk group in the CCLSG ALL 2000/2004 protocols. The times to relapse after the start of the therapy were 21 months, 26 months, 34 months and 38 months. There were two in S2 category and two in S3 category according to the BFM-S classification. Although all patients responded to the salvage chemotherapy and achieved a second morphological remission, their molecular MRD levels varied. In S2 group, the case with negative MRD levels ($10^{-3}>$) remained in the remission status without stem cell transplantation (SCT), but the other case with positive MRD levels($10^{-3}\leqq$) died due to the complications of SCT. The two cases in S3 showed positive levels of molecular MRD assays, and were assigned to the therapy group with SCT. The patient who received SCT was still alive without disease, but another patient progressed into overt relapse before SCT. These results suggest that the PCR-based MRD levels were important to predict the outcome of the children with relapse ALL and decide the salvage therapy including SCT.

要　旨　今回われわれは小児再発 ALL 症例の治療に際し，Ig/TCR 遺伝子再構成を用いた PCR にて MRD を定量し，その結果と臨床経過について検討した．対象とした 4 症例の発症年齢は 4 歳から 9 歳，全員が標準危険群に属し，再発時期は治療開始後 20 カ月から 46 カ月で，3 例が治療終了後，1 例は維持療法中の再発で，BFM-S 分類では 2 例が S2 群，2 例が S3 群であった．全例化学療法により形態学的再寛解を得たが，その MRD 量は異なっていた．S2 群については，MRD 陰性例は化学療法のみで寛解を維持しているが，MRD 陽性例は骨髄移植を施行後合併症にて死亡した．S3 群はともに MRD が陽性であったため造血幹細胞移植を計画，1 例は移植後 MRD の陰性化を認め寛解維持に至ったが，1 例は移植前に第 2 再発をきたし腫瘍死した．これらの結果は，再発後の予後予測や造血幹細胞移植を含む治療法の選択に PCR による分子生物学的 MRD 定量が有用であることを示唆していると考えられた．

Key words: acute lymphoblastic leukemia, relapse, minimal residual disease, Ig/TCR gene rearrangement

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I. はじめに

小児急性リンパ性白血病（ALL）における治療成績の進歩はめざましく，長期生存率は約 85%に達している[1,2]．しかし再発例の予後はいまだ不良で，その生存率は 50%にも達していない[3,4]．本邦では年間推定約 600 例の新規発症 ALL[5] のうち約 100 例が再発すると考えられ，これら再発症例に対してさまざまな治療が試みられているが，標準的治療法はいまだ確立されておらず，造血幹細胞移植（stem cell transplantation: SCT）の適応も各施設の判断に委ねられているのが現状である．

近年，免疫関連遺伝子（Immunogloblin/T-cell receptor: Ig/TCR）再構成を利用した polymerase chain reaction（PCR）による微小残存病変（minimal residual disease: MRD）定量の結果と小児 ALL の長期予後との関連について多くの報告がなされ[6-8]，Berlin-Frankfurt-Munster（BFM）グループを中心に骨髄の MRD 定量結果による治療層別化が，初発だけでなく再発例に対しても試みられている[8]．本邦では，この PCR-MRD を用いた小児 ALL の多施設共同研究は小児癌白血病研究グループ（Childhood Leukemia and Cancer Study group: CCLSG）において行われてきたが[9]，再発 ALL に関する報告はない．

今回，われわれは 4 例の骨髄単独再発の ALL 症例を対象として経時的な骨髄 MRD 定量を実施し，その結果に基づき造血細胞移植を含めた再発治療法を選択した．この 4 症例の経時的な MRD 量の推移と治療反応性および予後との関連について報告する．

II. 方　　法

1. 対　象

対象は 2004～2006 年に当科で診断治療した小児再発 ALL 患者 4 例で，Table 1 にその詳細を示す．初発時年齢は 4 歳から 9 歳で初発時の白血球数は 1,400/μl から 45,200/μl，全例が男児で FAB 分類は L1，フローサイトメトリー（flow cytometry: FCM）による表面マーカー測定にて precursor-B ALL と診断され，特異的な染色体異常は認めなかった．全例に対して患者家族から MRD 測定を含めた臨床試験参加同意取得後に CCLSG ALL2000MRD 研究[10] 低・中間危険群あるいは CCLSG ALL2004 パイロット研究標準危険群の治療（Fig. 1）を実施し，治療開始 4 週後の骨髄にて完全寛解を確認した．寛解判定時の骨髄の MRD は症例 2，4 で陽性であったが，治療層別化を行っている治療開始後 12 週目には全例で陰性で，治療アームの変更なく治療を継続し，問題となる治療の変更や遅延はなかった．治療開始から再発までの期間は 20 カ月から 46 カ月，1 例は維持療法中で，他の 3 例は治療終了後それぞれ 2 カ月，9 カ月，16 カ月後であった．全例が骨髄単独再発で，BFM グループの提唱した再発 ALL における S（strategy）分類（Table 2）[11] では 2 例が S2，2 例が S3 に相当した．

2. Ig/ TCR 遺伝子再構成を利用した MRD 定量

ヘパリンを加えて採取した再発時骨髄検体より高分子 DNA を抽出し[12]，Ig/TCR 遺伝子再構成（TCR δ 6 種類，TCR γ 6 種類，Ig κ 5 種類）[9] を標的としたプライマーを用い，multiplex PCR method[13] に従って再構成スクリーニング PCR を行った．続いて，得られた PCR 産物のク

Table 1　Patient characteristics

	Age & WBC of onset, Gender	FAB classification immunophenotype Risk groups	Initial treatment	Time of relapse* (months)	Site of relapse	BFM-study group S classification
Case 1	5Y 13,900/μl, Male	L1・PrecursorB intermediate	CCLSG ALL2000 LR/IR	46/9	Bone marrow	S2
Case 2	4Y 4,500/μl, Male	L1・PrecursorB low	CCLSG ALL2000 LR/IR	42/16	Bone marrow	S2
Case 3	4Y 45,200/μl, Male	L1・PrecursorB standard	CCLSG ALL2004 Pilot SR	27/2	Bone marrow	S3
Case 4	9Y 1,400/μl, Male	L1・PrecursorB standard	CCLSG ALL2004 Pilot SR	20/**	Bone marrow	S3

* Time of relapse is counted from the start of the initial treatment/the end of the initial treatment.
** Case 4 relapsed during maintenance therapy.

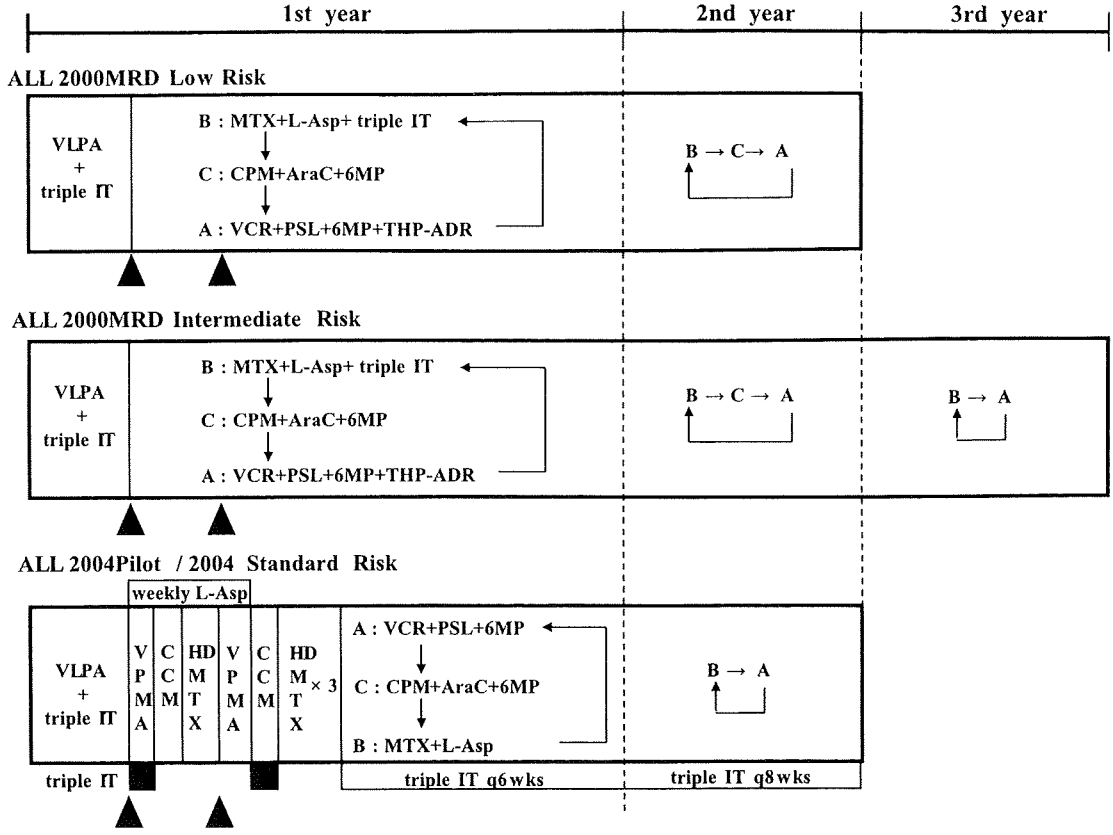

Fig. 1 Schema of CCLSG ALL2000MRD low & intermediate risk and ALL 2004 pilot/2004 standard risk
The scale in the figure is not the precise time. MTX: methotrexate, L-Asp: L-asparagenase, CPM: cyclophosphamide, AraC: cytarabine, VCR: vincristine, PSL: prednisolone, 6MP: 6-mercaptopurine, THP-ADR: pirarubicin, VLPA: VCR, L-Asp, PSL, and THP-ADR, VPMA: VCR, PSL, 6MP, and THP-ADR, CCM: CPM, AraC, and 6MP, HD-MTX: high dose MTX, IT: intrathecal injection, triple IT: MTX, AraC, and hydrocortisone. Black triangles indicate the points for bone marrow sampling of prospective MRD analysis.

Table 2 Definition of risk group S1–S4 of ALL-REZ BFM study[11]

Immunophenotype	Non T-ALL			T-ALL		
Location / Timepoint	Extra-medullary	BM combined	BM isolated	Extra-medullary	BM combined	BM isolated
Very early	S2	S4	S4	S2	S4	S4
Early	S2	S2	S3	S2	S4	S4
Late	S1	S2	S2	S1	S4	S4

Very early: ＜18 months after the start of the therapy, Early: ≧18 months after the start of the therapy and ＜6 months after the end of the therapy, Late: ≧6 months after the end of the therapy, CNS: central nervous system, BM: bone marrow.

ロナリティの確認のため，heteroduplex analysis[14]を用いてpolyacrylamide gel electrophoresis（PAGE）を行った．これは，94°C 5分の加熱で1本鎖になったDNAが（denaturation），4°Cに冷却されて再び2本鎖にもどる過程で（renaturation），モノクローナルなDNAはhomoduplexを，ポリクローナルな場合はheteroduplexを形成し，PAGEにてそれぞれ明瞭なバンドとスメアに分かれる原理を利用している．モノクローナルな再構成バンドを確認後，その塩基配列を解析して塩基の欠失，挿入を含む特異的な配列を同定し，3′側がこれを含むようにallele-specific oligonucleotide（ASO）プライマーを設計した．なお，塩基配列の決定はタカラバイオ社に依頼して行った．

再寛解導入療法の開始後，経時的に行われた骨髄穿刺時の骨髄検体（Point 1：治療開始後4週，Point 2：治療開始後17週）から同様に高分子DNAを抽出し，再発