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話　題

間葉系幹細胞による移植片対宿主病の治療*

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MSCsとは

間葉系間質細胞〔mesenchymal stromal cells（間葉系幹細胞；mesenchymal stem cells）いずれの場合も略はMSCsで，以後MSCsと記載する〕は，元来は骨髄微小環境を形成し造血を支持する間質細胞として同定された[1]．その後MSCsは増殖能が高く，コロニー形成し，線維芽細胞コロニー形成細胞となることが判明し，さらにはin vitroで骨，軟骨，脂肪細胞（中胚葉由来）などにも分化することが示された．さらには外胚葉系細胞（神経細胞），内胚葉系細胞（肝細胞など）への分化能と，自己増殖能という特質から，MSCsは幹細胞としての特質を持っていると理解され，今日ではさまざまな再生医療や細胞治療分野で応用されるに至っている．実際には臓器再生に加えて，組織修復や，免疫抑制などの場に広く用いられているのが現状である[2]．

国際細胞治療学会の定義ではプラスチックへの接着性，3系統への分化に加えて表面抗原として，CD73, CD90, CD105陽性，CD11b, CD14, CD34, CD19, CD79a, HLA-DR陰性であることをあげている[3]（表1）．MHC class II分子の発現がないことや，共刺激分子の発現がないことなどから，リンパ球刺激能に弱く，HLAが一致していなくても拒絶されないという点も大きな特徴である．

本稿では，造血細胞移植（hematopoietic cell transplantation；HCT）におけるMSCs細胞治療につき，GVHDに対する治療に焦点を当てて概説する．

作用機序（免疫抑制作用）の本体

MSCsが人体に投与されるようになってから15年以上が経過するが，その作用機序については不明な点が多い．まず修復（活動）部位で生着するMSCsはきわめて少ないかあるいは検出されないという特性がある．MSCsの作用にはサイトカイン・ケモカインなどの生理活性物質と細胞間相互作用の両者が重要であろうとされている．また，MSCsが分泌する物質は刺激の種類や強度によって異なり，相互作用する細胞も多岐にわたるために複雑である（表2, 3）．このような状況の中で，HCTの臨床現場においてMSCsが用いられるのは，①生着促進，②組織修復，③GVHD抑制を含む過剰免疫反応抑制を目的とした場面である[4]．

まず，MSCsはhematopoietic stem cells（HSCs）nicheにおいて重要な役割を果たす．骨髄のnicheには，古典的にはendosteal nicheとperivascular nicheがあり，前者はHSCsのつなぎ止め，維持，休止期制御に関与し，後者の場には活性化し自己複製するHSCsが認められるとされる．Nestin陽性MSCsとそこから派生したCXCL12-abundant

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表1 MSCsの発現する分子

表面抗原				
	陽性			
	CD73	CD90	CD105	
	陰性			
	CD34	CD45	他のリンパ球マーカー	
	MHC class II	CD40	CD80	CD86
分泌する生理活性物質				
	恒常的に産生			
	IL-6, IL-7, IL-8, IL-11, IL-12, IL-14, IL-15, M-CSF, FLt-3 ligand, stem cell factor, stromal derived factor			
	刺激後に産生			
	IL-1a, LIF, G-CSF, GM-CSF			
	ケモカインリガンド			
	CCL2, CCL4, CCL5, CCL20, CXCL1, CLCL8			

reticular(CAR) cellsはHSCsの機能を制御する分子，stem cell factor(SCF)，VCAM1などを発現し，さらに骨芽細胞などにも分化する[5)~7)]．

免疫系に対するMSCsの作用機序は多岐にわたる．獲得免疫系ではCD4$^+$，CD8$^+$エフェクター細胞の増殖を抑制し[8)9)]，TH1/TH2への偏りを是正する一方[10)11)]，調節性T細胞を誘導するとされている[10)12)](表2)．HSC nicheに存在するような休止期T細胞の生存を支持し，一方活性化T細胞の増殖は抑制するとともにアポトーシスを誘導するとのデータがある[8)13)14)]．

自然免疫系ではいわゆる抑制性マクロファージと呼称されるM2マクロファージを誘導し，それにはMSCsによるCOX2発現→PGE_2産生，IDO発現→Kynurenine産生が重要とされている．誘導されたM2マクロファージは活性化T細胞を抑制し，調節性T細胞を誘導する(表3)[11)15)16)]．

これらの造血促進作用，免疫抑制あるいは免疫調節作用から，HCTにおいて当初は自己由来MSCsで，次いで第三者のMSCsで治療を展開しようとする試みは理に適っているように思われる．作用機序についてはすべてに触れることは困難なため，以後の項にも織り込みつつ話を進めたい．

MSCsの臨床効果

1．生着促進効果

GVHD治療の観点からは副次的な内容であるが，治療に際して留意すべき事項でもあり(GVHD治療によりドナー免疫細胞を抑制し，ホストによる拒絶に至ることも稀ではない)，生着促進に対する作用につき最初に紹介する．MSCsはHSCsの増殖促進に加えて，HSCsおよびnicheへの損傷回避に関与しているのではとされている．実際にin vitro, in vivoともにMSCsにより造血能が増強することが証明されている[5)6)17)]．臨床の場では臍帯血移植やハプロ移植などでMSCsとの共移植により，よりよい生着が得られたとの報告があり，また部分的生着(混合キメラ)となった患者において2年後にハプロ一致MSCsを移植したところ，完全キメラとなり造血能が回復したとの報告もある[18)~20)]．一方，MSCsとの共培養でHSCsを増幅する方法もとられている．共培養でCD34陽性細胞は30倍近くまで増幅することが可能で，2つの臍帯血ユニットを移植する際に，片方の臍帯血を増幅し共に移植すると，好中球の生着(median)が24日→15日に，血小板の生着が49日→42日になったと報告されている[21)]．興味深いことに，54%では操作を加えなかった臍帯血の方が生着し，46%では両者が生着，6か月後に増幅した臍帯血は13%でのみ検出され，majorな集団とはならなかったとされている．

2．急性GVHD(aGVHD)に対する効果

GVHDに対しての治療は2004年のLe Blancによるパイオニア的論文が最初の報告である[22)]．通常のaGVHD治療に反応のなかったgrade IV GVHDに対して第三者(母親)のMSCs(2×10^6/kg)を投与し，特に消化管や肝臓のGVHDに対してはめざましい治療効果をあげた．この白血病患者ではGVL効果を誘導する目的でシクロスポリン(CyA)

表2　MSCsと獲得免疫

細胞間相互作用	
T細胞	
	1) $CD4^+$, $CD8^+$ T細胞の増殖，IFN-γ産生，細胞傷害活性の抑制
	2) NKT細胞，γδ T細胞の増殖と機能の抑制
	3) TH1/TH2バランスの是正(TH1優位，TH2優位の病態の改善)
調節性T細胞	
	4) 調節性T細胞の誘導 $FoxP3^+$Tregの誘導，IL-10/TGF-β産生細胞の誘導，CD8 Tregの誘導
樹状細胞	
	5) $CD34^+$造血幹細胞や単球からのDCへ分化の阻害
	6) DCの抗原提示，共刺激分子発現，リンパ節移動の阻害
B細胞	
	7) B細胞反応の阻害 細胞増殖，分化，免疫グロブリン産生，走化能の阻害
液性因子	
	IL-10, TGF-β, galectin 1, galectin 3, LIF, PGE_2など

を中止したところGVHDが再燃し，CyAの再開とMSCsの二度目の輸注を必要とした．実際にその施設ではgrade IV GVHDを起こした過去25名の中で，唯一の生存者となったという報告である．患者生着リンパ球は母親由来のMSCsに対して反応(増殖)せず，一方患者リンパ球のアロMLRは母親由来のMSCsの添加により90％以上抑制された．

この結果を受けてEuropean Blood and Marrow Transplant MSC Consortiumは非ランダム多施設研究を実施した[17]．ここでは25名の小児患者と30名の成人患者がエントリーされ，ステロイド抵抗性aGVHD(grade II 5名，III 25名，IV 25名)に対して骨髄由来MSCs(HLA一致同胞，ハプロ一致親族，あるいは第三者由来)が投与された．投与量(中央値)は1.4×10^6 cells/kgで，27名は単回投与，28名では複数回投与を行った．第1回投与後の効果は70％で認められ(30名がCR，9名がPR)，効果を示すまでの中央値は18日であった．39名のうち19名は以後6週まで効果の持続を認めた．第1回投与に反応した群は1年後の治療関連死亡率が有意に低く(37％ vs 72％，$P=0.002$)，また2年生存率も高いこと(53％ vs 16％，$P=0.018$)が明らかになった．GVHDのgradeとの関連では30名のII, III aGVHDでは22名が，25名のIV aGVHDでは17名が反応しており，II, IIIとIVの間に有意差は認めなかった．また，小児患者は成人に比べて反応が良好で，小児では17/25名が，成人では13/30名がCRとなった．輸注後の生存率も成人に比べて高いことが示されている(これ以降の研究の大半でも小児における良好な成績を指示する結果が得られている)．大半の症例では第三者からのMSCsを輸注されており，統計学的処理はないもののMHC適合は不要であることを強く示唆している．解析した時点で21名が生存中である．55名の患者の最終的な死因の第一はaGVHDで18名であった．3名は腫瘍の再発で，1名は新たなレシピエント由来のAMLを発症し，最終的に死亡に至った．また，MSCsに反応した患者のうち9名は感染症(クレブシエラ，大腸菌，緑膿菌，アデノウイルス，水痘・帯状疱疹ウイルスなど)で亡くなっている．

第三者からのMSCsのみ(Prochymal®)を用いて59名の小児患者でステロイド抵抗性aGVHDに対するphase II 臨床試験も行われている[23]．この試験では週に2回，2×10^6 cells/kgのMSCsを4週間(計8回)投与され，PRを示した患者では維持療法として追加で4週間，週1回投与(4回の追加)が行われた．大半の患者は重症の腸管あるいは肝臓のGVHDであり，中央値では3.2種類のaGVHD治療に不応であった．28日目における反応率(臓器症状が少なくとも1 stage低下し，その他の臓器病変の悪化なし)は64％であった．治療反応を認めた患者では100日目の生存率に大きな差を認めた(76％ vs 9％)．その後の同様の臨床試験でも同様の結果が認められ，それぞれ既

表3　MSCsと自然免疫

単球，マクロファージ	
1)	微生物や炎症に反応してCCL3, CSCL2, CCL12分泌→M1マクロファージのリクルート
2)	過剰炎症反応の抑制(TSG6の分泌)
3)	COX2, IDO(indoleamine 2,3-deoxygenase)の発現増強
4)	PGE_2, kynurenineの産生→M1マクロファージからM2マクロファージへの転換
5)	M2マクロファージによる免疫抑制(Treg誘導，effector T抑制，IL-10産生など)
好中球	
6)	TLR刺激によるIL-6, IL-8, GM^CSM, MIFの分泌→好中球のリクルート
NK細胞	
7)	未刺激NKではIL-2, IL-15による増殖を抑制
8)	IL-2/IL-15で刺激されたNK細胞には細胞傷害活性の抑制
9)	HLA-G5分泌→NK細胞の抑制性受容体刺激
TH17細胞	
10)	naïve Tからの新規TH17細胞誘導の抑制
11)	アポトーシス細胞と培養時にはTH17細胞の誘導
	MSC自体はIL-17Rを高発現し，IL-17により増殖

存の治療に比べて有効で，また明らかな有害事象がなかったことを提示している．

未治療のGVHDに対してはどのような成績を示すであろうか？ KebriaeiらはII～IV aGVHDに対してProchymal®を2(あるいは8)×10^6 cells/kgの用量で通常量のステロイドとともに用いた[24]．対象は32名の成人患者でMSCsは2度投与され，また投与後はGVHD予防としてTacrolimus, CyAあるいはMMFが継続投与された．31名の評価可能患者において94%で反応が認められ(24名がCR，5名がPR)，CRを示した24名のうち19名ではその後最低90日はGVHDの悪化を認めなかった．ステロイド単独の既報告よりも高い反応性を示している．ここでは2つの異なる用量を用いているが，優劣を比較するような試験デザインとはなっていない．しかし，2群での差は認めず，2×10^6 cells/kgで効果を示すことは他の試験からも明らかであろう．

これらの結果を受けてその後Prochymal®はphase III臨床試験にまで入り2つのスタディーはエンドポイントに達しなかったものの，MSCsの効果は検証されつつあり，日本においても臨床試験が走っている．特に肝臓や腸管GVHDに効果を示す結果が提出されている．

3．生着促進とaGVHDの予防

さらに，生着促進とGVHD予防(軽減)を目的とした臨床研究も行われている[25]．20名の造血系腫瘍患者でHLA-mismatched非血縁ドナーからの骨髄非破壊的前処置(フルダラビン＋2Gy TBI)・末梢血幹細胞移植においてMSCsを末梢血幹細胞移植の30～120分前に輸注された．19名で生着を認めた．100日目のII～IV GVHD頻度は35%で，慢性GVHD(cGVHD)の頻度は65%であった．GVHDの頻度には有意差を認めなかったが，1年時の再発以外による死亡率は10%，再発は30%，全生存率は80%，GVHDおよびGVHD＋感染症による死亡は10%で，いずれも同様の骨髄非破壊的前処置・HLA非合致末梢血幹細胞移植の成績よりも良好であったと報告されている．

4．cGVHDに対する効果

cGVHDに対する報告はaGVHD治療に比べて少ない．3名の小児cGVHDでの投与経験では，1例で移植後7か月目に3×10^6 cells/kgで投与を受け，さらに26か月目に投与されて若干の改善を認めたとの報告がある[26]．Le Blancの施設からは皮膚苔癬と肝機能異常を示すcGVHD患者に移植後153日目に1×10^6 cells/kgのMSCsが投与され，皮膚症状の改善はない一方，肝酵素は改善したと報告されている．患者はその後EBVによる移植後リンパ増殖症候群で亡くなっている[27]．Zhouらは4名の皮膚硬化症を示す治療抵抗性cGVHDに使用し，投与後徐々に改善を認めたとしている[28]．さらに，最近では19名の治療抵抗性cGVHDにMSCsが使用され(中央値：0.6×10^6 cells/kg)，14名(74%)で反応(CR 4名，PR 10名)を認めたとしている．8名では第1回投与に反応があり，

反応までの中央値は29日であった．cGVHDの治療効果判定は難しいがNIH基準が用いられている．皮膚での反応は78％であった．また，最終的な2年生存率は78％で，cGVHDのない対照群に比べて遜色のないデータを得ている．眼病変，口腔病変や腸管，肝臓cGVHDに対する効果は示されているが，呼吸器系に対する情報はいまだに乏しい．

cGVHDではB細胞が重要な働きを果たすとされている．表2に示すようにMSCsはB細胞に対しても直接的な増殖抑制やIgG産生抑制効果を示すことが示されている．しかし，実際にはB-T-DC相互作用の中で決定される抗体産生，自己抗体産生に対する効果についてはこれからモデル動物系などでの検証が必要であろう．

5．GVHDに合併するその他の病変に対する効果

移植後にはしばしばGVHDとともに出血性膀胱炎や腸管出血などを伴うことがある．MSCsはこれらの病態に対しても有効なことがあり，重症出血性膀胱炎では8/12名で投与後1～14日（中央値3日）に肉眼的血尿が消失したとの報告がある．まだ症例数が少ないが腸管穿孔や大量の小腸出血に対しても有効性を示した報告が認められる[28]．

MSCsの有害事象

今までの大半の研究からはMSCsは第三者のMSCsであっても安全に投与可能であることが示されている．実際にアロ反応惹起や，投与自体でひき起こされる有害な免疫応答に対する懸念はほぼ払拭されている．重篤な有害事象についての報告もないが，一方その免疫調節（免疫抑制）効果から，原疾患（特に白血病）再発を惹起する危険性や，ウイルス感染症を含む日和見感染症が増加する危険性が指摘されている．実際にはGVHDに対する免疫抑制薬はどのようなものであっても強力になるにつれて（特異的なものがない限り），原疾患再発や日和見感染症の頻度を高くする可能性が高く，比較検討は困難であろう．この中で今まで報告されている論文について簡単に紹介する．

1．病原体特異的T細胞反応への影響

MSCsは骨髄微小環境にある休止期T細胞の生存を支持し，一方活性化T細胞の増殖は抑制する（アポトーシスを誘導する）とされている．末梢血ではMSCsはナイーブ，記憶T細胞の両者の増殖を抑制する．CTLに対してはアロ抗原誘導増殖に対しては抑制効果を示す一方，ウイルス抗原に対する増殖には影響を与えず，またウイルス抗原刺激後のIFN-γ産生にも影響を与えない．MSCsとの共培養系においてはウイルス特異的CTL株の増殖，IFN-γ産生，標的細胞への細胞傷害活性を抑制しない．実際にaGVHDに対してMSCsを投与した2名の小児患者でCMV特異的T細胞反応を検証したところ，抑制は認めなかったようである．さらに，MSCsは臨床的に問題となるさまざまな病原体に対して防御的に働くことも示されている[13]．

最近の論文では臍帯血移植でMSCsを生着促進目的で投与した群では，移植後6,9か月後のT cell receptor excision circles（TRECs：T細胞新生能を反映）が低く，また9か月後のIgMレベルが低かったと報告されている[29]．これらが低値であった群はMSCs非投与群に比べて長期生存率が低下していた．MSCsは胸腺上皮に対する影響もあり，ナイーブT細胞のpositive selectionに際しても影響を与え，胸腺からのT細胞新生に影響を与えるのではという推察がなされている．ここで注意すべき点は生着促進目的で投与されていることであり，なんらかの免疫抑制薬が必要なGVHDなどの状況とは異なる点である．ステロイド単独でもTRECsは激減することはよく知られており，免疫抑制薬投与群とMSCs投与群での比較が必要であろう．

一方，691名の造血細胞移植後患者を後方視的に解析した論文では，55名がaGVHDや出血性膀胱炎などに対してMSCs治療が行われており，投与を受けた患者では多変量解析により有意に肺炎による死亡が多かったことが示されている[30]．その他の因子としてはII～IV aGVHDがCMV感染症との関連性が認められた．明らかにGVHDは感染症のリスクになり，またMSCsで治療を受けた群は生存が延長し，それが逆に感染症による死亡率を上げたのではというとらえ方もできる．しかし，臍帯血移植後に生着促進を目的にMSCs治療を受けた患者では有意にTRECsが低いとの報告がある．また，ステロイド抵抗性GVHDに

MSCsを投与した群では，非投与群に対して有意に真菌感染症が多いようである．いずれにせよMSCsを用いる際には適切な感染症モニタリングと治療が必要である．

2．原疾患再発への影響

白血病再発に対する影響の評価は難しい．生着促進とGVHD予防を目的でMSCsが骨髄あるいは末梢血幹細胞とともに投与された症例(10名)では非投与群(15名)に比べて，aGVHD, cGVHDの頻度は低かったものの，再発はそれぞれ6名，3名であったとの報告がある[31]．いずれにせよGVHD治療に用いられる免疫抑制薬とMSCsの白血病再発に対する影響の比較は，GVHDが白血病再発の抑制と相関する疾患群の存在などからも，疾患や移植源を層別化した大規模な研究が必要になるものと思われる．現実的に重症GVHDが発症した際に治療の選択は乏しく，GVHD治療に関しては現時点でMSCsの懸念材料とはならないという印象が強い．

どのMSCsを選ぶべきか（MSCsの多様性）

MSCsの作用機序やGVHDに対する治療効果などにつき概説してきたが，今後まだいくつか基礎的にも臨床的にも詰めていくべき課題が残されている．至適投与量については少なくとも$1\sim2\times10^6$ cells/kg以上は必要がなさそうである．一方，MSCsは多様であり，その由来や調製方法により性質が大きく異なることが指摘されている[4)8)28)]．

MSCs作製に用いる細胞の由来については現在，骨髄，脂肪細胞，臍帯などがあるが，それ自体の特性に差があり，どの細胞がGVHD抑制に効果があるのかは検証されていない．MSCs作製にあたり用いる培地によっても，また添加する血清(ウシ胎児血清，濃厚血小板血漿，無血清)によっても差異が生じる．何回継代したものがベストなのかも不明である[4)8)28)]．

さらに，作用機序に立ち返れば，MSCsの効果が直接的なのか間接的なのかも明らかになっていない．GVHD治療においての検証では少なくとも局所でMSCsドナー細胞のDNAが検証されるとの報告があり，局所へのMSCsの到達は重要に思われる．一方，MSCs(あるいは分化細胞)のみならずDNAさえ検証されないという症例もありこの場合の解釈(局所への到達は少なくとも重要なのかどうかの判断)は難しい．

最近のHorwitzらの知見によればMSCsから分泌される因子が重要であり，secretome解析によってそれが明らかになりつつあるということである．彼らは分泌因子の中でもexosomeが最も重要であり，そのprofileは由来細胞，調製方法，調製試薬により大きく影響を受け，さらには培養容器によっても異なっているとしている(私信)．

将来展望

骨髄由来MSCsの寿命は短いためにiPS細胞から効率よくMSCsを作製し均一な質を有するMSCsを作製しようとする試みがある[32)]．実際にこのようにして作製したiPS-MSCsはNK細胞の増殖や細胞傷害活性を抑制し，またiPS-MSCsはBM-MSCsに比べてNK細胞による細胞傷害に抵抗性であったという．現在はすでに各国でProchymal®という製剤が開発されているが，将来的には機能を最適化したMSCsが市場に出る可能性があり，iPS-MSCsは多くの中の一つの候補と思われる．

MSCsは明らかにGVHDコントロールに有用であるが，最大の懸案事項はやはり免疫抑制による感染症リスクの増加であろう．「炎症によるMSCのライセンシング(inflammatory MSC licensing)という概念があり，その強弱によりMSCsが免疫抑制にも強化にも働くとされている．実際に炎症性サイトカインであるIFN-γ, TNF-α, IL-1などによりMSCsはCOX2, IDO, iNOSの発現が高くなり，T細胞抑制機能をフルに装備するようになり，一方より低いレベルのIFN-γはclass II MHCの発現を誘導し，MSCsを活性型にするという[15)33)~35)]．一方，MSCsはTLR1～10を発現しており，TLRやNLRなどのパターン認識受容体(PRR)刺激によりMSCsの可塑性を誘導できるという論文も多く，特定のPRR刺激(＋炎症性サイトカイン)によってMSCsを活性型にも抑制型にも変化させることができる可能性がある．

また，MSCs自体に抗菌活性があるという論文も認められる．抗菌ペプチドであるLL-37を分泌したり，ウイルス感染症を感知してIFN-γ, IL-2,

IL-15を産生したりするとされている[36]．また，BM-MSCsはCMVやParvovirus B19に感染し，in vitroでは感染MSCsは特異的T細胞免疫応答を誘導する[8]．

作用機序を含め不明な点が多いMSCsであるが基礎的検討から均一な特性を有するMSCsを誘導できるようになれば，GVHDへのより有効かつ安全な治療法が確立するものと思われる．

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Brief Article

Effect of ABO Blood Group Incompatibility on the Outcome of Single-Unit Cord Blood Transplantation after Myeloablative Conditioning

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A B S T R A C T

ABO blood group incompatibility between donor and recipient has been associated with poor transplant outcomes in allogeneic hematopoietic stem cell transplantation. However, its effect on the outcome of cord blood transplantation (CBT) has yet to be clarified. We retrospectively analyzed 191 adult patients who received single-unit CBT after myeloablative conditioning for malignant disease in our institute. Major mismatch showed a significantly lower incidence of platelet engraftment compared with ABO match as a reference (hazard ratio, .57; $P = .01$). Nevertheless, there was no increase in graft-versus-host disease, transplant-related mortality, and overall mortality after ABO-incompatible CBT. These data suggested that donor–recipient ABO incompatibility does not have a significant impact on outcome after myeloablative CBT for hematological malignancies.

INTRODUCTION

In contrast to solid organ transplantation, ABO blood group incompatibility between donor and recipient is reportedly a more common situation after allogeneic hematopoietic stem cell transplantation (allo-HSCT). It is well known that ABO incompatibility of allo-HSCT can cause an increased risk of delayed erythroid reconstitution, pure red cell aplasia, and acute and delayed hemolysis [1,2]. However, the association between ABO incompatibility and transplantation outcomes, such as neutrophil and platelet recovery, graft-versus-host disease (GVHD), and survival, is controversial [1,2]. Moreover, most of these studies analyzed patients receiving allo-HSCT using bone marrow or mobilized peripheral blood as a stem cell source from related and unrelated donors [1-5].

Cord blood transplantation (CBT) from an unrelated donor is increasingly used as an alternative transplant method for adult patients without HLA-compatible related or unrelated donors. Although most patients receive an HLA-mismatched cord blood unit, the lower risk of GVHD without compromising graft-versus-leukemia effects is one of the most attractive advantages of CBT. We previously reported that ABO incompatibility influenced platelet engraftment and transfusion requirement of RBCs and platelets in CBT [6]. However, the effects of ABO incompatibility on GVHD and survival after myeloablative CBT are limited. In the present study, we analyzed the neutrophil and platelet recovery, GVHD, transplant-related mortality (TRM), relapse, and survival in myeloablative CBT in adult patients with malignant disease in our institute.

METHODS

This retrospective study included data from 191 adult patients who underwent unrelated first allogeneic transplantation using single-unit CBT at our institute between August 1998 and February 2013. Donor–recipient ABO compatibility was categorized as follows: ABO match in 55 patients, major mismatch in 47, minor mismatch in 58, and bidirectional mismatch in 31. All patients received 12 Gy total body irradiation (TBI)-based myeloablative conditioning regimens and cyclosporine with or without short-term methotrexate as a GVHD prophylaxis, and cord blood units were selected as reported previously [7,8]. The institutional review board of the Institute of Medical Science, The University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

The primary study endpoint was overall survival (OS), defined as the time from the date of transplantation to the date of death or last contact. Secondary endpoints were relapse, TRM, GVHD, and neutrophil and platelet recovery. Relapse was defined by morphologic evidence of disease in peripheral blood, bone marrow, or extramedullary sites. TRM was defined as death during a remission. Both acute GVHD (aGVHD) and chronic GVHD (cGVHD) were graded according to previously published criteria [9,10]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days. Neutrophil engraftment was defined as being achieved on the first of 3 consecutive days during which the absolute neutrophil count was at least 0.5×10^9/L. Platelet engraftment was defined as being achieved on the first of 3 days when the platelet count was higher than 50×10^9/L without transfusion support.

Baseline patient and transplant characteristics were compared using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. The probability of OS was estimated according to the Kaplan-Meier method, and groups were compared using the log-rank test. The probabilities of the others were estimated based on a cumulative incidence method to accommodate competing risks. Multivariate analysis was

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performed with a Cox proportional hazard model adjusted for OS, and a Fine and Gray proportional hazards model for the others.

The following variables for multivariate analysis were considered: age (<45 versus ≥45 years), disease status at CBT (standard risk versus high risk), cord blood nucleated cell count (<2.5×10^7 versus ≥2.5×10^7/kg), cord blood CD34 + cell count (<1×10^5 versus ≥1×10^5/kg), HLA disparities based on antigen level HLA-A and -B and allele level HLA-DRB1 (1 versus 2 versus ≥3), sex compatibility between donor and recipient (female donor to male recipient versus other), year of CBT (1998 to 2005 versus 2006 to 2013), and ABO compatibility between donor and recipient (match versus major mismatch versus minor mismatch versus bidirectional mismatch). The ABO match was considered the reference group in the multivariate analyses.

All statistical analyses were performed with EZR, a graphic user interface for R 2.13.0 [11]. $P < .05$ was considered significant. Analysis of data was performed in August 2013. The median follow-up of surviving patients was 92 months (range, 5 to 181) after CBT in the entire cohort.

RESULTS

The characteristics of patients and cord blood units are shown in Table 1. There were no significant differences among the 4 groups, except for HLA disparities. The major mismatch group contained a slightly higher number of HLA disparities as compared with the minor mismatch group ($P = .07$) or the bidirectional mismatch group ($P = .08$), although these were not statistically significant.

The probability of OS at 5 years significantly differed among the 4 groups in univariate analysis ($P = .03$) (Figure 1A). However, multivariate analysis of mortality adjusting for other variables showed no significant difference between ABO match and major (hazard ratio [HR], 1.20; $P = .62$), minor (HR, .72; $P = .41$), or bidirectional (HR, 1.76; $P = .14$) mismatch (Table 2). In univariate analysis, ABO incompatibility was not associated with cumulative incidence of TRM (Figure 1B) or relapse (Table 2). In multivariate analysis, a trend toward a higher incidence of TRM was observed in the major mismatch compared with the match group, but this was not significant ($P = .05$).

In univariate analysis, there was no significant difference in the cumulative incidence of grades II to IV aGVHD among the 4 groups ($P = .91$) (Figure 1C). In multivariate analysis, a higher number (≥3) of HLA disparities (HR, 1.56; 95% confidence interval [CI], 1.05 to 2.32; $P = .02$), a higher cord blood CD34 + cell count (HR, 1.51; 95% CI, 1.05 to 2.18; $P = .02$), and older year of CBT (HR, 1.85; 95% CI, 1.30 to 2.65; $P < .01$) were associated with a higher incidence of grades II to IV aGVHD, but ABO incompatibility was not associated with the incidence of grades II to IV aGVHD (Table 2). The cumulative incidence of grades III to IV aGVHD significantly differed among the 4 groups in univariate analysis ($P = .02$). However, multivariate analysis adjusting for other variables showed no significant difference in the cumulative incidence of grades III to IV aGVHD between ABO match and major (HR, 2.56; $P = .19$), minor (HR, .59; $P = .56$), or bidirectional (HR, 1.46; $P = .67$) mismatch (Table 2). In univariate analysis, there was no significant difference in the cumulative incidence of extensive cGVHD among the 4 groups ($P = .86$) (Figure 1D). In multivariate analysis, older age (HR, 1.85; 95% CI, 1.06 to 3.23; $P = .03$) and female donor to male recipient (HR, 1.79; 95% CI, 1.02 to 3.15; $P = .04$) were associated with a higher incidence of extensive cGVHD, but ABO incompatibility was not associated with the incidence of extensive cGVHD (Table 2).

Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

	Total	Match	Major Mismatch	Minor Mismatch	Bidirectional Mismatch	P
Number (%)	191	55 (28)	47 (24)	58 (30)	31 (16)	
Age, yr, median (range)	40 (16-55)	40 (16-55)	40 (16-53)	40 (16-53)	41 (18-52)	.94
Disease type, n (%)						.61
AML	101 (52)	30 (54)	24 (51)	30 (51)	17 (54)	
ALL	45 (23)	17 (30)	10 (21)	11 (18)	7 (22)	
MDS	25 (13)	5 (9)	5 (10)	10 (17)	5 (16)	
CML	11 (5)	1 (1)	4 (8)	4 (6)	2 (6)	
NHL	9 (4)	2 (3)	4 (8)	3 (5)	0 (0)	
Disease status at CBT,* n (%)						.09
Standard risk	79 (41)	24 (44)	17 (36)	30 (51)	8 (25)	
High risk	112 (58)	31 (54)	30 (64)	28 (48)	23 (74)	
Conditioning regimen, n (%)						.36
TBI12Gy+Ara-C/G-CSF+CY	131 (68)	34 (61)	33 (70)	40 (68)	24 (77)	
TBI12Gy+Ara-C+CY	31 (16)	9 (16)	11 (23)	9 (15)	2 (6)	
TBI12Gy+CY	16 (8)	6 (10)	1 (2)	5 (8)	4 (12)	
TBI12Gy+others	13 (6)	6 (10)	2 (4)	4 (6)	1 (3)	
GVHD prophylaxis, n (%)						.10
Cyclosporine A + methotrexate	188 (98)	55 (100)	47 (100)	57 (98)	29 (93)	
Cyclosporine A	3 (1)	0 (0)	0 (0)	1 (2)	2 (6)	
Number of nucleated cells, $\times 10^7$/kg, median (range)	2.43 (1.32-5.69)	2.52 (1.32-5.50)	2.47 (1.65-4.92)	2.38 (1.51-5.69)	2.58 (1.65-5.07)	.79
Number of $CD34^+$cells, $\times 10^5$/kg, median (range)	.92 (.17-7.75)	.88 (.28-3.15)	.93 (.17-1.99)	.91 (.28-7.75)	1.14 (.44-2.84)	.20
HLA disparities,† n (%)						.05
1	23 (12)	4 (7)	7 (14)	8 (13)	4 (12)	
2	106 (55)	32 (58)	16 (34)	37 (63)	21 (67)	
3	57 (29)	17 (30)	23 (48)	12 (20)	5 (16)	
4	5 (2)	2 (3)	1 (2)	1 (1)	1 (3)	
Sex compatibility, n (%)						
Female donor to male recipient	58 (30)	19 (34)	13 (27)	17 (29)	9 (29)	.88
Other	133 (69)	36 (65)	34 (72)	41 (70)	22 (70)	
Year of CBT, n (%)						.58
1998-2005	102 (53)	28 (50)	22 (46)	33 (56)	19 (61)	
2006-2013	89 (46)	27 (49)	25 (53)	25 (43)	12 (38)	

AML indicates acute myelogenous leukemia; ALL, acute lymphoblastic leukemia, MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; NHL, non-Hodgkin lymphoma, Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide.

* For disease status at CBT, patients in complete remission (CR) 1 or CR2 without poor prognostic karyotype for AML and ALL, refractory anemia for MDS, chronic phase for CML, and CR1 or CR2 for NHL were classified as standard risk, whereas patients in all other situations were classified as high risk.

† The number of HLA disparities defined as low resolution for HLA-A and -B and high resolution for HLA-DRB1.

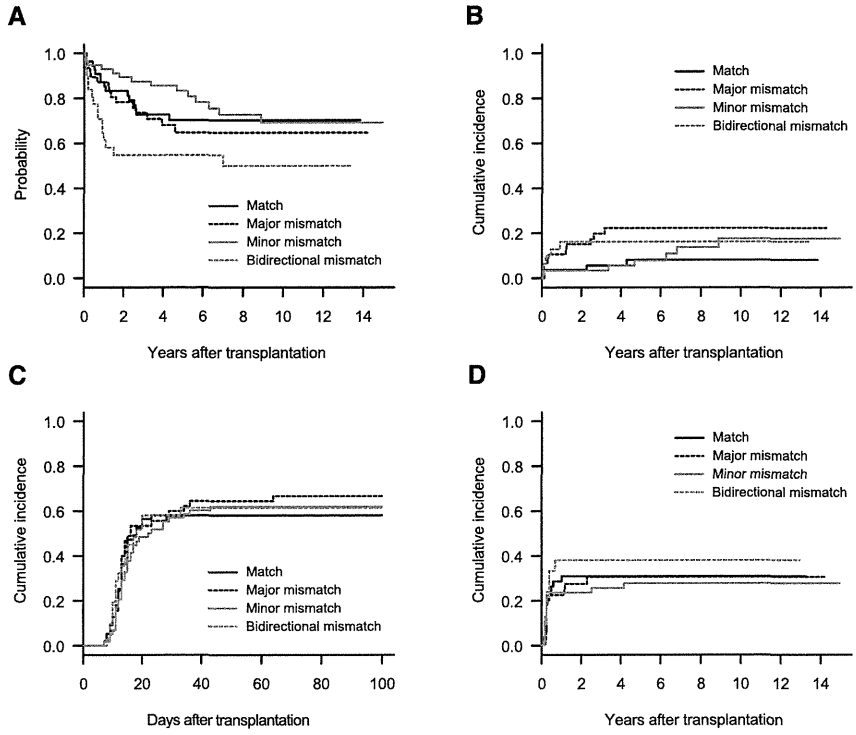

Figure 1. Probability of OS (A), cumulative incidence of TRM (B), grades II to IV aGVHD (C), and extensive cGVHD (D) according to donor–recipient ABO incompatibility after myeloablative CBT.

ABO incompatibility was not associated with cumulative incidence of neutrophil engraftment among the 4 groups in univariate analysis ($P = .73$). In multivariate analysis, a lower cord blood CD34 + cell count (HR, .51; 95% CI, .37 to .70; $P < .001$), high risk of disease status at CBT (HR, .68; 95% CI, .50 to .93; $P = .01$), and older year of CBT (HR, .71; 95% CI, .53 to .96; $P = .02$) were associated with a lower incidence of neutrophil engraftment, but ABO incompatibility was not associated with neutrophil engraftment (Table 2). The cumulative incidence of platelet recovery was not significantly different among the 4 groups in univariate analysis ($P = .30$). In multivariate analysis, major mismatch (HR, .57; $P = .01$) showed a significantly lower incidence of platelet engraftment when compared with ABO match (Table 2). In addition, a lower cord blood CD34 + cell count (HR, .63; 95% CI, .45 to .88; $P < .01$), lower cord blood nucleated cell count (HR, .70; 95% CI, .52 to .94; $P = .01$), and high risk of disease status at CBT (HR, .65; 95% CI, .45 to .94; $P = .02$) were associated with a lower incidence of platelet engraftment.

We also analyzed the effect of major/bidirectional mismatch group defined as combined group of major and bidirectional mismatch. However, we were unable to find any impact of major/bidirectional mismatch on outcomes in multivariate analysis, except for platelet engraftment (Supplemental Table 1).

DISCUSSION

The ABO blood group antigens consist of oligosaccharide glycoproteins and are expressed not only in erythrocytes but also in neutrophils, platelets, and, vascular endothelial and epithelial cells. The ABO antigens could be immunological targets for ABO-incompatible donor or recipient lymphocytes, affecting GVHD and engraftment. Many previous studies have reported an increased risk of aGVHD after ABO-incompatible allogeneic bone marrow transplantation from related and unrelated donors, particularly in minor and bidirectional mismatch [3-5]. Igarashi et al. [12] reported an association between the anti-host isohemagglutinin produced by donor-derived B lymphocytes and the development of aGVHD after minor and bidirectional mismatched allogeneic bone marrow transplantation and peripheral blood stem cell transplantation from related and unrelated donors. These effects might be associated with ABO-incompatible immune responses against ABO antigens in vascular endothelial and epithelial cells of recipients. However, it has been reported that donor-derived isohemagglutinin was not identified in patients after minor and bidirectional mismatched CBT [12,13]. The higher proportion of naïve B lymphocytes in cord blood grafts might contribute to defective isohemagglutinin production after ABO-incompatible CBT, which might have contributed to the low incidence of severe GVHD even after ABO-incompatible CBT. Therefore, the effect of ABO incompatibility on transplant outcome might differ depending on the kinds of stem cell sources in allo-HSCT.

Several studies have reports on associations between ABO incompatibility and outcomes after CBT [14-19]. Romee et al. [14] reported no impact of ABO incompatibility on aGVHD and cGVHD in 503 CBT recipients. Berglund et al. [15] reported an increased incidence of grades II to IV aGVHD in major mismatch recipients (n = 23) of CBT. Moreover, previous studies demonstrated lower survival for major mismatch recipients of single-unit CBT [16,17], whereas other studies did not [14,18,19]. However, these studies included a relatively heterogeneous group of patients receiving single or double CBT after reduced-intensity or myeloablative conditioning regimen. In most of these studies, 3 groups of ABO mismatch, namely, major, minor,

Table 2
Univariate and Multivariate Analysis of ABO Compatibility for the Outcomes of CBT

	Univariate Analysis			Multivariate Analysis		
	Number	Percent (95% CI)	*P*	HR	95% CI	*P*
OS*		At 5 yr	.03			
Match	55	70.2 (55.3-81.0)		1.00		Reference
Major mismatch	47	64.8 (48.0-77.3)		1.20	.57-2.50	.62
Minor mismatch	58	83.2 (70.1-90.9)		.72	.33-1.57	.41
Bidirectional mismatch	31	54.6 (35.7-70.1)		1.76	.82-3.77	.14
Relapse†		At 5 yr	.09			
Match	55	26.9 (15.6-39.6)		1.00		Reference
Major mismatch	47	15.8 (6.8-28.2)		.54	.20-1.42	.21
Minor mismatch	58	14.4 (6.6-24.9)		.54	.22-1.32	.18
Bidirectional mismatch	31	32.5 (16.7-49.3)		1.08	.43-2.71	.86
TRM‡		At 5 yr	.19			
Match	55	8.1 (2.5-18.1)		1.00		Reference
Major mismatch	47	22.2 (11.3-35.4)		3.19	.97-10.46	.05
Minor mismatch	58	7.9 (2.5-17.6)		1.34	.34-5.33	.67
Bidirectional mismatch	31	16.1 (5.7-31.2)		1.99	.49-8.03	.33
Grades II-IV aGVHD		At 100 d	.91			
Match	55	58.2 (43.9-70.1)		1.00		Reference
Major mismatch	45	66.7 (50.5-78.6)		1.06	.64-1.73	.81
Minor mismatch	58	62.1 (48.1-73.3)		1.11	.68-1.80	.66
Bidirectional mismatch	31	61.3 (41.4-76.2)		1.28	.73-2.24	.37
Grades III-IV aGVHD		At 100 d	.02			
Match	55	5.5 (1.4-13.7)		1.00		Reference
Major mismatch	45	20.0 (9.8-32.8)		2.56	.63-10.37	.19
Minor mismatch	58	3.4 (.6-10.7)		.59	.10-3.46	.56
Bidirectional mismatch	31	9.7 (2.4-23.2)		1.46	.25-8.44	.67
Extensive cGVHD		At 5 yr	.86			
Match	49	28.6 (16.7-41.6)		1.00		Reference
Major mismatch	40	30.5 (16.9-45.3)		1.18	.56-2.47	.65
Minor mismatch	55	27.9 (16.5-40.4)		1.24	.57-2.72	.58
Bidirectional mismatch	21	38.1 (17.8-58.3)		1.56	.67-3.63	.30
Neutrophil engraftment		At 60 d	.73			
Match	55	96.4 (83.6-99.2)		1.00		Reference
Major mismatch	47	92.6 (75.2-98.0)		.82	.56-1.20	.33
Minor mismatch	58	94.8 (83.3-98.5)		1.09	.78-1.53	.59
Bidirectional mismatch	31	88.7 (64.1-96.8)		1.06	.66-1.68	.80
Platelet engraftment		At 100 d	.30			
Match	55	88.9 (76.0-95.0)		1.00		Reference
Major mismatch	47	70.0 (53.6-81.6)		.57	.36-.90	.01
Minor mismatch	58	93.1 (81.2-97.6)		.92	.66-1.28	.64
Bidirectional mismatch	31	73.3 (51.5-86.4)		.78	.45-1.34	.37

* HR for overall mortality. In multivariate analysis, there were no significant variables, but there was a trend toward a higher mortality among those with a high risk of disease status at CBT (HR, 1.60; 95% CI, .88-2.89; $P = .11$) and female donor to male recipient (HR, 1.64; 95% CI, .94-2.85; $P = .07$).

† In multivariate analysis, there were no significant variables, but there was a trend toward a higher relapse among those with a high risk of disease status at CBT (HR, 1.71; 95% CI, .85-3.44; $P = .13$).

‡ In multivariate analysis, there were no significant variables, but there was a trend toward a higher TRM among those with female donor to male recipient (HR, 2.05; 95% CI, .87-4.81; $P = .09$).

§ In multivariate analysis, there were no significant variables, but there was a trend toward a higher incidence of grades III-IV aGVHD among those with a lower cord blood CD34 + cell count (HR, 2.75; 95% CI, .84-9.00; $P = .09$) and a high risk of disease status at CBT (HR, 3.98; 95% CI, .80-19.65; $P = .08$).

and bidirectional mismatch, were not evaluated separately. Of note, the advantage of our study is the relatively homogeneous adult patient population with hematological malignancies treated with single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens and a cyclosporine-based GVHD prophylaxis. Moreover, 3 groups of ABO mismatch were evaluated separately. Therefore, we were able to determine the potential effect of ABO incompatibility in CBT.

In conclusion, our data showed that ABO incompatibility affected the incidences of platelet engraftment but did not have a significant effect on the incidence of GVHD, relapse, TRM, and OS after CBT. These results should be interpreted with caution because this retrospective study included a relatively small number of Japanese patients who received single-unit CBT after 12 Gy TBI-based myeloablative conditioning regimens for hematological malignancies. Although these findings should be confirmed in large prospective studies, ABO incompatibility does not appear to have had a significant impact on the outcome after CBT in our study.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2013.12.563.

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Biol Blood Marrow Transplant 20 (2014) 396–401

Single-Unit Cord Blood Transplantation after Granulocyte Colony-Stimulating Factor–Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission

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A B S T R A C T

High disease burden in myeloablative allogeneic hematopoietic stem cell transplantation is associated with adverse outcomes in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). Quiescent leukemia stem cells could be induced to enter cell cycle by granulocyte colony-stimulating factor (G-CSF) administration and become more susceptible to chemotherapy. We report on the outcome of unrelated cord blood transplantation (CBT) using a conditioning regimen of 12 Gy total body irradiation, G-CSF–combined high-dose cytarabine, and cyclophosphamide in 61 adult patients with AML or advanced MDS not in remission. With a median follow-up of 97 months, the probability of overall survival and cumulative incidence of relapse at 7 years were 61.4% and 30.5%, respectively. In multivariate analysis, poor-risk cytogenetics and high lactate dehydrogenase values at CBT were independently associated with inferior survival. These data demonstrate that CBT after G-CSF–combined myeloablative conditioning is a promising curative option for patients with myeloid malignancies not in remission.

INTRODUCTION

The prognoses of patients with acute myelogenous leukemia (AML) and advanced myelodysplastic syndrome (MDS) who have not achieved remission after chemotherapy have been poor. Although allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only potentially curative therapy for such patients, high disease burden has been reported to be associated with increased relapse or poor survival rate after allo-HSCT [1-9]. Recently, cord blood (CB) has been considered an acceptable alternative as a source of hematopoietic stem cells in unrelated allo-HSCT for adult patients without HLA-identical related or unrelated donors [9-16]. In comparison with other sources of allo-HSCT, one of the main advantages of using CB for patients with a high disease burden who require urgent transplantation is its rapid and convenient availability. Because it was shown that administration of granulocyte colony-stimulating factor (G-CSF) increased the susceptibility of cell-cycle–specific agent cytarabine in leukemia cells in vitro [17], we administered G-CSF–combined high-dose cytarabine in myeloablative conditioning for allo-HSCT [18,19] and reported that a G-CSF–combined conditioning regimen provided better engraftment and survival results in cord blood transplantation (CBT) for myeloid malignancies [13-16]. The objective of this retrospective study was to confirm the effects of CBT after G-CSF–combined myeloablative conditioning in adult patients with myeloid malignancies not in remission and to identify variables influencing long-term outcomes.

PATIENTS AND METHODS

Patients and Transplantation Procedures

This retrospective study included 61 consecutive adult patients who underwent unrelated transplantation using single-unit CB for AML or advanced MDS not in remission at our institute between 1998 and 2013. Thirty-two patients were included in our previous study [15,16] and extended the follow-up. The diagnoses of AML and MDS were made according to the World Health Organization classification. Advanced MDS was defined as having refractory anemia with excess blasts type 1 or refractory anemia with excess blasts type 2 by World Health Organization classification. Myeloid malignancies not in remission were defined as more than 5% blasts in the bone marrow (BM), or circulating blasts in peripheral blood (PB) or central nervous system. The cytogenetic subgroups were defined according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML [20] and International Prognostic Scoring System criteria for MDS [21]. All patients received 12 Gy total body irradiation (TBI) in 4 divided fractions on days −8 and −7, cytarabine on days −5 and −4 (total dose 12 g/m2, and 3 g/m2 every 12 hours for 2 days) with 5 μg/kg G-CSF (lenograstim) from 12 hours before the first dose of cytarabine to the end of cytarabine dosing, and cyclophosphamide (total dose 120 mg/kg) on days −3 and −2 [15,16]. Fifty-eight patients received cyclosporine (CSP) (3 mg/kg/day) with a short course of methotrexate (15 mg/m2 on day +1 and 10 mg/m2 on days +3 and +6), and 3 patients received CSP only as graft-versus-host disease (GVHD) prophylaxis. CB units were obtained from the Japanese Cord Blood Bank Network. Donor-recipient HLA-matching status was based on antigen level HLA-A and -B and on allele level HLA-DRB1 typing. All patients received similar supportive care and CB units were

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Table 1
Characteristics of Patients, Cord Blood Units, and Transplantation

Characteristic	Value
No. of patients	61
Sex	
Male	36 (59)
Female	25 (41)
Age, median (range), yr	41 (18-55)
CMV serostatus	
Positive	54 (86)
Negative	7 (11)
Disease type	
De novo AML	24 (39)
AML secondary to MDS	24 (39)
Advanced MDS*	13 (21)
Cytogenetics†	
Good	1 (2)
Intermediate	27 (44)
Poor	30 (49)
Unknown	3 (5)
Bone marrow blasts at CBT, median (range), %	17.7 (1.4-86.0)¶
< 25%	39
≥ 25%	22
Peripheral blood blasts at CBT, median (range), %	6.5 (0-68.5)
Absent	12
Present	49
LDH at CBT	
≤ ULN	41 (67)
> ULN	20 (33)
Disease status at CBT‡	
Untreated	31 (51)
Primary refractory	14 (23)
Refractory relapse	16 (26)
Time from diagnosis to CBT, median (range), mo	7 (1-219)
Conditioning regimen	
TBI12Gy+Ara-C/G-CSF+CY	61
GVHD prophylaxis	
CyclosporineA+methotrexate	58 (95)
CyclosporineA	3 (5)
Number of nucleated cells, median (range), $\times 10^7$/kg	2.43 (1.32-5.50)
Number of CD34 cells, median (range), $\times 10^5$/kg	1.03 (.21-2.27)
HLA disparities§	
1	13 (21)
2	32 (52)
3	14 (22)
4	2 (3)

CMV indicates cytomegalovirus; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; TBI, total body irradiation; Ara-C, cytosine arabinoside; G-CSF, granulocyte colony-stimulating factor; CY, cyclophosphamide; GVHD, graft-versus-host disease; HLA, human leukocyte antigen.
Data presented are n (%) unless otherwise indicated.

* Advanced MDS are defined as having refractory anemia with excess blasts-1 (RAEB-1) or RAEB-2 by WHO criteria.

† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.

‡ Untreated was defined as no treatment before conditioning regimen, indicating that the majority of patients with AML secondary to MDS or advanced MDS received CBT as an up-front treatment. Primary refractory was defined as failure to achieve complete remission with induction chemotherapy. Refractory relapse was defined as failure to achieve complete remission with salvage chemotherapy after first or subsequent relapse.

§ The number of HLA disparities, defined as the low resolution for HLA-A and -B and the high resolution for HLA-DRB1.

¶ The 5 patients with less than 5% blasts in the bone marrow included circulating blasts in peripheral blood (n = 3) or central nervous system (n = 2).

selected, as previously reported [15,16]. The institutional review board of the Institute of Medical Science, University of Tokyo approved this study. This study was conducted in accordance with the Declaration of Helsinki.

End Points and Statistical Analysis

The primary study end point was overall survival (OS), defined as time from the date of transplantation to the date of death or last contact. Secondary end points were relapse, including disease progression before engraftment; transplantation-related mortality (TRM); neutrophil and platelet engraftment; acute graft-versus-host disease (aGVHD); and chronic GVHD (cGVHD). Relapse was defined as morphologic evidence of disease in PB, BM, or extramedullary sites. TRM was defined as death during remission. Neutrophil engraftment was defined as the first of 3 consecutive days during which the absolute neutrophil count was at least .5 $\times$ 10^9/L. Platelet engraftment was achieved on the first of 3 days when the platelet count was higher than 50 $\times$ 10^9/L without transfusion support. Both aGVHD and cGVHD were graded according to the previously published criteria [22,23]. The incidence of aGVHD was evaluated in all engrafted patients, whereas the incidence of cGVHD was evaluated in engrafted patients surviving more than 100 days.

The probability of OS was estimated according to the Kaplan-Meier method, and the groups were compared using the log-rank test. The probabilities of relapse, TRM, neutrophil and platelet engraftment, and acute and chronic GVHD were estimated based on a cumulative incidence method to accommodate competing risks [24]. Multivariate analysis was performed with a Cox proportional hazard model adjusted for OS and Fine and Gray proportional hazards model for relapse [25]. The following variables were considered: age (< 45 versus ≥ 45 years), disease type (de novo AML versus AML secondary to MDS versus advanced MDS), cytogenetic risk (other than poor versus poor), proportion of blasts in BM (< 25 versus ≥ 25%), the presence of blasts in PB (absent versus present), lactate dehydrogenase (LDH) at CBT (≤ upper limit of normal versus > upper limit of normal), disease status at CBT (untreated versus primary refractory versus refractory relapse), cord blood nucleated cell count (< 2.5 versus ≥ 2.5 $\times$ 10^7/kg), and HLA disparities based on antigen level HLA-A and -B and allele level

HLA-DRB1 (≤ 2 versus ≥ 3). All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), a graphical user interface for R 2.13.0 (R Foundation for Statistical Computing, Vienna, Austria) [26]. $P < .05$ was considered significant. Analysis of data was performed in August 2013.

RESULTS

Patient and CB unit characteristics are shown in Table 1. The median age was 41 years (range, 18 to 55 years), the median number of nucleated cells was 2.43×10^7/kg (range, 1.32 to 5.50×10^7/kg), and the median number of CD34+ cells was 1.03×10^5/kg (range, .21 to 2.27×10^5/kg). Disease types were de novo AML in 24 patients, AML secondary to MDS in 24, and advanced MDS in 13. The majority of patients with de novo AML with multilineage dysplasia (n = 2), AML secondary to MDS (n = 19), or advanced MDS (n = 10) received CBT as an up-front treatment, which was classified as untreated group (n = 31). Among patients with primary refractory status (n = 14), 3 patients received CBT after the first cycle of induction chemotherapy. The median number of prior chemotherapy treatments before CBT for primary refractory status was 3 (range, 1 to 5). The median time from diagnosis to CBT was 7 months (range, 1 to 219 months), and the median period of follow-up for survivors after CBT was 97 months (range, 5 to 181 months).

The cumulative incidence of neutrophil recovery was 93.4% (95% confidence interval [CI], 81.0% to 97.8%) at 60 days after CBT with a median time to achieve greater than $.5 \times 10^9$/L neutrophils of 22 days (range, 18 to 41 days). Disease progression before engraftment occurred in 2 patients. The cumulative incidence of platelet recovery was 78.7% (95% CI, 65.7% to 87.2%) at 100 days after CBT with a median time to an untransfused platelet count greater than 50×10^9/L of 50 days (range, 30 to 179 days). The cumulative incidences of grade II to IV acute GVHD and extensive chronic GVHD were 62.3% (95% CI, 48.7% to 73.2%) at 100 days and 32.9% (95% CI, 21.4% to 44.9%) at 3 years after CBT, respectively. The probability of OS at 7 years was 61.4% (95% CI, 47.1% to 72.9%). The cumulative incidence of relapse at 7 years was 30.5% (95% CI, 19.2% to 42.6%). The cumulative incidence of TRM at 100 days and at 1 year was 6.6% (95% CI, 2.1% to 14.7%) and 8.2% (95% CI, 3.0% to 16.9%), respectively (Figure 1).

In multivariate analysis, poor-risk cytogenetics (hazard ratio [HR], 7.14; 95% CI, 2.33 to 21.80; $P < .001$) and high LDH value (HR, 4.00; 95% CI, 1.33 to 12.07; $P = .013$) were associated with inferior survival (Figure 2, Table 2). De novo AML (HR, 9.66; 95% CI, 1.06 to 87.75; $P = .044$), primary refractory status at CBT (HR, 6.47; 95% CI, 1.86 to 22.51; $P = .003$), and high LDH value (HR, 3.75; 95% CI, 1.11 to 12.57; $P = .032$) were associated with an increased relapse incidence (Table 3, Supplemental Figure 1). In contrast, the proportion of blasts in BM and the presence of blasts in PB did not show any impact on survival and relapse incidence.

DISCUSSION

Previous reports have suggested that the only potentially curative therapy for patients with myeloid malignancies not in remission is allo-HSCT. However, the incidence of relapse has been reported to be high, and several reports showed long-term survival rates of only 10% to 30% [1-6]. Several factors, including blasts in BM or PB, cytogenetics, and donor availability, have been associated with outcome. In this study, poor-risk cytogenetics and high LDH value were significantly associated with inferior OS. De novo AML, primary refractory status, and high LDH value were associated with increased relapse. However, we found no impact of disease burden on survival and relapse. In fact, several retrospective studies did not show any advantage of induction chemotherapy before allo-HSCT to reduce the disease burden for patients with advanced MDS or AML secondary to MDS [27-29]. Therefore, the majority of patients with advanced MDS or AML secondary to MDS received G-CSF–combined myeloablative conditioning followed by CBT without prior induction chemotherapy in our institute.

After physicians have decided that allo-HSCT is appropriate for patients with myeloid malignancy not in remission, the elective timing of the transplantation is the main advantage of CBT. In fact, CBT timing is decided depending on the patient's conditions, such as control of infection and disease burden. Such elective timing of CBT might have contributed to disease burden not being shown to influence outcome in our study. On the other hand, the use of CB as a source of hematopoietic stem cells could offer the opportunity for patients to receive allo-HSCT without related or unrelated donors. Moreover, the lower incidence of severe GVHD without compromising graft-versus-leukemia effects in CBT may also have contributed to long-term survival in our study.

Relapse is the most important cause of treatment failure after allo-HSCT, particularly in patients with myeloid malignancies not in remission. This is mainly due to the residual leukemic cells that have escaped the cytotoxic effect of conditioning before transplantation. To reduce disease relapse, the role of a more intense conditioning regimen has been analyzed extensively [30]. Since chemosensitization of leukemia cells with G-CSF enhances the cytotoxicity of the cell-cycle–specific agent cytarabine [17], we administered G-CSF–combined high-dose cytarabine in the standard conditioning regimen of TBI/cyclophosphamide. The clinical efficacy of concomitant use of G-CSF with chemotherapy has remained controversial in newly diagnosed or relapsed refractory AML and MDS [31,32]. Recently, Pabst et al. reported

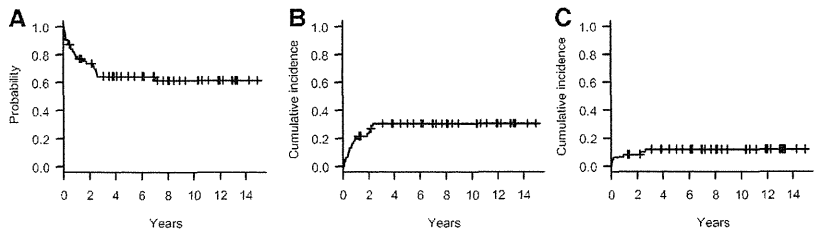

Figure 1. Probability of overall survival and cumulative incidences of relapse and transplant-related mortality after G-CSF–combined myeloablative CBT. Overall survival (A), relapse (B), and transplantation-related mortality (C) in 61 patients with AML or advanced MDS not in remission after G-CSF–combined myeloablative CBT.

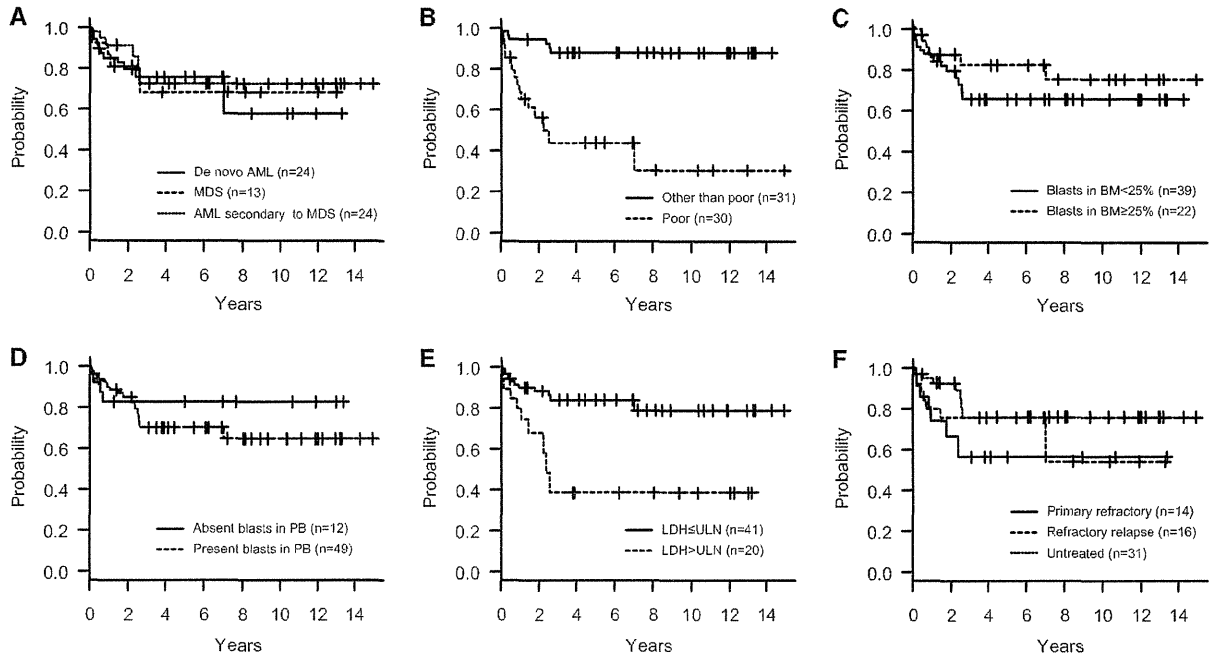

Figure 2. Adjusted probabilities of overall survival in 61 patients with AML and advanced MDS not in remission after G-CSF–combined myeloablative CBT. The adjusted probabilities of overall survival grouped according to the disease type (A), cytogenetic risk (B), the proportion of blasts in bone marrow (BM) (C), the presence of blasts in peripheral blood (PB) (D), the lactate dehydrogenase (LDH) value at cord blood transplantation (CBT) (E), and disease status at CBT (F). Multivariate analysis for overall survival is shown in Table 2.

Table 2
Univariate and Multivariate Analysis of Prognostic Factors for Survival

Variable	Univariate Analysis			Multivariate Analysis		
	Number	7-year OS (95% CI)	*P*	Hazard Ratio*	95% CI	*P*
Age						
< 45 years	36	63.5 (44.1-77.7)		1		
≥ 45	25	58.7 (36.7-75.4)	.555	.69	.25-1.86	.464
Disease type						
Advanced MDS	13	59.3 (27.5-81.0)		1		
AML secondary to MDS	24	74.4 (51.6-87.6)		.58	.13-2.54	.471
De novo AML	24	47.4 (23.0-68.4)	.234	.97	.18-5.16	.978
Cytogenetics†						
Other than poor	31	80.3 (61.3-90.6)		1		
Poor	30	38.9 (18.8-58.6)	.002	7.14	2.33-21.80	<.001
Bone marrow blasts at CBT, %						
< 25	39	58.0 (40.8-71.8)		1		
≥ 25	22	68.2 (41.2-84.7)	.297	.59	.16-2.09	.418
Peripheral blood blasts at CBT						
Absent	12	66.7 (33.7-86.0)		1		
Present	49	60.2 (44.0-73.1)	.983	1.18	.34-4.10	.787
LDH value at CBT						
≤ ULN	41	67.4 (48.9-80.4)		1		
> ULN	20	50.0 (27.1-69.2)	.147	4.00	1.33-12.07	.013
Disease status at CBT						
Untreated	31	71.1 (50.1-84.5)		1		
Primary refractory	14	50.0 (22.9-72.2)		2.76	.78-9.77	.114
Refractory relapse	16	50.0 (20.2-74.1)	.234	1.75	.30-10.22	.530
Number of nucleated cells, $\times 10^7$/kg						
≥ 2.5	29	59.2 (37.9-75.3)		1		
< 2.5	32	64.1 (44.3-78.4)	.989	.99	.38-2.58	.989
HLA disparities‡						
≤ 2	45	60.3 (43.7-73.4)		1		
≥ 3	16	65.0 (35.1-83.7)	.597	.98	.30-3.18	.975

MDS indicates myelodysplastic syndrome; AML, acute myelogenous leukemia; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; HLA, human leukocyte antigen; OS, overall survival; CI, confidence interval.

* Hazards ratio for overall mortality.

† The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.

‡ The number of HLA disparities defined as the low resolution for HLA-A and -B and the high resolution for HLA–DRB1.

Table 3
Univariate and Multivariate Analysis of Prognostic Factors for Relapse

Variable	Univariate Analysis			Multivariate Analysis		
	Number	7-year Relapse (95% CI)	*P*	Hazard Ratio	95% CI	*P*
Age						
< 45	36	29.3 (15.0-45.2)		1		
≥ 45	25	32.0 (14.9-50.6)	.567	1.62	.50-5.17	.420
Disease type						
Advanced MDS	13	7.7 (.4-30.5)		1		
AML secondary to MDS	24	29.8 (12.9-49.0)		4.37	.38-49.80	.230
De novo AML	24	43.4 (22.4-62.7)	.096	9.66	1.06-87.75	.044
Cytogenetics*						
Other than poor	31	23.0 (9.9-39.2)		1		
Poor	30	38.2 (20.5-55.7)	.163	2.33	.90-5.97	.078
Bone marrow blasts at CBT, %						
< 25	39	26.0 (13.3-40.6)		1		
≥ 25	22	39.2 (18.0-59.9)	.397	1.72	.57-5.16	.330
Peripheral blood blasts at CBT						
Absent	12	16.7 (2.3-42.8)		1		
Present	49	33.8 (20.6-47.4)	.309	3.08	.40-23.70	.280
LDH value at CBT						
≤ ULN	41	25.6 (13.1-40.1)		1		
> ULN	20	40.0 (18.5-60.8)	.240	3.75	1.11-12.57	.032
Disease status at CBT						
Untreated	31	17.8 (6.3-34.1)		1		
Primary refractory	14	50.0 (21.4-73.3)		6.47	1.86-22.51	.003
Refractory relapse	16	37.5 (14.5-60.7)	.043	1.36	.26-7.05	.71
Number of nucleated cells, $\times 10^7$/kg						
≥ 2.5	29	35.5 (18.3-53.1)		1		
< 2.5	32	25.3 (11.7-41.5)	.525	.54	.14-2.12	.380
HLA disparities†						
≤ 2	45	34.0 (20.4-48.1)		1		
≥ 3	16	20.3 (4.5-43.9)	.306	.53	.11-2.49	.420

MDS indicates myelodysplastic syndrome; AML, acute myelogenous leukemia; CBT, cord blood transplantation; LDH, lactate dehydrogenase; ULN, upper limit of normal; HLA, human leukocyte antigen; CI, confidence interval.

* The cytogenetic subgroups according to the Southwest Oncology Group/Eastern Cooperative Oncology Group criteria for AML and International Prognostic Scoring System criteria for MDS.

† The number of HLA disparities defined as the low resolution for HLA-A and -B and the high resolution for HLA–DRB1.

significantly improved survival with concomitant use of G-CSF with escalated-dose, but not with conventional-dose cytarabine [31]. In the setting of allo-HSCT, the conditioning regimen consisting of G-CSF–combined high-dose cytarabine and TBI 12 Gy was feasible and might reduce post-transplantation relapse in patients with AML [18,19]. The presence of quiescent leukemia stem cells (LSCs), which are thought to be resistant to chemotherapy, might contribute to relapse after treatment. Recently, a xenograft model demonstrated that cytarabine with G-CSF recruited quiescent LSCs into a phase of the cell cycle, leading to enhanced elimination of LSCs within the niche [33]. This effect might have contributed to reduced relapse in our study. Although these findings should be confirmed in prospective studies, the combination of G-CSF–combined myeloablative conditioning with CBT offered a promising curative option for patients with myeloid malignancies not in remission.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbmt.2013.12.555.

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Visceral varicella zoster virus infection after allogeneic stem cell transplantation

N. Doki, S. Miyawaki, M. Tanaka, D. Kudo, A. Wake, K. Oshima, H. Fujita, T. Uehara, R. Hyo, T. Mori, S. Takahashi, S. Okamoto, H. Sakamaki, for the Kanto Study Group for Cell Therapy. Visceral varicella zoster virus infection after allogeneic stem cell transplantation. Transpl Infect Dis 2013: **15**: 314–318. All rights reserved

Abstract: *Introduction.* Varicella zoster virus (VZV) disease is one of the major infectious complications that can occur after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Many reports have shown visceral VZV infection, a special type of VZV disease, to be rare. However, few studies so far have included a large number of patients.
Findings. Visceral VZV infection was found in 20 (0.8%) of 2411 patients who underwent allo-HSCT at our hospitals. Seventeen (85%) patients were taking immunosuppressive agents at the time of presentation with zoster. The presenting symptom was abdominal pain in 16 patients (80%), unconsciousness in 3 patients (15%), and no symptoms in 1 patient. The mean time interval from allo-HSCT to symptomatic visceral VZV infection was 273 days (103–800 days). The eruptions appeared within 3 days (0–13) after the first symptoms. Treatment with intravenous acyclovir was initiated before the appearance of eruptions in 3 of 18 patients (all 3 survived) with vesicular eruptions, the same day in 12 patients (11 survived, 1 died), and after the appearance in 3 patients (1 survived, 2 died). The overall mortality was 20%.
Conclusion. In conclusion, these data confirm that the incidence of visceral VZV infection is infrequent, but this disease is serious. When patients being treated with immunosuppressive agents demonstrate abdominal pain or unconsciousness, the possibility of visceral VZV infection should be considered as well as earlier therapeutic intervention.

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Key words: visceral varicella zoster virus (VZV) infection; allogeneic hematopoietic stem cell transplantation; abdominal pain; chronic graft-versus-host-disease (GVHD)

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Varicella zoster virus (VZV) infection is a common complication after hematopoietic stem cell transplantation (HSCT), and affects about 18–50% (1, 2) of HSCT recipients. The majority of these infections are the result of reactivation of a preexisting infection among adult recipients. Approximately 20% (1, 3) of these cases subsequently develop cutaneous dissemination, whereas visceral dissemination occurs in only