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   竹腰久容、松井利充　他 5 名
   造血幹細胞移植時の口腔ケアにおける歯科衛生士早期介入の有用性
   八木夏紀、松井利充　他 3 名
   同種造血幹移植後患者に対する継続看護

H. 知的財産権の出願・登録状況

1. 特許取得
   該当なし
2. 実用新案登録
   該当なし
3. その他
   該当なし

厚生労働科学研究費補助金　ヒトゲノム・再生医療等研究事業
分担研究報告書

『 腫瘍細胞の細胞周期とＧＶＬ効果 』

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研究要旨

細胞回転が普通数％と言われている多発性骨髄腫は、細胞増殖がゆっくりであるため GVL 効果の期待は大きい。多発性骨髄腫は systemic cytokine & chemokine disease の様相を見せる。MM とその関連疾患について、cytokine & chemokine（サイトカイン、ケモカイン）の測定を行った。腫瘍細胞の接着と増殖に主たる役割を果たすと考えられている TNFα や IL6 は測定範囲以下であったが、sVCAM-1,sIL2R,VEGF と RANTES が有意に変動し、腫瘍細胞の増殖や、副次的病態である骨病変に対する指標となる可能性を示した。今後 MM とその関連疾患の allo-PBSCT 治療開始時期の決定や経過の観察に有益な監視指標となる可能性が考えられた。

A．研究目的

多発性骨髄腫の治療は 1962 年の MP 療法から始まった。長期予後の改善に向けて 1996 年から autoPBSCT、mini-allo-PBSCT が積極的に行われる様になり、1997 年には tandem auto-BMT の CR 率、生存期間の良い成績が発表された。しかし長期予後の大きな改善はみられていない。1999 年から thalidomide の有効性が発表され、それに続いていくつかの新薬による新しい化学療法の開発が始まっている。遺伝学的研究や分子生物学的研究の進歩は多発性骨髄腫の病態の解明をもたらしてくれることが期待される。多発性骨髄腫は非常に heterogeneous な疾患であり、その病態の対する治療の反応も多様なものとなる。私は今までなされてきた層別化の方法、すなわち Durie & Salmon と International Staging System の方法で層別化での治療結果を検討し、今後の新しい層別化の可能性を検討した。

多発性骨髄腫細胞は多くのサイトカイン産生する機能性腫瘍である。他の骨髄内間質細胞とはサイトカイン、サイトカイン受容体、接着分子を介したネットワークを形成している。血清サイトカインは骨髄内のダイナミズムを反映していると思われる。下記の機能を持つ cytokine,chemokine が知られている。

Growthに関与するもの ：IL6, IGF-1, VEGF
Survivalに関与するもの ：IL6, IGF-1
薬剤耐性に関与するもの ：IL6, IGF-1, VEGF
migrationに関与するもの ：IGF-1, VEGF, SDF-1α
angiogenesisに関与するもの ：VEGF
stroma cellへのadhesion：TNFb
骨破壊に関与するもの：RANK,RANKL,MIP-1
BMSCs上の接着因子：sVCAM-1

この研究で注目するRANTESはRegulated upon Activation, Normal T cell Expressed and Secreted(*Schall* 1990)の短縮語。。RANTESはCC chemokine/intercrine に属する(*Gallard* 1994) 。RANTESはTリンパ球、顆粒球、マクロファージ、線維芽細胞、mesangial cells, 腎尿細管上皮細胞、血小板などが遊離する。人遺伝子部位は17q11-q21 と言われる(*Gallard* 1994) 。T細胞、好酸球、好塩基球に Chemotactic で炎症部位への白血球の浸潤を促進する。骨折治癒経過で Osteoblast,Osteoclast に RANTES 産生が見られる。Osteoblast は CCR1,CCR3,CCR5 を受容体として持ち、osteoclast は CCR1 を受容体として持つ。

血清学的検査で測定可能な、病態に呼応して動くサイトカイン、ケモカインから腫瘍細胞の増殖や、副次的病態である骨病変に対する指標となる可能性を検討する。

B．研究方法

E-1 方法：京阪血液研究会参加施設 7 施設の 2001 年から 2005 年の 5 年間に新しく診断された多発性骨髄腫の症例を集計した。D&S と ISS とによる層別化を行い、各層の治療内容と生存期間の関係を K-M 法で解析した。

結果：登録症例数は 204 名

年齢　40 歳以下　9 人
　　41 歳以上　60 歳未満　40 人
　　61 歳以上　155 人
　　（　内 71 歳以上　71 人　）
女性　97 人
男性　107 人

IgG	106 人
IgA	45 人
IgD	5 人
BJ	25 人
非分泌	5 人
不明	4 人
以上 190 人（多発性骨髄腫）	
Plasmacytoma	7 人
PCL	1 人
Macroglobulinemia	1 人
MGUS	3 人
Amyloidoisis	2 人

多発性骨髄腫と診断された 190 人について検討した。データが揃い統計的解析が可能な症例は 181 例 ®2MG が計測されていて ISS 判定ができた症例は 128 例であった。

E-2 MGUS 3 例、骨髄内形質細胞が 5%以下の good control Myeloma2 例と、骨髄内形質細胞が多い poor control Myeloma 2 例の計 7 例について測定した。

以下の cytokine, chemokine を測定した。

sIL2R	IGF-1	IL3	IL6	IL8	VFGF
sIL6R	IL1b	TNFb	sVCAM-1	RANTES	

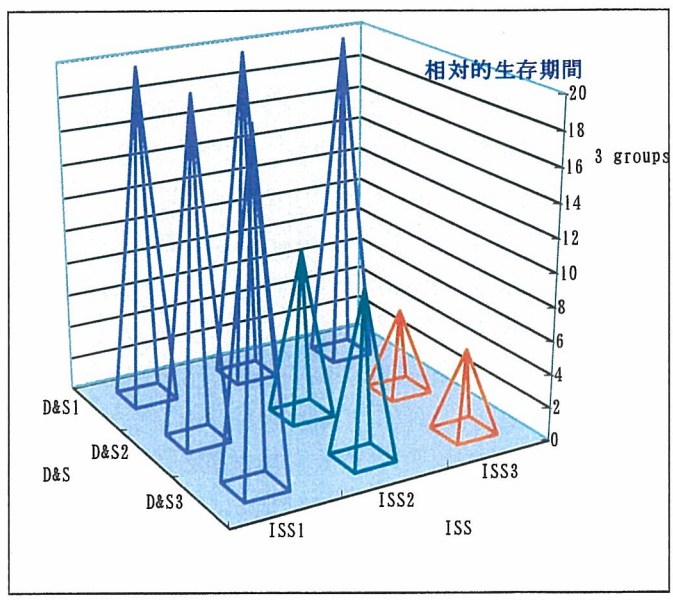
＜倫理面への配慮＞

多発性骨髄腫の病態はまだまだ未知の部分が多く、今後の研究に待たねばならない段階である。しかし我々の研究はその取っかかりとなる研究であり、今後の治療に寄与するものであるとして研究の意味を説明した。検査結果の報告を行う。

検査費用は研究費で支払い、患者負担でないことも説明している。

C．研究結果

G-1. D&S と ISS と で 9 分割し、各項での相対的生存期間を図示した。D&S か ISS かで stage1 となる群、どちらかが stage2,3 の群、stage3,3 の群に分けられた。



G-2.　Cytokine,Chemokine 測定結果

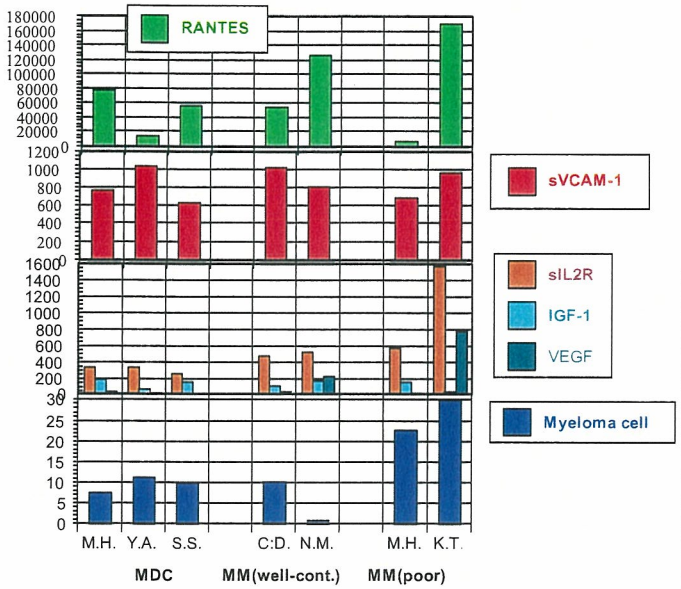
測定結果を図示するが、上昇を示す Cytokine,Chemokine は VEGF,IGF-1,sIL2R, sVCAM-1 と RANTES である。

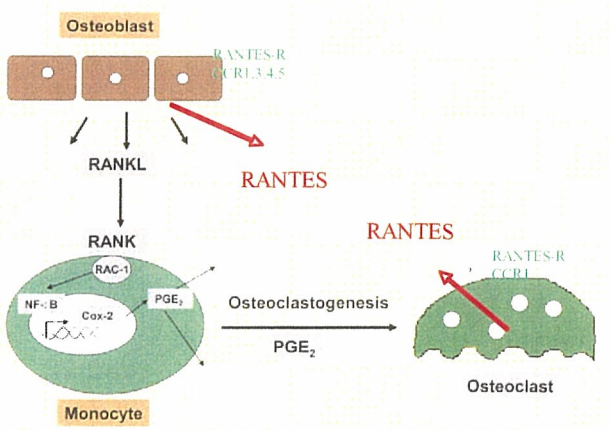
Stroma cell と myeloma cell の接着因子の産生を促す cytokine TNFαおよび stroma cell から産生される IL6 の上昇は認めなかった。

sVCAM-1 は MGUS から poor control myeloma まで同じように上昇している。Stroma cells にある Myeloma cell の receptor であり、MM 関連疾患の基礎的 chemokine といえる。IGF-1 も全ての病態で上昇が見られる。

sIL2R、VEGF と RANTES は MGUS と MM で差が認めらた。sIL2R は骨髄内 Myeloma cell%と相関

している。VEGF は poor controlled MM で高く、骨髄内腫瘍細胞の量や増殖に関連する可能性が示唆される。RANTES は骨折治癒過程における Osteoclast,Osteoblast で発現が認められる Cytokine である。今回の MGUS と MM とで差が認められる。MM 内での差は、punced-out lesion や pathological bone fracture がある MM で高値であった。これまで RANK,RANKL 系、MIP-1 系が骨病変と関連があることが認められてきたが、RANTES もまた骨病変に関連する cytokine であると推定出来る。





サイトカイン、ケモカイン個人差があるもので、病態差を明確に出そうと思うと多数の
症例で測定して統計的有意差を検討する必要がある。また治療経過での変化を追うことも
必要と考える。

D．考察

MM細胞から産生させるサイトかインTNFαの作用によって間質細胞膜に表現されるレセプターである s VCAM-1 は、MGUSから高い値で、MMになってもその値は変わらず、また病態のコントロールの良し悪しにも関係がない。形質細胞の間質細胞と接着によって初めて形質細胞の増殖が始まるが、さらに病状が進むには他の追加変化が必要と考えられる。血管新生をうながすVEGF の上昇、腫瘍の量を反映すると考えられる s IL2Rの上昇がMMに認められるのは興味深い。血清学的に確認できたこの２つのサイトカインが細胞増殖を反映すると考えられる。骨折後の治癒過程で活性化されるRANTESがMMにおいて増加、圧迫骨折・溶骨のあるMMに高い。Myelomaの骨破壊の病態を反映している可能性のあるRANTESは、多発性骨髄腫とその関連疾患の病態の理解及び治療効果の判定に有効な指標となるケモカインである可能性が高い。サイトカイン・ケモカインの測定結果の特徴は個人差があることである。従って今後MGUS, MM で多くの検査結果を集めて統計学的評価をする必要が出てくる。

E．結論

多発性骨髄腫に関する層別化で Durie & Salmon と International Staging Sysytem では何れも治療法の適応や長期予後について必ずしも適切な指標とならないことを経験する。両方法を組み合わせた層別化の可能性を提示できた。一方、腫瘍細胞の Cell kinetics を確認のため腫瘍活性に関連するサイトカインの量的評価を行う試みを行った。血清学的に有意に確認できたサイトカイン、ケモカインは sVACAM-1,sIL2R,VEGF,RANTES であった。s VCAM-1 は MM 関連疾患の基本的サイトカインのようである。sIL2R,VEGF は腫瘍細胞の量やその増殖活性を反映している。多発性骨髄腫の主要なる病的合併症である骨病変の動きを反映するサイトカインの内、今まで指摘されてきた RANK/RANKL 系や MIP 系以外に RANTES が関与している可能性があることが考えられる。これらは骨髄内形質細胞％や M 蛋白量とともに病態の確認をする手段となり、治療方法の選択と効果の判定に有効な指標となると考えられる。

F．健康危機情報

該当なし

G．研究発表

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「多発性骨髄腫病期層別化の再検討」

麥谷安津子1)、北山等2)、魚嶋伸彦3)、手島博文4)、野村昌作5)、長谷川稔6)、林邦雄7)：1）府中病院、2）星ヶ丘厚生年金病院、3）松下記念病院、4）大阪市立総合医療センター、5）岸和田市民病院、6）大阪医大第二内科、7）京阪奈病院（(京 阪 血 液 研 究 会 グループ）

H．知的財産権の出願・登録状況

1. 特許取得

該当なし

2. 実用新案登録

該当なし

3.その他

該当なし

厚生労働科学研究費補助金　ヒトゲノム・再生医療等研究事業
分担研究報告書

『 成人T細胞白血病に対する同種造血幹細胞移植 』

分担研究者　　宇都宮 與　慈愛会 今村病院分院／院長

研究要旨

成人T細胞白血病（ATL）29例（年齢中央値49歳）に対して32回の同種造血幹細胞移植を実施した。100日以上の生存例は24例であり、そのうち12例が再発し、再発した12例を中心に検討した。再発部位は皮膚9例、末梢血5例、リンパ節4例、その他2例であった。再発後免疫抑制剤の中止にて12例中10例に急性GVHD（Grade II-IV）が認められ、その後CR 7例が得られ、graft-versus-ATL（GvATL）効果と考えられた。寛解の得られた症例でもGVHDの出現とその治療後に起こるATL再発と感染症などで4例が死亡し、再発から死亡までの期間の中央値は3.5カ月と短かった。ATLの移植後再発においては免疫抑制剤の中止で再寛解が得られてもGVHDやATLの再再発の問題があり、免疫抑制剤の調整とともに積極的な化学療法の実施を適切に組み合わせる必要がある。

A．研究目的

近年ATLに対する同種造血幹細胞移植での予後の改善が報告され、種々の幹細胞源を用いて様々な移植が行われている。ATLに対しては骨髄非破壊的移植の割合が高く、移植後の再発が問題となっている。今回当科で同種造血幹細胞移植を施行し、移植後再発したATL症例の背景とその後の経過を明らかにすることを目的とした。

B．研究方法

対象は1998年6月から2006年9月までに同種造血幹細胞移植を施行したATL 29例（移植回数32回）であった。移植後100日以上生存した24例のうち再発した12例について臨床的背景、移植方法、移植成績、再発やその後の経過などを検討した。

＜倫理面への配慮＞

患者の自由意志による治療同意を文書にて得た。

C．研究結果

男性15例、女性14例、移植時の年齢の中央値は49歳（37ー62歳）、ATLの臨床病型は急性型26例、リンパ腫型2例、慢性型1例であった。移植前の寛解状態はCR 14例、PR 2例、NC 4例、PD 9例で、骨髄破壊的移植が18例、骨髄非破壊的移植が11例であった。骨髄移植が11例、末梢血幹細胞移植が14例、臍帯血移植が4例で、HLA適合移植が20例、不適合移植が9例（臍帯血4例、血縁noninherited maternal antigen不一致4例、非血縁1例）であった。同種移植後の全生存期間の中央値は9カ月（1-104+カ月）であった。100日以内の早期死亡は18例中5例にみられ、それらの死因は腫瘍死2例、血栓性微小血管障害 2例、急性GVHD 1例であった。100日以上の生存例は24例であり、そのうち12例が再発・再増悪した。再発・再増悪部位やその後の臨床経過を表1に示した。再発・再増悪までの期間の中央値は3カ月（1-13カ月）で、再発部位は皮膚9例、末梢血5例、リンパ節4例、骨1例、眼1例であった。再発後全例免疫抑制剤を中止したが、10例にGrade II-IVの急性GVHD認められた。免疫抑制剤中止後GVHDの出現と連動して多くの例で抗腫瘍効果（GvATL 効果）が得られた（CR 7例、NC 4例、PD 1例）。再発後9例が死亡し、生存は3例のみであった。再発後再寛解の得られた7例のうち4例が死亡し、死因は腫瘍死2例、感染症1例、GVHD 1例であった。また再発から死亡までの期間の中央値は 3.5 カ月（1-30カ月）と短かった。

表1. 移植後再発・再増悪した12例の移植効果と転帰

No.	CST/RIST	移植前寛解状態	移植後寛解状態	再発部位	移植から再発再燃までの期間(M)	免疫抑制中止後のGVHD	免疫抑制中止後の寛解状態	死因	再発から死亡までの期間(M)
1	RIST	PD	CR	Skin,PB,LN	3	0	PD	ATL	2
2	RIST	PD	CR	Skin,PB	2	2	CR	生存	
3	RIST	PD	PR	Skin,PB,LN	3	4	CR	GVHD	2
4	RIST	PD	PR	Skin,PB,LN	1	4	NC	GVHD	4
5	RIST	SD	PR	Skin	3	2	NC	ATL	7
6	RIST	PR	CR	Skin	1	2	CR	ATL	1
7	CST	SD	PR	Skin,PB	3	2	NC	感染症	6
8	CST	PR	CR	LN	3	2	CR	感染症	5
9	CST	CR	CR	Skin	3	2	CR	生存	
10	CST	CR	CR	Skin	13	2	CR	ATL	30
11	CST	CR	CR	Bone	9	1	CR	生存	
12	CST	PD	NC	Orbita	2	3	NC	感染症	2

D. 考察

ATL の同種造血幹細胞移植においては骨髄非破壊的移植の割合も増加し、移植後の再発症例も多くなっている。再発後免疫抑制剤の中止にて、再寛解の得られる症例が多く、GvATL 効果の存在が示唆された。しかしながらその後に合併する GVHD や ATL の再再発、免疫抑制剤使用による感染症などで、寛解の得られた例ですら 7 例中 4 例が死亡し、再発から死亡までの期間の中央値は 3.5 カ月であり、予後は不良であった。ATL の同種移植後の再発例においては免疫抑制剤の調整と積極的な化学療法を適切に組み合わせる必要があるものと思われる。

E. 結論

ATL の同種移植後の再発に対しては、免疫抑制剤中止後に増悪する例に対しては適当なタイミングで化学療法を用いるべきである。

F. 健康危機情報

該当なし

G. 研究発表

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H. 知的財産権の出願・登録状況

1. 特許取得
該当なし
2. 実用新案登録
該当なし
3.その他
該当なし

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ORIGINAL ARTICLE

Population pharmacokinetics of intravenous busulfan in patients undergoing hematopoietic stem cell transplantation

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A population pharmacokinetic analysis was performed in 30 patients who received an intravenous busulfan and cyclophosphamide regimen before hematopoietic stem cell transplantation. Each patient received 0.8 mg/kg as a 2 h infusion every 6 h for 16 doses. A total of 690 concentration measurements were analyzed using the nonlinear mixed effect model (NONMEM) program. A one-compartment model with an additive error model as an intraindividual variability including an interoccasion variability (IOV) in clearance (CL) was sufficient to describe the concentration–time profile of busulfan. Actual body weight (ABW) was found to be the determinant for CL and the volume of distribution (*V*) according to NONMEM analysis. In this limited study, the age (range 7–53 years old; median, 30 years old) had no significant effect on busulfan pharmacokinetics. For a patient weighting 60 kg, the typical CL and *V* were estimated to be 8.87 l/h and 33.8 l, respectively. The interindividual variability of CL and *V* were 13.6 and 6.3%, respectively. The IOV (6.6%) in CL was estimated to be less than the intraindividual variability. These results indicate high interpatient and intrapatient consistency of busulfan pharmacokinetics after intravenous administration, which may eliminate the requirement for pharmacokinetic monitoring.
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Keywords: intravenous; busulfan; population pharmacokinetics; NONMEM

Introduction

A high dose of busulfan in combination with cyclophosphamide is a widely used myeloablative conditioning regimen before both allogenic and autologous bone marrow transplantation (BMT).[1,2] In most cases, busulfan is administered every 6-h over four consecutive days with a total standard dose of 16 mg/kg.[1] As with most alkylating agent, busulfan has a narrow therapeutic window. The dose-limiting toxicity of busulfan in the myeloablative conditioning regimen is hepatic veno-occlusive disease (VOD), which can lead to fatal liver failure.[3,4] Following administration of the oral formulation, very wide inter- and intraindividual systemic exposure has been reported,[5] which may be linked to erratic intestinal absorption, variable hepatic metabolism, circadian rhythm, genetics, diagnosis, drug–drug interaction and age.[5–9] Recently, the intravenous formulation of busulfan has been developed in order to minimize variations of the inter- and intrainidividual systemic exposure and to provide complete dose assurance. The intravenous busulfan is registered in the USA (Busulfex™) and in Europe (Busilvex™) for adults. The recommended dosage was 0.8 mg/kg/dose for 16 consecutive doses in adults.[10–12] There have been several reports about intravenous busulfan pharmacokinetics,[10,13] with only a few applying population pharmacokinetic analysis.[13] We report here, the results of the population pharmacokinetic modeling of intravenous busulfan. The aim of this analysis was to characterize the pharmacokinetics of intravenous busulfan, including the IOV and covariate relationships in patients.

Materials and methods

Patients

A total of 30 Japanese patients (27 adults and three children) receiving a first BMT entered in a Phase 2 study were investigated. These patients received busulfan at 0.8 mg/kg as a 2 h infusion every 6 h for four consecutive days. Following busulfan therapy, patients were given cyclophosphamide at 60 mg/kg as a 3 h infusion daily for 2 days. In order to prevent seizures, phenytoin (5–10 mg/kg/day) was administered orally for 8 days, starting 2 days before the start of busulfan therapy. The following demographic and physiopathological data were considered in the analysis: diagnosis, acute myeloid leukemia (13), acute lymphocytic leukemia (5), chronic

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myelogenous leukemia (5), myelodysplastic syndrome (3), non-Hodgkin's lymphoma (4); gender, male (20), female (10); age, 7–53 years (median = 30 years); actual body weight (ABW), 18.5–82.7 kg (median = 64.1 kg); height, 111–180 cm (median = 165.5 cm); body mass index, 14.40–29.10 kg/m^2 (median = 22.65 kg/m^2); serum albumin, 3.5–4.8 g/dl (median = 4.3 g/dl); creatinine, 0.2–1.2 mg/dl (median = 0.7 mg/dl); serum alanine transaminase (ALT), 8.0–109.0 IU/l (median = 21.0 IU/l); history of hepatic disease, no (27), yes (3); concomitant antifungal treatment, no (7), yes (23); concomitant 5-HT$_3$ antiemetic treatment, no (16), yes (14). The study was approved by an independent Ethical Committee at each center. All patients provided written informed consent before enrollment.

Pharmacokinetic sampling and busulfan determination
Serial blood samples were drawn from each patient immediately before the first and ninth busulfan dose and then 0.25, 0.5, 0.75, 1.92, 2.25, 2.5, 3, 4, 5 and 6 h after the start of the first and ninth dose. The 13th dose sampling of each patient was made immediately before the infusion and at 1.92 h from the start of infusion, respectively. Plasma samples obtained by centrifugation were stored frozen until analysis. Busulfan was assayed by a validated gas chromatographic-mass selective detection (GC-MSD) assay technique.[14] The calibration curves were linear over concentrations ranging from 62.5 (quantification limit) to 2000 ng/ml. Samples with a concentration higher than 2000 ng/ml were diluted such that the concentration fell within the range of the calibration curve. Acceptance criteria for validating the analytical results of each run were as follows. Quality control (QC) samples in duplicate at three concentrations (125, 500, and 1500 ng/ml) were incorporated into each run. The results of the QC samples provided the basis for accepting or rejecting the run. At least four of six QC samples had to be within $\pm$20% of their respective nominal values, and two of six QC samples (both at the same concentration) had also to be within the $\pm$20% respective nominal value. The GC-MSD for pharmacokinetic investigation was performed at BML Inc. (Saitama, Japan). A total of 690 concentration measurements were available.

Population pharmacokinetic analysis and model validation
Data were analyzed using the nonlinear mixed effect model (NONMEM) program (version 5.0, Globomax LLC, Hanover, MD, USA). As the population pharmacokinetic model is used for prediction, it is important to develop a model with validation.[15] Owing to the limited number of patients in this study, external validation of the population pharmacokinetic model could not be applied; therefore, the model was evaluated using bootstrapping, one of the internal validation techniques.[15,16]

Population pharmacokinetic modeling steps were as follows: (1) a basic pharmacokinetic modeling using the NONMEM program and obtaining Bayesian individual parameter estimates, (2) validation of a basic model using the bootstrap resampling technique, (3) generalized additive modeling (GAM) for the selection of covariate candidates, (4) final pharmacokinetic modeling to determine the covariate model, and (5) validation of the final model. The NONMEM program and PREDPP package were used throughout the analysis. The first-order conditional estimation with interaction method was used in all analysis processes because of the extensive sampling design in the study. Initial pharmacokinetic parameter estimates for NONMEM modeling were calculated using the mean data obtained from all the patients by WinNonlin (version 3.3, Pharsight Corp., Mountain View, CA, USA).

Step 1: basic pharmacokinetic modeling without bootstrapping. One-compartment structural model with constant rate infusion was fitted to the busulfan concentration–time data. Interindividual variability in clearance (CL) was modeled using an exponential error model, as follows:

$$CL_i = CL \cdot \exp(\eta_i),$$

where CL_i represents the hypothetical true CL for the ith individual, CL is the typical population value of CL and η is independent, identically distributed random variables with mean 0 and variance ω^2. Interindividual variability in volume of distribution (V) was similarly modeled.

Residual intraindividual variability was identically distributed and was modeled using the additive error, constant coefficient of variation (CCV) error or the combination of the additive and CCV error models. The additive error model is described by the following equation:

$$Cp_{ij} = Cp_{mij} + \varepsilon_{ij},$$

where Cp_{ij} is the ith measured concentration in the jth individual and Cp_{mij} is the ith concentration predicted by the model at the ith observation time for the jth individual. ε is independent random variable with mean zero and variance σ^2. The magnitude of residual intraindividual variability usually depends on measurement, dosing, sampling and model misspecification errors.

IOV was introduced into the model as previously proposed.[17] The following expression was used for CL

$$CL_{ij} = CL \cdot \exp(\eta_i + \kappa_{ij}),$$

where CL_{ij} represents the hypothetical true CL for the ith individual at occasion j, CL is the typical population value of CL and η and κ are independent, identically distributed random variables both with mean 0 and variance ω^2 and π^2, respectively. IOV in V was similarly modeled.

With the fixed and random effects chosen, empirical Bayes estimates of pharmacokinetic parameters were subsequently obtained using POSTHOC option within the NONMEM program. The choice of a basic population model was based on monitoring the Akaike's information criterion (AIC). The reliability of the model selection was checked by the analysis of residual and by the visual inspection of plots of predicted versus measured concentrations.

Step 2: validation of a basic model using the bootstrap resampling technique. Resampling the original data with replacements generated 100 bootstrap samples. The resampling unit comprises samples obtained from each

patient. The appropriate structural model that best describes the data from each sample was determined. This was performed to ensure that the model, which best described the bootstrap data was not different from the basic used for developing the population pharmacokinetic model in the subsequent step. In addition, density plots of each pharmacokinetic parameter estimate were used to examine the adequacy of the basic model.

Step 3: selection of covariate candidates. Exploratory data analysis was performed on the empirical Bayesian parameter estimates from step 2 and treated as data to examine the distribution, shapes and relationships between covariates and individual pharmacokinetic parameter estimates.

The data were subjected to a stepwise (single term addition/deletion) procedure using the GAM procedure in the Xpose program (version 3.1)[18] running on the S-PLUS statistical software package (version 6.0, Insightful Corp., Seattle, WA, USA). Each covariate was allowed to enter the model in any of several functional representations. AIC was used for model selection.[19] At each step, the model was changed by the addition or deletion of the covariate that results in the largest decrease in AIC. The search was stopped when AIC reached a minimum value.

Step 4: population model building using NONMEM. For each NONMEM analysis, the improvement in fit obtained upon the addition of a covariate selected from step 3 to the regression model was assessed by changes in the NONMEM objective function. Minimization of the NONMEM objective function, equal to twice the negative log-likelihood of the data, is equivalent to maximizing the probability of the data. The change in the objective function of the NONMEM value is approximately χ^2 distributed. A difference in the NONMEM objective function value of 3.84, associated with a P-value of less than 0.05, was considered statistically significant.

The construction of the regression model for each structural model parameter was performed in three steps using the original data set. Covariates were first screened individually. The full model was then defined as incorporating all significant covariates. Lastly, the final model was elaborated by backward elimination from the full model.

Step 5: validation of the final population pharmacokinetic model. Two hundred bootstrap samples were generated by resampling with replacements and used for the evaluation of the stability of the final model built in step 4. The final population pharmacokinetic model was fitted repeatedly to the 200 additional bootstrap samples. The mean parameter estimates obtained from these bootstrap replications were compared with those obtained from the original data set.

The area under the plasma concentration–time curve
The area under the plasma concentration–time curve (AUC) in each patient was calculated according to the linear trapezoidal rule using WinNonlin. The AUC at the steady state was calculated for the ninth dose from dosing interval (from zero to last sampling time). The AUC in one of 30 patients after the ninth administration was not calculated because the last sample at the ninth dose was collected after the start of the next dose.

Results

Determination of a basic pharmacokinetic model
Plasma concentration versus time curves are shown in Figure 1. Parameter estimates of various structural models are given in Table 1. The models including IOV gave lower AIC values than the models not including IOV. The Additive model, including IOV and the combination of the additive and CCV error models (the combination error model) including IOV gave similar AIC values. Analysis of residuals and plots of observed versus predicted concentrations were performed to check the reliability of the basic model selection. The residuals calculated in the additive model including IOV were not obviously different from those obtained in the combination error model including IOV (data not shown). The stability of these two models was examined in a subsequent step.

Stability of the basic model as assessed using the bootstrap resampling technique
One hundred bootstrap replicates were generated from the original data and used for the evaluation of the stability of

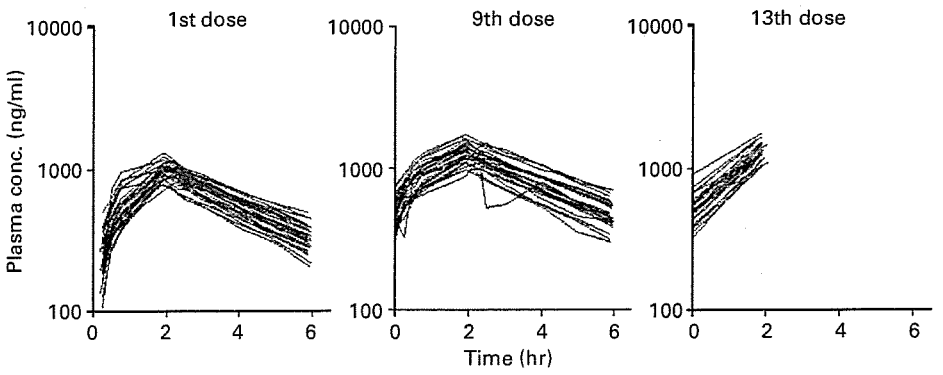

Figure 1 Observed plasma busulfan concentrations versus time.

Table 1 Parameter estimates of various models

Residual error	*IOV*	*CL (l/h)*	V *(l)*	*AIC*
CCV	No	8.56	28.9	7489
Additive	No	8.73	33.9	7235
Combination	No	8.73	33.8	7236
CCV	Yes	8.72	30.0	7421
Additive	Yes	8.77	33.4	7232
Combination	Yes	8.78	33.3	7233

IOV = interoccasion variability; CL = clearance; V = volume of distribution; AIC = akaike's information criterion; CCV = constant coefficient of variation model; additive = additive error model; combination = combination of the additive and CCV.

Table 2 Parameter estimates of the basic model

Parameter	*Estimates*
θ_{CL} (l/h)	8.77
θ_V (l)	33.4
ω_{CL} (%)	27.1
ω_V (%)	26.0
π_{CL} (%)	7.4
π_V (%)	1.4×10^{-3}
σ (ng/ml)	93.9

the basic pharmacokinetic model selected in the previous step. The parameter estimates could be obtained from all bootstrap data sets using the additive error model including IOV; however, one of 100 bootstrap data sets using the combination error model including IOV did not result in convergence. It was found that the additive model including IOV was more stable than the combination error model including IOV. Each parameter distribution of the additive error model including IOV is in a narrow range and almost unimodal (data not shown). Therefore, the additive error model including IOV was selected as the optimum basic model and was used in subsequent steps. Parameter estimates of the basic model are given in Table 2. As can be seen, the value of IOV in V is small, the decision was made whether the IOV introduces into V or not in subsequent steps. Plots of observed versus predicted concentration for the basic model are shown in Figure 2a.

Selection of covariate candidates

GAM analysis indicated that CL and V are functions of ABW (data not shown).

Population model building and stability of the final population models

The population model with covariates was built using the NONMEM program on the basis of the result of GAM analysis. ABW was found to be the predictor of both CL and V with a log-likelihood difference (LLD) of more than 10.83 ($P<0.001$) between each model in which ABW was introduced singly, and the basic model of each pharmacokinetic parameter modeled without ABW (data not shown). The full regression model was that following the allometric equations: $CL = \theta_1 \cdot (ABW/60)^{\theta 2}$, $V = \theta_3 \cdot (ABW/60)^{\theta 4}$, where θ_1 and θ_3 are the population values of CL and volume of distribution for the 60-kg patients. The IOV was not introduced into V in the population model since the IOV values obtained from each covariate model were negligible and the other parameter estimates were not changed by the introduction of IOV in V (data not shown). The full model was tested against the reduced models (Table 3).

The final population pharmacokinetic model obtained from the previous step was fitted repeatedly to the 200 bootstrapped samples. The parameter estimates of the final model using the original data and the mean parameter estimates obtained from the 200 bootstrap replicates are provided in Table 4. The mean parameter estimates were within 15% of those obtained with the original data set. Plots of observed versus predicted concentrations for the final model are shown in Figure 2b. Plots of individual parameter values obtained from the model-independent technique versus ABW are shown in Figure 3. The final

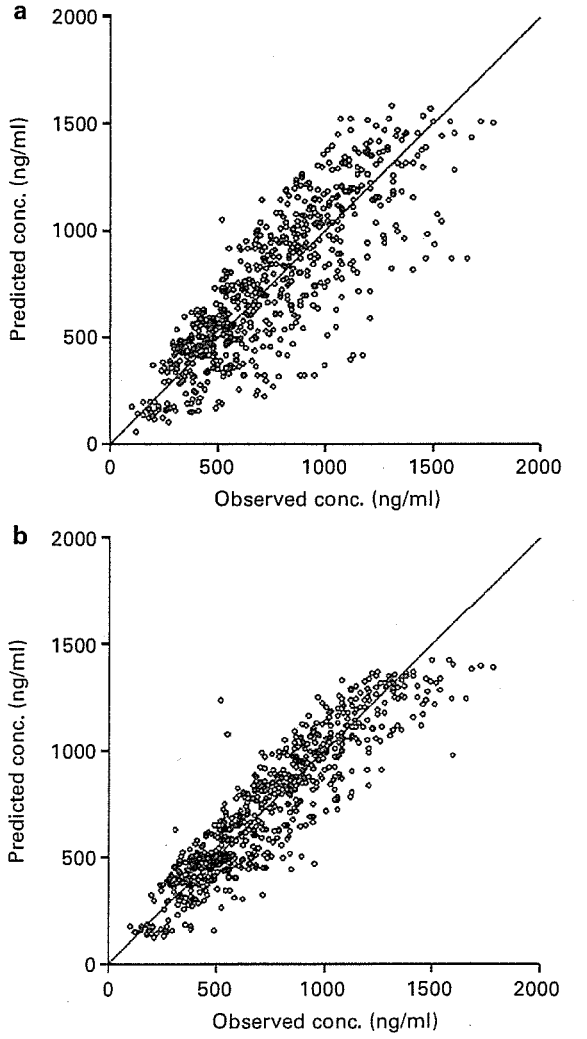

Figure 2 Plots of observed versus predicted concentration for the basic model (**a**) and for the final model (**b**).

Table 3 Comparison of the full and reduced model

Regression model	*LLD (versus full model)*
Full model	
$CL = \theta_1 \cdot (ABW/60)^{\theta 2}$	0
$V = \theta_3 \cdot (ABW/60)^{\theta 4}$	
Reduced model	
$\theta_2 = 0$	38.2*
$\theta_4 = 0$	64.9*

*$P < 0.001$.
LLD = log-likelihood difference.

Table 4 Typical population parameter estimates and stability of the final model

Parameters	*Typical population parameter estimate (s.e.)*[a]	*Mean population parameter estimate (s.e.)*[b]	*Difference (%)*[c]
θ_1[d] (l/h)	8.87 (0.23)	8.86 (0.23)	−0.1
θ_2[d]	0.833 (0.077)	0.833 (0.103)	0.0
θ_3[e] (l)	33.8 (0.6)	33.8 (0.7)	0.1
θ_4[e]	0.889 (0.049)	0.889 (0.060)	0.0
ω_{CL} (%)	13.6	13.2 (1.6)	−3.6
ω_V (%)	6.3	5.6 (2.3)	−12.3
π_{CL} (%)	6.6	6.1 (2.4)	−7.9
σ (ng/ml)	94.3	94.0 (8.3)	−0.3

[a]Obtained from the original data.
[b]Mean (s.e.) calculated from 200 bootstrap replicates.
[c](Bootstrap mean value-typical value from final model)/bootstrap mean value × 100(%).
[d]$CL = \theta_1 \cdot (ABW/60)^{\theta 2}$.
[e]$V = \theta_3 \cdot (ABW/60)^{\theta 4}$.

population model was well described the relationships between the pharmacokinetic parameters and ABW.

Discussion

The objective of population pharmacokinetic analysis was to characterize the pharmacokinetics of the intravenous busulfan including IOV and covariate relationships in patients. Reliability of results obtained from population analyses depends on the modeling procedure. Therefore, the evaluation of basic (covariate-free model) and final (covariate model) population pharmacokinetic models was performed using bootstrap resampling because of the limited number of patients in the study.

The one-compartment model with an additive error model including IOV in CL was selected as the population model during model development. The final population pharmacokinetic model built in the study was fitted to the 200 bootstrap samples. The mean parameter estimates obtained with the 200 bootstrap replicates of the data were within 15% of those obtained from original data. This indicates that the final model is stable.

With regard to the effect of covariates investigated in this analysis on the pharmacokinetic parameters of busulfan after intravenous infusion, the ABW was found to be a

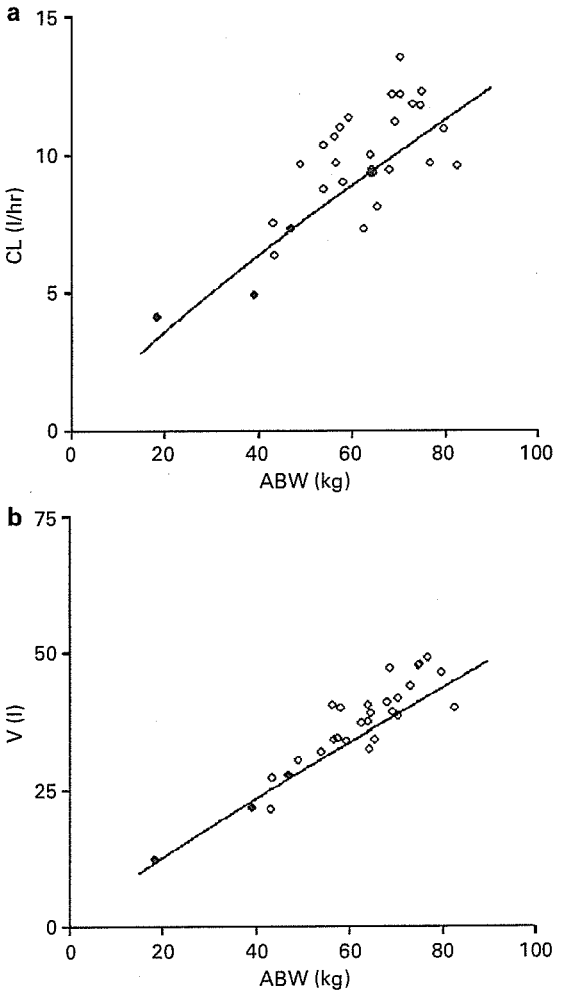

Figure 3 Plots of individual parameter values versus actual body weight. ABW, actual body weight. Clearance (CL) (**a**) and the volume of distribution (V) (**b**) after the first administration were calculated according to noncompartmental analysis using WinNonlin. Lines represent the estimates predicted the proposed allometric equations. Open and closed circles represent the value in adults and children, respectively.

determinant of CL and V. In the previous studies, age, ABW, body surface area (BSA), ALT and concomitant phenytoin treatment were reported as possible covariates of oral busulfan pharmacokinetics.[5,9,20,21] After the intravenous administration of busulfan, the relationships between ABW and pharmacokinetic parameters were reported.[13] Since physiological function was relatively well controlled in our study, variation of covariates was in a narrow range or within the normal limits. The limitation of developing population models based on such a small, relatively uniform patient population has been reported.[22] Therefore, the relationships between covariates and the pharmacokinetic parameters of intravenous busulfan need further investigation in a larger population, especially in younger children.

In general, a nomogram based on the population approach is a useful tool for dose adjustment, and therapeutic drug monitoring (TDM) is another powerful

tool. With TDM, some of the unknown interindividual variations can be quantified and total fluctuations in drug exposure can be reduced after dose adjustment. There are several reports about TDM techniques of oral busulfan.[20,23,26]

To compare our data with the oral busulfan studies in adult patients, we used previously published data.[23] The selected study has similar sampling points to our study and individual AUC were reported. Several reports indicate that an AUC of 900–1500 μmol min/l[4,6,24,25] in patients receiving a conventional busulfan regimen prevents treatment failure and the risk of fatal toxicities. In the previous study, the observed AUC at a steady state in 8/12 (66.7%) patients without dose adjustment fell in the above range. The percentage of patients within the range increased to 92.9% (13/14) with the dose adjustment according to the TDM results. In our study, the observed AUCs at steady state in 25/29 (86.2%) patients were within the range.

TDM requires blood sampling, drug concentration measurement and pharmacokinetic analysis. In the case of TDM after the administration of busulfan, it takes 1 or 2 days to adjust the dosage regimen using TDM data. Therefore, 25–50% of busulfan exposure remains uncontrollable in the standard regimen of 16 doses four times per day for 4 days. The contribution of the TDM for busulfan therapy is limited because of the reason described above. Intravenous busulfan may have an advantage over the oral busulfan in the limited term therapy since the systemic exposure of intravenous busulfan is expected to be reproducible throughout the treatment as compared with that of oral busulfan with TDM.

Oral busulfan is generally used at a dose of 1 mg/kg in adults, but the recommended dosage of intravenous busulfan is 0.8 mg/kg.[10 12] The previous report suggests that 1 mg/kg oral busulfan is a slight over-dosage.[27] According to the previous report, the benefit of the intravenous busulfan administration is not only the decrease in the variability of systemic exposure (AUC), but also the optimizing of average exposure during busulfan dosing.[28] In order to come to a definite conclusion of the clinical benefit of the intravenous busulfan, a prospective comparison of exposure of intravenous versus oral administration might be needed.

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ORIGINAL ARTICLE

Peripheral blood stem cell mobilization by granulocyte colony-stimulating factor alone and engraftment kinetics following autologous transplantation in children and adolescents with solid tumor

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In 56 pediatric and adolescent patients (median age 7 years, range 1–21) with various solid tumors, peripheral blood stem cells (PBSC) were mobilized with granulocyte colony-stimulating factor (G-CSF) alone, and the yields of PBSC and engraftment kinetics following autologous peripheral blood stem cell transplantation (PBSCT) were evaluated retrospectively. Granulocyte colony-stimulating factor (10 μg/kg) was injected subcutaneously for mobilization when patients showed no influence of previous chemotherapy, and administration was continued for 5 days. The peaks of CD34$^+$ cells and colony-forming units-granulocyte/macrophage in the blood were observed on days 4 through 6 of G-CSF administration in all patients. Peripheral blood stem cell harvest was commenced on day 5 of G-CSF treatment. Compared to the results in patients mobilized by chemotherapy plus G-CSF ($N=18$), the progenitor cell yields were lower in patients mobilized with G-CSF alone. However, there were no significant differences in WBC and ANC engraftment compared to the chemotherapy plus G-CSF mobilization group. Platelet recovery following autologous PBSCT was delayed in patients mobilized with G-CSF alone. The median time taken for ANC and platelet counts to reach 0.5×10^9 and 20×10^9/l was 12 days (range: 9–28) and 15 days (8–55), respectively, in all patients who received PBSC mobilized by G-CSF alone. In summary, mobilization with G-CSF alone can mobilize sufficient CD34$^+$ cells for successful autografting and sustained hematological reconstitution in pediatric and adolescent patients with solid tumors, and even in heavily pre-treated patients.
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Introduction

Autologous hematopoietic stem cell transplantation is increasingly performed for the treatment of various pediatric and adolescent cancers,[1,2] and currently most centers use peripheral blood stem cells (PBSC) as the source of stem cells to support high-dose chemotherapy. The advantages of PBSC include rapid hematopoietic recovery following transplantation, and less of a potential for tumor cell contamination of the grafts.[3] The dose of PBSC administered determines the success of engraftment,[4] which makes effective PBSC mobilization a critical step for the entire procedure. Methods for PBSC mobilization that are available for cancer patients include chemotherapy-induced mobilization, and mobilization by hematopoietic growth factor with or without chemotherapy.[5–8]

Although chemotherapy plus granulocyte colony-stimulating factor (G-CSF) is a standard mobilization regimen for patients with cancer,[9] there is great variability in predicting the optimal day to start collecting PBSC. In particular, it takes longer to reach the peak of circulating CD34$^+$ cells following pre-mobilization chemotherapy, especially in heavily pre-treated patients. Although the daily measurement of blood CD34$^+$ cells from the day of rising WBC to the day of apheresis has been used to determine the time to start collection,[10] the frequent measurement of CD34$^+$ cells is both time consuming and labor intensive. We sometimes miss the opportunity for collection when WBC is rising very slowly. Furthermore, owing to a paucity of technical staff, we seek to avoid collection procedures on weekends. These considerations motivated us to adopt mobilization with G-CSF alone for patients undergoing autologous transplantation. Mobilization with G-CSF alone is exclusively used for healthy donors, and the mobilization kinetics have been well studied.[11] Whether or not mobilization with chemotherapy plus G-CSF is preferable to mobilization with G-CSF alone remains to be established in the autologous setting. Mobilization with G-CSF alone in cancer patients might have advantages, such as ease of making a collection schedule without a daily determination of CD34$^+$ cells in

the blood, and the avoidance of neutropenic fever and additional transfusion. In addition, mobilization with G-CSF alone can be performed on an outpatient basis.

In this study, we examined the effectiveness of PBSC mobilization with G-CSF alone in pediatric and adolescent patients with solid tumors, including heavily treated patients.

Materials and methods

Patients

Data from 74 patients who were 21 years of age or younger and who underwent autologous PBSC harvest for primary cancer treatment between April 1997 and June 2005 were analyzed retrospectively. Their characteristics are shown in Table 1. The patients, who were mobilized with G-CSF alone ($N=56$), were categorized according to prior treatment: patients who received four or fewer cycles of chemotherapy with/without local irradiation (Group 1; $N=21$); patients who received more than four cycles of chemotherapy, or three or more cycles of chemotherapy with extended irradiation such as cerebrospinal irradiation (Group 2; $N=23$); and patients who have been mobilized previously and received high-dose chemotherapy with PBSC support following conventional chemotherapy, and then they had a recurrent disease, and mobilization was attempted again (Group 3; $N=12$). In Group 3, the median time from the last high-dose chemotherapy to PBSC harvest was 8.5 months (range: 3–48). Patients who were mobilized with chemotherapy followed by G-CSF ($N=18$) during the same period in our institute were also evaluated as a control group. Groups 1–3, and the control group were comparable in age. The clinical protocols for peripheral blood stem cell transplantation (PBSCT) were approved by the Institutional Review Board at Tokushima University Hospital. Written informed consent was obtained from the patients (if the patient was more than 10 years old) or their guardians.

Peripheral blood stem cell mobilization and harvest

For mobilization with G-CSF alone, G-CSF was initiated during the steady state, when blood cells had recovered from the influence of chemotherapy. The time from the last day of chemotherapy to the commencement of G-CSF averaged 19 days (range: 0–35; 0 means that patients did not receive chemotherapy before mobilization) in Group 1, 19 days (11–41) in Group 2 and 29 days (15–85) in Group 3 (Table 1). Granulocyte colony-stimulating factor (10 μg/kg) was administered for 5 consecutive days. Principally, G-CSF was injected subcutaneously once a day at 0900 each day. Blood samples for daily analysis of complete blood count, colony-forming unit-granulocyte/macrophage (CFU-GM) assay and $CD34^+$ cell assay were drawn immediately before the injection of G-CSF. On day 5 of G-CSF treatment, after the fifth injection of G-CSF, PBSC harvest was commenced using a continuous cell separator.

For mobilization with chemotherapy plus G-CSF, the patients received disease-oriented chemotherapy; G-CSF was started when WBC reached a nadir, and complete blood counts were followed. The dose of G-CSF was 5 μg/kg intravenously, and was continued daily until the completion of PBSC harvest. We usually started to harvest PBSC when the platelet count reached over 100×10^9/l without transfusion support, with a concomitant rising WBC. Chemotherapeutic drugs and dosages used for mobilization were etoposide 500 mg/m^2 and cisplatin 90 mg/m^2 ($N=8$), carboplatinum 800 mg/m^2 and etoposide 375 mg/m^2 ($N=5$), doxorubicin 75 mg/m^2 and ifosphamide 7.5 g/m^2 ($N=3$), and high-dose cytosine arabinoside 20 g/m^2 ($N=2$). Circulating $CD34^+$ cells and CFU-GM were not determined in this group.

Apheresis was usually initiated at 1000 and 150–300 ml/kg (max. 10 l) was processed per session. The details of the harvesting procedure have been described previously.[12] Briefly, mostly in children less than 10 years old, blood was drawn from a radial artery using a 20–24 gauge catheter and given back to an antecubital vein using an 18–24 gauge catheter. For donors weighing less than 20 kg, the extracorporeal circuit was pre-primed with donated WBC-depleted blood, depending on the machine and separating chambers. In adolescents, we used antecubital veins to collect PBSC. Calcium gluconate was continuously infused at a dose of 1 ml per processed 200 ml blood during apheresis. The target doses were set at $>2 \times 10^6$/kg $CD34^+$ cells for one course of high-dose therapy. In 19 of 56 patients who were mobilized with G-CSF alone, double PBSCT was planned, along with repeated aphereses to support two rounds of high-dose chemotherapy. Two aphereses were performed on day 5 in most patients, and additional aphereses were performed on days 6 and 7. Peripheral blood stem cells were cryopreserved in 10% dimethylsulfoxide in a deep freezer (−150°C) until use.

Mobilization failure was defined as a patient who never reached a $CD34^+$ cell level in the blood of 20×10^6/l, or a patient from whom a proper $CD34^+$ cell level was not collected in the first apheresis product, and in such cases we did not attempt further apheresis.

Flow cytometer analysis for CD34+ cells

One hundred microliters of cell suspension were added to a test tube containing isotype control (phycoerythrin-mouse IgG1) and phycoerythrin-conjugated CD34 monoclonal antibody (anti-HPCA2 antibody; Becton Dickinson, San Jose, CA, USA) at a concentration of 1 μg antibody/10^6 cells. MNCs from samples were incubated for 30 min, and then washed twice. Samples were analyzed with a FACScan flow cytometer (Becton Dickinson). After the function was verified, samples were drawn into the flow cytometer using forward scatter and side scatter, as gating parameters, along with debris subtraction techniques to determine the characteristics of the cells. A total of 20 000 events were counted to identify the mononuclear cell fraction. The flow cytometric data were analyzed using a gated analysis via a set of SSC-FL parameters for $CD34^+$ cells to calculate the percentage of positive cells.

The number of circulating $CD34^+$ cells was calculated using the following formula:

$$\begin{aligned} CD34^+ \text{ cells/l} = &\text{WBC } (\times 10^6/\text{l}) \\ &\times \text{percentage of MNC } (\%/100) \\ &\times \text{frequency of positive cells } (\%/100) \end{aligned}$$