HLA に関する資料集

（ 医 師 の 方 へ ）

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コーディネート過程において、適切なドナーを早い段階で選択することが、迅速な移植を実現する上で重要な鍵の一つとなっております。この度、ドナー選択に役立てていただきたい資料を一冊にまとめましたので、どうぞご活用ください。＊過去に配布済みの資料については当時の日付にて掲載しております。

なお、ドナー選択には患者さんのアリルデータが欠かせません。患者確認検査は登録後すぐに実施願います。化学療法中で白血球数が不足している、近日中の外来予定がないなど、採血までに日数を要する場合にはお問い合わせください。

お問い合わせ先：移植調整部　TEL03-5280-4771／FAX03-5280-3856

1. ドナー検索時の評価点

ドナーは評価点に基づき優先順位が付けられ検索されます。
評価点は解析結果をもとに、点数配分されています。

1-1. ランク評価点

ランク	評価点
6 抗原マッチ	400,000
DR ミスマッチ	300,000
B ミスマッチ	200,000
A ミスマッチ	100,000

＊　現在、C 座の血清ミスマッチに関してはランク評価点がありません。
＊　ただし、アリルが判明している場合は下記のように評価対象となります。

1-2. HLA 型評価点

	HLA-A	HLA-B	HLA-DR	HLA-C
アリルマッチ(抗原ごと)	700	700	100	200
コードマッチ(抗原ごと)	650	650	50	150
抗原マッチ				0
アリルミスマッチ(ローカスごと)	-24,000	-24,000	-8,000	-12,000
抗原ミスマッチ				0
血清型 split match	30			

A、B、DR 3 ローカス全て
スプリットマッチなら 30 点加算
(患者が血清のみの場合)

1-3. その他評価点

ランク	評価点
血液型 ABO 一致	4
血液型 Rh 一致	3
体重比率	120% ＜ 3
性別一致	0
年齢	20～29 歳:2 30～39 歳:1
居住地域	0

1-4.ドナープールの階層状況と HLA 型評価点　～血清フルマッチを例として～

対象ドナー	ドナーの HLA	アリルの適合度	評価点
血清フルマッチ	アリル 4 桁 判明	A,B,C,DR アリルフルマッチ	①403,400
	血清 2 桁のみ(アリル未検査)	不明	②400,000
	アリル 4 桁 判明	DR1アリルミスマッチ	③395,300
		・ 中略 ・	・ 中略 ・
		A,B,C,DR4座 8 抗原全て アリルミスマッチ	④332,000

ミスマッチ検索対象ドナーについても、同様に、マッチなら加点、ミスマッチなら減点され、順位が決まります。

2. 患者の HLA 抗原と検索されるドナーの HLA 抗原

ブロード抗原とスプリット抗原

Broad specificities	Split and associated antigens#
A10	A25, A26, A34, A66
A19	A29, A30, A31, A32, A33, A74
A2	A203#, A210#
A28	A68, A69
A9	A23, A24, A2403#
B12	B44,B45
B14	B64,B65
B15	B62,B63,B75,B76,B77
B16	B38,B39,B3901#,,B3902#
B17	B57,B58
B21	B49,B50,B4005#
B22	B54,B55,B56
B27	B2708#
B40	B60,B61
B5	B51,B52,B5102#,B5103#
B7	B703#
B70	B71,B72
C3	C9, C10
DR1	DR103#
DR2	DR15, DR16
DR3	DR17, DR18
DR5	DR11, DR12
DR6	DR13, DR14, DR1403#, DR1404#
DQ1	DQ5, DQ6
DQ3	DQ7, DQ8, DQ9

(WHO Nomenclature for Factors of the HLA System, 1996 **より**)

実際の例

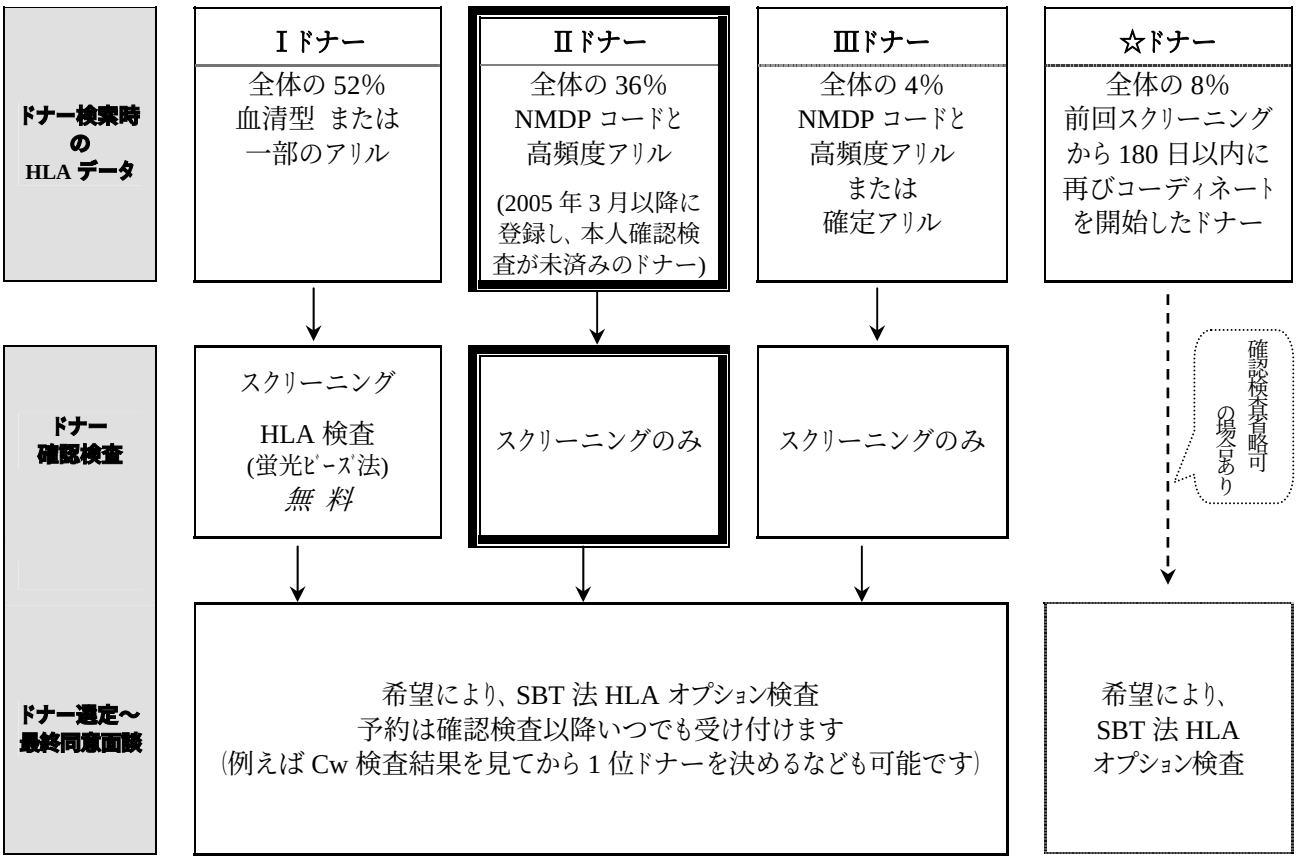
患者の抗原	検索されるドナーの抗原	
	スプリット	ブロード
B16		B16 B38 B39 B3901 B3902
B38	B38	B16
B39	B39	B3901 B3902 B16
B3901	B3901	B39 B16 B3902
B3902	B3902	B39 B16 B3901
DR2		DR2 DR15 DR16
DR5		DR5 DR11 DR12
DR6		DR6 DR13 DR14 DR1403 DR1404
DR11	DR11	DR5
DR12	DR12	DR5
DR13	DR13	DR6
DR14	DR14	DR6 DR1403 DR1404
DR1404	DR1404	DR6 DR14 DR1403
DR15	DR15	DR2
DR16	DR16	DR2

例 1 患者 HLA-DR2 で検索する場合
↓
HLA-DR2／DR15／DR16 の
ドナーが検索されます

例 2 患者 HLA-DR15 で検索する場合
↓
HLA-DR2／DR15 の
ドナーが検索されます
(HLA-DR16 は検索されません)

3. ドナーの確認検査とコーディネートの流れ

ドナーが検索された時点で保持している HLA データによって、ドナー確認検査の検査項目が異なります。



Ⅰ・Ⅱ・Ⅲ・☆ドナーの%は 2006/10～2007/3 に検索されたドナーを全体数としたデータです。

3-1. SBT 法による HLA オプション検査

① SBT 法 HLA 検査を実施するかどうかは、蛍光ビーズ法 HLA 検査結果が判明した後に、「蛍光ビーズ法による高頻度アリルと SBT 法による確定アリルの比較表」(P.10～12 参照)を参照するなどして、高頻度アリルの意味をよくご理解いただいた上で、担当医師がご判断ください。現時点のデータでは、高頻度アリルと確定アリルは 99%以上の確率で一致しています。

② SBT 法 HLA 検査は HLA-A、B、DRB1 の他に、C、DP.B1、DQB1 も受付けております。高頻度アリルの結果を見た上で、さらに高精度の検査(SBT 法)をご希望の場合はご連絡ください。

③ スクリーニング検査時に採血した残検体を使用するため、ドナーの方から改めて採血する必要はありません。

④ SBT 法 HLA 検査は有料(患者負担)です。

4. HLA に関する詳細説明

4-1. HLA 検査結果レベル

HLA 検査で出される結果のレベルは、検査方法･試薬によって決定されます。下表は検査で 出された結果の例です。

		A locus	B locus	DR locus
血清学的検査	ブロード抗原	A19	B40	DR5
	スプリット抗原	A31	B61	DR11
DNA 検査	Low Resolution	A＊31	B＊40	DRB1＊11
	Middle Resolution	A＊3101/3102	B＊4001/4006/4009	DRB1＊1101/1104/1105/1106
	High Resolution	A＊3101	B＊4006	DRB1＊1104

SBT 法は このレベル

蛍光ビーズ法は このレベル

「 / (スラッシュ)」は 「または」の意

4-2. 用語説明

血清対応型	検査の結果得られた DNA 型について対応する血清型。
確定アリル	SBT 法で検査した結果。
NMDP コード	アリルの組み合わせをアルファベットで コード化したもの。Middle Resolution レベル。 NMDP が定義し世界各国で使用。 (システム構築が完了するまでは、表記されません。)
高頻度アリル	確定したアリルではない。NMDP コードで表記したアリルのなかで、日本人に最も高頻度に見られるアリル。 日本人で 0.1%以下の頻度で存在するアリルは無視した上で 4 桁表記したもの。
座（ローカス） と 抗原	HLA の適合度を表現する際に「1座不一致」、「2座不一致」ということをみかけますが、これは正確な表現法ではありません。「座」とは遺伝子座、つまり A 座、B 座、DR 座などを意味します。HLA の適合度については「抗原の不一致」なのか「アリルの不一致」なのかを明確にして表現する必要があります。抗原での表現法を例に取ってみると、「1座不一致」といっても同一遺伝子座では2つの抗原がありますので、一方のみが不一致である「1抗原不一致」と両方が不一致である「2抗原不一致」の場合があります。「2座不一致」では2～4抗原不一致までが含まれることになります。

HLA 検査の結果は、4 つ(血清型･確定アリル･NMDP コード･高頻度アリル)に分類して表示します。

5. 検査方法

5-1. 蛍光ビーズ法(PCR-rsso 法)

蛍光ビーズ法では、多くの場合アリル(DNA)データを1つに特定することはできませんが、検査結果はNMDP コードで表記し、可能性があるアリルを絞り込むことが可能です。

NMDP コード	NMDP コードの要素 (可能性があるアリル)	高頻度アリル
例 1) DRB1＊01EW	DRB1＊0101/05/07/08/11	0101
例 2) DRB1＊15GEP	DRB1＊1501/06/13	1501

＊ NMDP コードが意味する具体的なアリルは、NMDP のホームページにある“NMDP Allele Code List“でご覧になれます。　URL ： http://bioinformatics.nmdp.org/HLA/allele_code_lists.html

＊ 各コードの要素は次ページ Lookup Tool　をご利用ください

URL ： http://bioinformatics.nmdp.org/cgi-bin/HLA/ALLELE/dnatyp.pl

5-2. SBT 法

High resolution レベルの DNA タイピングで、遺伝子の塩基配列を幅広く解析するため、非常に精密な測定が可能です。

5-3. コード検索の例

＜B75（B1507）を持つ患者さんに B62(高頻度アリル：1501)を持つドナーが検索される理由＞

高頻度アリル：B1501、血清対応型：B62（NMDP コード：B15BNUF）のドナーがコードマッチドナーとして上位に検索される場合があります。これは、次のような仕組みによります。

ドナーの HLA 検査（蛍光ビーズ法）の結果である B15BNUF は、NMDP コードの要素として次ページに示すとおり、1501/1507・・など 31 種類のアリルの可能性を持ちます。そのうち日本人と仮定した場合に最も高頻度で見られるアリルは B1501（血清対応型 B62）で高頻度アリルとしています。このとき、31 種類のアリルは B15BNUF のコードマッチとして評価されるため全て同等であり、高頻度アリル B1501 を特別に高く評価はしておりません。

5-4. DNA Type Lookup Tool　：　NMDPコードからアリル要素を調べる方法

Locus:

☐ A ◉ B ☐ C

☐ DRB1 ☐ DRB3 ☐ DRB4 ☐ DRB5

☐ DQA1 ☐ DQB1 ☐ DPA1 ☐ DPB1 ☐ None

Enter a list of alleles and/or allele codes:

15BNUF

クエリ送信 リセット

Allele Code:

B*15BNUF -> B*1501/1501N/1504/1505/1507/1508/1512/1514/1515/1519/1520/1523/1524/1525/1532/1533/1534/1537/1550/1556/1557/1570/1575/1578/1579N/1581/1582/1585/1592/1594N/1596

BNUF -> 1501/1501N/1504/1505/1507/1508/1512/1514/1515/1519/1520/1523/1524/1525/1532/1533/1534/1537/1550/1556/1557/1570/1575/1578/1579N/1581/1582/1585/1592/1594N/1596

ACTIVATED for use at:

B*15BNUF

6. その他 ドナー検索に関する参考情報

6-1.個別のドナープール状況

希望するドナーが検索されない場合などには、移植調整部までお問い合わせください。検索されたドナー以外にどんなドナーがいるかご案内できます。

例えばこんなことをご案内できます。

例1　血清フルマッチドナーが 100 人もいるのに、確認検査を実施してみるとどのドナーもアリルが 2 つ以上異なる。時間と費用ばかりかかっている。
↓
アリルが判明しているドナーの中に、1アリルミスマッチドナーがいるか

例2　検索されるドナーはいずれもアリルまで判明しているが、どのドナーも患者と不適合。
↓
仮に、血清1抗原不適合検索を行った場合、6 分の 5 アリルマッチドナーがいるか

例3　患者さんが希なアリルを持つため、適合ドナーがなかなかみつからない
↓
希なアリルを適合させることを諦めた場合、他の 5 アリルがマッチしているドナーがいるか

例4　例えば B 座がホモの患者さん、フルマッチドナーがいない
↓
B 座がヘテロで、他の 5 アリルがマッチしているドナーがいるか

※お伝えするドナー状況は、あくまでもその時点での情報であり、検索の都度変わる可能性があることをご了承ください。

HLA 相談窓口

当財団では、専門家による「HLA 相談窓口」を設置し、主治医からの HLA に関する問い合わせを受付けています。HLA に関するご質問をお寄せください。回答には約 1 週間を要します。

URL ： http://www.jmdp.or.jp/pt/coordinat/HLA.html

- *依頼は医師からのみ受け付けています。*
- *コンサルティングの結果はあくまでも参考意見であることをご理解いただき、最終的には担当医師がご判断ください。*

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7. HLA適合度をみる場合の判断例

7-1. 珍しいアリルを持つ場合の判断例

① 患者が非常に珍しいアリルを持つ場合は、そのアリルと一致するドナーがみつかる可能性は非常に低いので、アリル型適合のドナー検索は困難と認識して、他の選択肢を考慮する。
② 逆にドナーが非常に珍しいアリルを持つ場合は、そのドナーは避けるか選ばないようにすることで、適合性を高めることができる。
③ P.10～12「SBT法による確定アリルと蛍光ビーズ法による高頻度アリルの比較表（以下比較表）」に、 高頻度アリルでは同定できない確定アリルとその頻度が示されている（緑色）が、この高頻度アリルが確定アリルと異なる可能性はきわめて低いが存在する。なお、この比較表で緑色で表示されている確定アリルと高頻度アリルの不一致の組合せは、あくまでも今回検討対象となった17，552例のサンプルにおけるもので、その他のアリルにおいてもそれぞれの頻度にしたがって両者の不一致はあり得るものである。したがって、緑色の表示がない場合でも両者の不一致がないことを保証するものではない。
④ ドナーの「SBT法によるHLAオプション検査」は、「特殊なアリル型（Nullアリル*を含む）をもつドナーを避けられる」意味がある。

＊Nullアリル
非発現型のアリル。DNA検査法ではアリルとして認められ表記もされるが、血清型は表記されない。

7-2. 個別の例（A座を例にして）

① 患者が0215Nを持つ場合、その遺伝子があってもA2抗原は患者に「表現」されないため、GVHD方向の不適合にならないので臨床的には無視してよい。ただし、選ばれたドナーがA2抗原を表現しているので、HVG方向の不適合になる。
② 逆に、ドナーが0215Nを持つ場合、患者のA2分子はGVHD方向のミスマッチ標的になり、そのドナーを選択することを避けなければならない。
③ 患者がA*2624を持つ場合は、A*2624をもつドナーの検索はほぼできない。
④ A2 アリル型は合わせることを原則にすべきである。GVHDの発症率と重症化傾向が高く、予後不良因子となり得る。
⑤ A26アリル型は合わせることを原則とすべきである。ドナーがまれなA26アリル（A*2605,*2606,*2624）であることがわかれば、それ以外の（患者さんに適合する）ドナーを選ぶことを推奨する。患者さんがまれなA26アリル型であるときは、アリル型適合ドナーの選択をあきらめ、次善のドナーを選択する。
⑥ A24については、ドナーがまれなアリル型を持っていて、患者（多くはA*2402)に不適合のときはそのドナーを除外して選択することでよいことが多い。患者にまれなA24 アリル型があったときは、その適合を図ることで、時間とコストをかける必要はない。
⑦ まれなA*1121N（Null allele）がドナーとして選択されないように気をつける。

参考：HLAアリルの表記法

HLA ＝ ヒトMHC領域にありHLA遺伝子として同定されている遺伝子の総称
HLA-DRB ＝ HLA遺伝子領域内の座位の名称、DR領域には他に、DRB1、DRB2（偽遺伝子）、DRB3（DRB1*11,*12,*13, *14などに連鎖して存在）、DRB4（DRB1*04,*07,*09に連鎖して存在）、DRB5（DRB1 *15, *16などに連鎖して存在）などがあり、それらの総称
HLA-DRB1 ＝ DRB遺伝子のうち、最初に同定された遺伝子座位の名称
HLA-DRB1*13 ＝ アリルの総称。DR13抗原を産生するアリルの総称。俗称：2桁アリル名
HLA-DRB1*1301 ＝ 通常のアリル名。俗称：4桁アリル名
HLA-DRB1*1301N ＝ Null（ナル）アリル。遺伝子は存在するが、完全な分子としてのHLA抗原が表現されない（途中に stop codonが入っている）。
HLA-DRB1*130102 ＝ *1301アリルの一種であるが、塩基に「同義置換」がある。アミノ酸変異を伴わないので、免疫学的（臨床的）にはDRB1*1301と同じに扱うことでよい。俗称：6桁アリル名
HLA-DRB1*13010102 ＝ *1301アリルの一種であるが、非翻訳領域に塩基置換がある。免疫学的（臨床的）にはDRB1*1301と同じに扱うことでよい。俗称：8桁アリル名
HLA-DRB1*13010101N ＝ DR13 Null アリルの一種であるが、非翻訳領域に塩基置換がある

8. SBT 法による確定アリルと蛍光ビーズ法による高頻度アリルの比較表

8-1. A ローカス

抗原	N=	遺伝子頻度	蛍光ビーズ法 高頻度アリル	SBT 法 確定アリル	N'=	抗原型内頻度
A1	67	0.38%	**0101**	**0101**	**14**	100.00%
A2	4,274	24.35%	**0201**	**0201**	**1,818**	43.88%
			0201	**0275**	**1**	0.02%
			0203	**0203**	**4**	0.10%
			0205	**0205**	**1**	0.02%
			0206	**0206**	**1,548**	37.36%
			0207	**0207**	**700**	16.90%
			0207	**0215N**	**3**	0.07%
			0210	**0210**	**51**	1.23%
			0211	**0211**	**1**	0.02%
			0218	**0218**	**10**	0.24%
			0228	**0228**	**5**	0.12%
			0242	**0242**	**1**	0.02%
A3	42	0.24%	**0301**	**0301**	**14**	100.00%
A11	1,445	8.23%	**1101**	**1101**	**545**	98.20%
			1101	**1121N**	**1**	0.18%
			1102	**1102**	**9**	1.62%
A24	6,971	39.72%	**2402**	**2402**	**2,473**	97.67%
			2402	**2425**	**1**	0.04%
			2402	**2449**	**1**	0.04%
			2408	**2408**	**2**	0.08%
			2420	**2420**	**55**	2.17%
A26	2,225	12.68%	**2601**	**2601**	**1,391**	64.97%
			2601	**2624**	**1**	0.05%
			2602	**2602**	**350**	16.35%
			2603	**2603**	**384**	17.94%
			2605	**2605**	**14**	0.65%
			2606	**2606**	**1**	0.05%
A30	31	0.18%	**3001**	**3001**	**9**	100.00%
A31	1,320	7.52%	**3101**	**3101**	**509**	99.80%
			3101	**3111**	**1**	0.20%
A32	1	0.01%				
A33	1,176	6.70%	**3303**	**3303**	**410**	100.00%
合計	17,552	100.00%			10,328	

＊exon4 の多型は下線で示した。
蛍光ビーズ法では exon2 および 3 を、SBT 法では exon2、3 および 4 について検査を行っていることから、exon4 のみに塩基置換を伴うアリルに関しては蛍光ビーズ法（高頻度アリル）と SBT 法（確定アリル）とで異なる結果となる。
＊蛍光ビーズ法と SBT 法とで異なるアリルを緑色で示した。
＊Nの合計 17,552 は、2003/10/27 以降に採血しＳＢＴ法ＨＬＡ確認検査を実施した、
患者･ドナーの合計 8,776 人のAローカス 2 抗原（17,552 件）を指す。

8-2. B ローカス

抗原	N=	遺伝子頻度	蛍光ビーズ法 高頻度アリル	SBT 法 確定アリル	N'=	抗原型内頻度
B7	1,069	6.09%	**0702**	**0702**	**351**	100.00%
B13	157	0.89%	**1301**	**1301**	**25**	83.33%
			1302	**1302**	**5**	16.67%
B27	28	0.16%	**2704**	**2704**	**8**	72.73%
			2705	**2705**	**3**	27.27%
B35	1,526	8.69%	**3501**	**3501**	**408**	99.51%
			3503	**3503**	**1**	0.24%
			3552	**3552**	**1**	0.24%
B37	78	0.44%	**3701**	**3701**	**23**	100.00%
B38	22	0.13%	**3802**	**3802**	**10**	100.00%
B39	655	3.73%	**3901**	**3901**	**556**	87.01%
			3902	**3902**	**42**	6.57%
			3904	**3904**	**41**	6.42%
B44	1,110	6.32%	**4402**	**4402**	**7**	2.41%
			4403	**4403**	**283**	97.59%
B46	928	5.29%	**4601**	**4601**	**281**	100.00%
B48	399	2.27%	**4801**	**4801**	**96**	100.00%
B50	1	0.01%				
B51	1,665	9.49%	**5101**	**5101**	**421**	98.83%
			5102	**5102**	**5**	1.17%
B52	2,176	12.40%	**5201**	**5201**	**597**	100.00%
B54	1,333	7.59%	**5401**	**5401**	**332**	100.00%
B55	380	2.16%	**5502**	**5502**	**100**	97.09%
			5504	**5504**	**3**	2.91%
B56	136	0.77%	**5601**	**5601**	**28**	73.68%
			5603	**5603**	**9**	23.68%
			5604	**5604**	**1**	2.63%
B57	2	0.01%				
B58	77	0.44%	**5801**	**5801**	**15**	100.00%
B59	314	1.79%	**5901**	**5901**	**81**	100.00%
B60	858	4.89%	**4001**	**4001**	**273**	99.27%
			4001	**4055**	**1**	0.36%
			4054	**4054**	**1**	0.36%
B61	2,583	14.72%	**4002**	**4002**	**1,465**	59.36%
			4002	**4056**	**1**	0.04%
			4002V8	**4002V8**	**1**	0.04%
			4003	**4003**	**73**	2.96%
			4004	**4004**	**1**	0.04%
			4006	**4006**	**926**	37.52%
			4050	**4050**	**1**	0.04%
B62	1,540	8.77%	**1501**	**1501**	**1,341**	89.70%
			1501	**1501V5**	**9**	0.60%
			1507	**1507**	**116**	7.76%
			1515	**1515**	**1**	0.07%
			1527	**1527**	**20**	1.34%
			1528	**1528**	**7**	0.47%
			1592	**1592**	**1**	0.07%
B67	144	0.82%	**6701**	**6701**	**36**	100.00%
B71	237	1.35%	**1518**	**1518**	**67**	100.00%
B72	1	0.01%	**1546**	**1546**	**1**	100.00%
B75	132	0.75%	**1502**	**1502**	**4**	3.05%
			1511	**1511**	**127**	96.95%
B77	1	0.01%				
合計	17,552	100.00%			8,206	

＊exon4 の多型は下線で示した。

蛍光ビーズ法では exon2 および 3 を、SBT 法では exon2、3 および 4 について検査を行っていることから、exon4 のみに塩基置換を伴うアリルに関しては蛍光ビーズ法（高頻度アリル）と SBT 法（確定アリル）とで異なる結果となる。

＊蛍光ビーズ法と SBT 法とで異なるアリルを緑色で示した。
＊Nの合計 17,552 は、2003/10/27 以降に採血しＳＢＴ法ＨＬＡ確認検査を実施した、
患者･ドナーの合計 8,776 人の B ローカス 2 抗原（17,552 件）を指す。

8-3. DR **ローカス**

抗原	N=	遺伝子頻度	**蛍光ビーズ法高頻度アリル**	**SBT 法確定アリル**	**N'=**	抗原型内頻度
DR1	1,053	6.00%	**0101**	**0101**	**1,053**	100.00%
DR4	4,420	25.18%	**0401**	**0401**	**207**	4.68%
			0403	**0403**	**582**	13.17%
			0403	**0452**	**1**	0.02%
			0404	**0404**	**53**	1.20%
			0405	**0405**	**2,447**	55.36%
			0406	**0406**	**632**	14.30%
			0407	**0407**	**84**	1.90%
			0410	**0410**	**413**	9.34%
			0413	**0413**	**1**	0.02%
DR7	48	0.27%	**0701**	**0701**	**48**	100.00%
DR8	2,162	12.32%	**0802**	**0802**	**650**	30.06%
			0803	**0803**	**1,505**	69.61%
			0809	**0809**	**6**	0.28%
			0823	**0823**	**1**	0.05%
DR9	2,700	15.38%	**0901**	**0901**	**2,700**	100.00%
DR10	72	0.41%	**1001**	**1001**	**72**	100.00%
DR11	389	2.22%	**1101**	**1101**	**386**	99.23%
			1123	**1123**	**3**	0.77%
DR12	660	3.76%	**1201**	**1201**	**474**	71.82%
			1202	**1202**	**185**	28.03%
			1205	**1205**	**1**	0.15%
DR13	1,096	6.24%	**1301**	**1301**	**51**	4.65%
			1302	**1302**	**1,043**	95.16%
			1307	**1307**	**2**	0.18%
DR14	1,548	8.82%	**1401**	**1401**	**621**	40.12%
			1402	**1402**	**4**	0.26%
			1403	**1403**	**307**	19.83%
			1405	**1405**	**362**	23.39%
			1405	**1445**	**1**	0.06%
			1406	**1406**	**235**	15.18%
			1407	**1407**	**12**	0.78%
			1412	**1412**	**5**	0.32%
			1429	**1429**	**1**	0.06%
DR15	3,299	18.80%	**1501**	**1501**	**1,232**	37.34%
			1502	**1502**	**2,065**	62.63%
			1506	**1506**	**1**	0.03%
			1515	**1515**	**1**	0.03%
DR16	99	0.56%	**1602**	**1602**	**99**	100.00%
DR17	6	0.03%	**0301**	**0301**	**6**	100.00%
合計	17,552	100.00%			17,552	

＊蛍光ビーズ法と SBT 法と異なるアリルを緑色で示した
蛍光ビーズ法では exon2 および 3 を、SBT 法では exon2、3 および 4 について検査を行っていることから、exon4 のみに塩基置換を伴うアリルに関しては蛍光ビーズ法（高頻度アリル）と SBT 法（確定アリル）とで異なる結果となる。

＊Nの合計 17,552 は、2003/10/27 以降に採血しＳＢＴ法ＨＬＡ確認検査を実施した、
患者･ドナーの合計 8,776 人の DR ローカス 2 抗原（17,552 件）を指す。

9. 解析資料集

＜目次＞

重症急性 GVHD ハイリスクな HLA 型の組み合わせについて

2007 年 3 月　骨髄移植推進財団　HLA 委員会

日本骨髄バンクを介した非血縁者間骨髄移植の成績とドナー･患者 HLA 型との解析から、重症急性 GVHD が起こりやすい特定の HLA 型の組み合わせが次第に明らかになってきました。骨髄移植推進財団 HLA 委員会では、ドナー選択の上で、これらの重症急性 GVHD が起こりやすい HLA 型の組み合わせの情報が有用であると判断しましたので、移植病院の先生方にお知らせすることとしました。

重症GVHDと生存に関与する不適合HLA型の組み合わせ （JMDP解析 2007年2月)

Mismatch Combination**	N	HR (95% CI) for aGVHD	P	HR (95% CI) for OS	P	
A*0206-A*0201	131	1.78 (1.32-2.41)	<0.001	1.41 (1.13-1.75)	0.002	●
A*0206-A*0207	27	3.45 (2.09-5.70)	<0.001	1.83 (1.16-2.90)	0.009	●
A*2602-A*2601	21	3.35 (1.89-5.91)	<0.001	1.58 (0.89-2.79)	0.115	
A*2603-A*2601	35	2.17 (1.29-3.64)	0.003	1.27 (0.83-1.94)	0.266	
B*1501-B*1507	19	3.34 (1.85-5.99)	<0.001	1.82 (1.07-3.12)	0.027	●
C*0303-C*1502	25	3.22 (1.75-5.89)	<0.001	1.50 (0.91-2.47)	0.111	KIR2DLリガンド
C*0304-C*0801	69	2.34 (1.55-3.52)	<0.001	1.26 (0.91-1.74)	0.158	
C*0401-C*0303	42	2.81 (1.72-4.60)	<0.001	1.95 (1.36-2.79)	<0.001	●KIR2DLリガンド
C*0801-C*0303	80	2.32 (1.58-3.40)	<0.001	1.52 (1.13-2.03)	0.004	●
C*1402-C*0304	23	3.66 (2.00-6.68)	<0.001	0.77 (0.38-1.56)	0.482	
C*1502-C*0304	27	3.77 (2.20-6.47)	<0.001	1.49 (0.90-2.45)	0.115	
C*1502-C*1402	50	4.97 (3.41-7.25)	<0.001	1.82 (1.28-2.59)	0.001	●KIR2DLリガンド
DRB1*0405-DRB1*0403	53	2.13 (1.28-3.53)	0.003	1.19 (0.79-1.77)	0.39	
DRB1*1403-DRB1*1401	23	3.19 (1.77-5.73)	<0.001	1.45 (0.86-2.46)	0.16	

**ドナーHLA型ー患者HLA型

[表の説明]

5,200 例の HLA-A,B,DR 血清型適合移植例につき、HLA-A,B,C,DRB1,DQB1,DPB1 のアリル型を後方視的に同定し、各 HLA 座の適合度と臨床因子を考慮に入れ、Cox hazard model による多変量解析を実施しました。それぞれの HLA 型不適合の組み合わせ別に、急性 GVHD の発症危険率を同一 HLA 座の適合症例との相対危険率（Hazard Ratio: HR）として計算しました。これらのうち、有意水準を P <0.005 としても GVHD 発症の相対危険率が高いと判断された HLA 型不適合の組み合わせを抽出し、さらに、ブートストラップ法でも有意差が確認されたものを、重症 GVHD ハイリスクな組み合わせと定義しました。合わせて、これらの組み合わせにつき、移植後死亡の相対危険率も示しました。

ドナー選択時の参考資料とする場合には、**以下の点に留意して**ご利用ください。

1．表で示した組み合わせは、重症 GVHD の発症リスクの高い組み合わせであり、生存への影響については有意でないものがあること。（生存も有意な組み合わせには●印を記載しました。）

2．これら以外にも、重症 GVHD の発症頻度が高い組み合わせが存在する可能性があること（組み合わせによっては、症例数が少ないため有意にならなかった可能性もあります）。したがって、この組み合わせ以外の HLA 型不適合が、GVHD が起こりにくい組み合わせとは断定できないこと。

3．この表の組み合わせは、様々な GVHD 予防法（T 細胞除去法を除く）や疾患を含んだ多変量解析の結果であること。

4．論文化前のデータであること。

5．2006 年 2 月資料（HLA 適合度に基づいた治療成績の分析、NK 細胞受容体リガンド適合度の同定）資料添付、ならびに、最近論文化された論文：Morishima Y, Yabe T, Matsuo K, Kashiwase K, Inoko H, Saji H, Yamamoto K, Maruya E, Akatsuka Y, Onizuka M, Sakamaki H, Sao H, Ogawa S, Kato S, Juji T, Sasazuki T, Kodera Y; Japan Marrow Donor Program: Effects of HLA Allele and Killer Immunoglobulin-Like Receptor Ligand Matching on Clinical Outcome in Leukemia Patients Undergoing Transplantation With T-cell-Replete Marrow From an Unrelated Donor. Biol Blood Marrow Transplant. 2007 Mar; 13(3):315-28.）も参照してください。

【HLA 相談窓口のご案内】

ドナー・患者の HLA ならびに HLA 適合度に基づくドナー選択に関して、HLA 委員会委員を中心にして HLA 相談窓口を設けています。何なりとご相談ください。

問い合わせ先：骨髄移植推進財団　移植調整部（電話 03-5280-4771）

非血縁者間骨髄移植における HLA-C 適合度の解析 ：update

2007 年 10 月 12 日　骨髄移植推進財団 HLA 委員会

HLA-A、B,　DR の血清型が適合した JMDP5210 症例につき解析した。T 細胞除去 GVHD 予防法症例で、解析は多変量解析（Cox regression model）を用い、変数として[症例]に示した臨床的な因子と他座の適合度を用いた。

[症例]　急性リンパ性白血病 1301 例 急性骨髄性白血病 1405 例、慢性骨髄性白血病 887 例 骨髄異形性症候群 597 例　悪性リンパ腫 453 例　再生不良性貧血 302 例 GVHD 予防法：ATG 使用 400 例　　ATG 未使用 4810 例
シクロスポリン使用 2733 例　タクロリムス使用 2437 例 移植前治療：全身放射線照射 4021 例　非照射 1189 例
全例骨髄破壊的全治療実施

HLA 座不適合の割合（GVH 方向）
HLA-A　13.4%　HLA-B　6.4%　HLA-C　29.2%
HLA-DRB1 19.6%　HLA-DQB1 22.5%　HLA-DPB1 65.7%.

HLA-A, B, DR血清型適合移植5210症例の臨床因子も含めた多変量解析の結果

	重症急性GVHD（III 度以上）		死亡	
HLA座適合度	HR (95% CI)	p	HR (95% CI)	p
A-1 locus mismatch	1.41 (1.20-1.66)	<0.001	1.31 (1.18-1.47)	<0.001
A-2locus mismatch	1.79 (1.03-3.13)	0.038	1.62 (1.08-2.43)	0.019
B-1 locus mismatch	1.50 (1.22-1.84)	<0.001	1.30 (1.12-1.51)	0.001
B-2locus mismatch	1.90 (0.58-6.21)	0.282	1.44 (0.52-3.94)	0.476
C-1 locus mismatch	1.93 (1.69-2.21)	<0.001	1.25 (1.14-1.36)	<0.001
C-2locus mismatch	1.78 (1.27-2.49)	0.001	1.40 (1.11-1.76)	0.004
DRB1 -1 locus mismatch	1.08 (0.88-1.32)	0.424	1.03 (0.90-1.18)	0.624
DRB1 -2locus mismatch	1.20 (0.69-2.11)	0.507	0.96 (0.63-1.45)	0.858
DQB1 -1 locus mismatch	1.10 (0.91-1.33)	0.315	1.08 (0.95-1.23)	0.195
DQB1 -2locus mismatch	1.61 (1.02-2.54)	0.040	1.33 (0.95-1.86)	0.089
DPB1 -1 locus mismatch	1.25 (1.09-1.45)	0.001	1.11 (1.01-1.21)	0.021
DPB1 -2locus mismatch	1.29 (1.08-1.54)	0.005	1.09 (0.97-1.22)	0.127

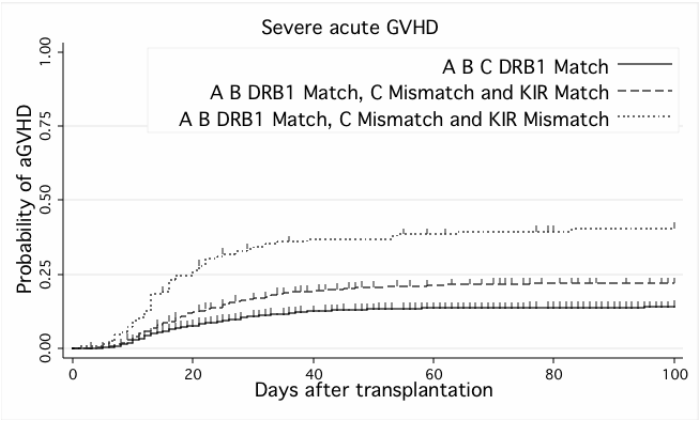
HLA-A, B, DRB1遺伝子型適合症例におけるHLA-C適合度の影響
（臨床データも加えた多変量解析による相対危険率(HR: Hazard Risk) ：HLA-A, B　DR血清型適合5210症例から抽出）

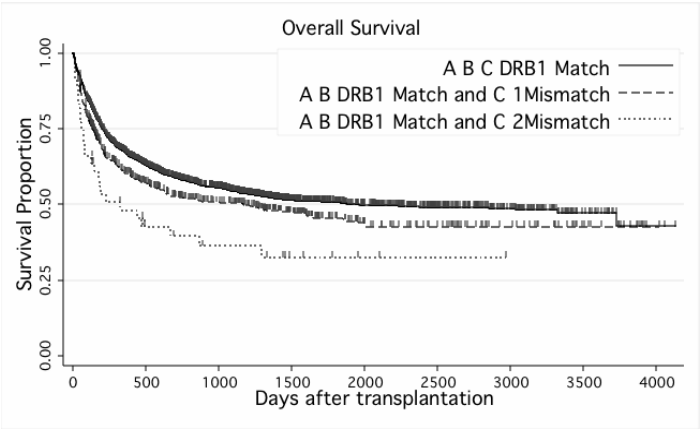
	重症GVHD			死亡		
HLA-A B DRB1 遺伝子型適合症例	N	HR	p	N	HR	p
HLA-C 適合**	2827			2865		
HLA-C 1座 不適合	752	1.97 (1.65-2.37)	<0.001	760	1.26 (1.11-1.42)	<0.001
HLA-C 2座不適合	44	2.57 (1.44-4.59)	0.001	44	1.84 (1.25-2.70)	0.002
HLA-C 適合***	2761			2799		
HLA-C 不適合 KIR*適合	726	1.68 (1.39-2.04)	<0.001	584	1.21 (1.06-1.39)	0.004
HLA-C 不適合 KIR*不適合	136	3.52 (2.61-4.76)	<0.001	286	1.42 (1.19-1.69)	<0.001
KIR*：NK細胞受容体(KIR2DL)リガンド適合度(GVH方向) **GVH 方向 ***GVH and/or HVG方向						
参考 1。HLA-A適合度の影響 （HLA-B C DRB1 適合症例）						
A B C DRB1 適合	2827			2865		
A-1 座不適合	314	1.59 (1.21-2.08)	0.001	316	1.35 (1.15-1.60)	<0.001
A-2座不適合	14	1.85 (0.58-5.83)	0.292	14	1.39 (0.65-2.94)	0.385
参考 2。HLA-B適合度の影響 （HLA-A C DRB1 適合症例）						
A B C DRB1 適合	2827			2865		
B-1 座不適合	50	2.47 (1.46-4.17)	0.001	50	1.36 (0.93-2.00)	0.112
B-2座不適合	-	-	-	-	-	-
					2007.5.15 JMDP	

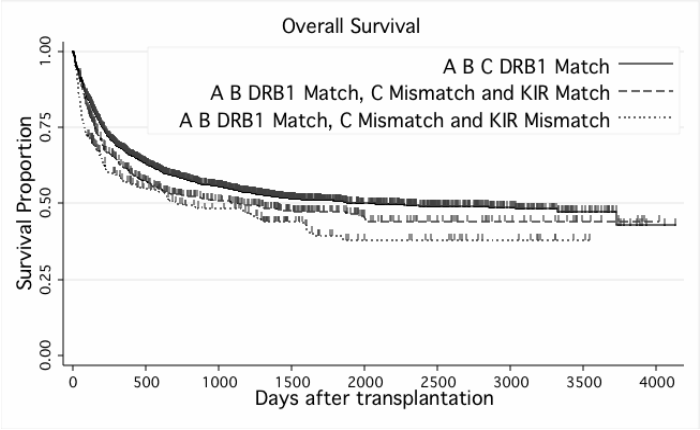
HLA-Cw血清型不適合と遺伝子型不適合の重症GVHDと生存に与える影響

	severe a GVHD			OS		
	N	HR	p	N	HR	p
C Geno-match Sero-match	3682			3660		
C Geno-1 M Sero-match	78	1.74 (1.11-2.74)	0.015	80	1.09 (0.78-1.52)	0.592
C Geno-1 M Sero-1 M	1480	1.94 (1.70-2.22)	<0.001	1506	1.26 (1.15-1.37)	<0.001

解説　HLA－Cw遺伝子型不適合症例(1576例)の中で血清型適合で遺伝子型不適合症例(Geno-1 M Sero-matc
78例(5%)と、重症GVHDと生存の影響を評価するには症例数が不足しているが、重症GVHDの発症頻度は
HLA-Cw遺伝子型適合症例に比べ有意に高かった。







HLA 適合度に基づいた治療成績の分析

2006 年 2 月 25 日

(財)骨髄移植推進財団　HLA 委員会

HLA-A, B, C, DRB1 の DNA 型が判明した場合の治療成績について、適合度別の 3 年生存率、6 年生存率、重症 GVHD 発症率を示した。HLA-C の適合度が臨床成績に影響している。JMDP を介して実施された最初から 3000 症例の中で HLA-A, B, DR の血清型が適合し DNA 型レトロスペクティブに解析された全 2500 症例を対象とした。異なった疾患・病期・GVHD 予防法が含まれることと、群によっては症例数が少ないことに留意されたい。また、この分析結果は、今後のドナー選択の際の参考資料となるものと考える。

○ HLA 遺伝子型適合　× HLA 遺伝子型 1 型不適合　P は全適合症例との比較。

	A	B	C	DR B1	*n*	3 年生存	6 年生存	P	A-GVHD (grade3,4)	P
1	○	○	○	○	*1128*	58%	52%	-	13%	-
2	○	○	○	×	*219*	55	53	0.745	14	0.536
3	○	○	×	○	*371*	54	45	0.052	20	0.001
4	○	○	×	×	*172*	49	48	0.011	28	<0.0001
5a	×	○	○	○	*155*	42	36	<0.0001	22	0.001
5b	○	×	○	○	*22*	34	-	0.013	28	<0.0001
6a	○	×	×	○	*84*	37	37	<0.0001	31	<0.0001
6b	×	○	×	○	*100*	31	30	<0.0001	31	<0.0001
6c	×	○	○	×	*30*	35	28	0.003	15	0.697
6d	○	×	○	×	*19*	26	20	0.001	28	0.046
6e	×	×	○	○	*7*	ne	ne		ne	
7a	○	×	×	×	*28*	42	18	0.009	38	0.0002
7b	×	○	×	×	*47*	31	31	<0.0001	35	<0.0001
7c	×	×	○	×	*6*	ne	ne		ne	
7d	×	×	×	○	*34*	30	27	0.0004	41	<0.0001
8	×	×	×	×	*19*	24	16	<0.0001	28	0.029

ne ：症例数が 9 例以下のため解析せず。a, b, c, d, e は順位ではない。

HLA-C 不適合移植（3. 4.）における NK 細胞受容体(KIR:NKG2L) ligand 適合の成績
KIR 不適合：GVHD 方向のデータ

	A	B	C	DR B1	KIR	*n*	3 年生存	6 年生存	P	A-GVHD (grade3,4)	P
HLA-C 単独不適合											
3a	○	○	×	○	○	***324***	**56%**	**47%**	**-**	**18%**	**-**
3b	○	○	×	○	×	***52***	**40**	**20**	**0.016**	**34**	**0.008**
HLA-C + DRB1 不適合											
4a	○	○	×	×	○	***147***	**53**	**53**	**-**	**25**	**-**
4b	○	○	×	×	×	***29***	**34**	**28**	**0.012**	**41**	**0.085**

NK 細胞受容体（KIR２DL）と移植との解説

NK 細胞受容体の一つである KIR２DL の KIR２DL２と KIR２DL３は HLA－C のアミノ酸配列 Ser77,Asp80（Ｃｗ３group エピトープ）と結合して NK 細胞の活性化を抑制している。

KIR２DL1 は HLA-C の配列 Asp77, Lys80（Ｃｗ４group エピトープ）と結合して NK 細胞の活性化を抑制している。 HLA-C 抗原は下表に示すようにＣｗ３group かＣｗ４group のどちらかのエピトープを有する。

造血細胞移植ではドナー由来の造血細胞に置き換わるため、HLA-C 不適合移植においては、以下に示すように患者（あるいはドナー）の KIR２DL と結合する HLA-C のエピトープが存在しない場合があり、このため患者（あるいはドナー）の KIR 陽性細胞が活性化され、GVHD（あるいは拒絶）が生じる可能性がある。KIR の GVHD 方向（拒絶方向）の不適合は通常のリンパ球による HLA 不適合とは逆方向になる。

HLA-C 型から KIR２DL リガンド適合度を見出す手順。

下表（A）を用いドナーと患者の HLA-C 型から HLA-C のエピトープ（Cw3 group か Cw4 group か）を決め、下表（B）に当てはめ、ligand の適合度を見出す。

表 A

HLA－C エピトープ	HLA 型	
Cw 4 group (Asp77, Lys80)	血清型	Cw2, Cw4, Cw5, Cw6
	遺伝子型	Cw*0401, Cw*0501, Cw*0602, Cw*1502
Cw 3 group (Ser77, Asp80)	血清型	Cw１, Cw3, Cw7, Cw8, Cw10
	遺伝子型	Cw*0102, Cw*0302, Cw*0303, Cw*0304, Cw*0702, Cw*0704,Cw*0801, Cw*0803, Cw*1202 Cw*1402 Cw*1403

参考:JMDP での頻度 **Cw4 group** 7.3% **Cw3 group** 92.7%

表 B

適合度	患者	ドナー	JMDP での頻度
GVHD 方向の不適合	**Cw3 Cw3** **Cw4 Cw4**	**Cw3 Cw4** **Cw3 Cw4**	4.6%
拒絶方向の不適合	**Cw3 Cw4** **Cw3 Cw4**	**Cw3 Cw3** **Cw4 Cw4**	5.8%
両方向の不適合 .	**Cw3 Cw3** **Cw4 Cw4**	**Cw4 Cw4** **Cw3 Cw3**	0.5%
適合	**Cw3 Cw4** **Cw3 Cw3** **Cw4 Cw4**	**Cw3 Cw4** **Cw3 Cw3** **Cw4 Cw4**	89.2%

Cw3 : Cw3 group　Cw4 : Cw4 group

High-risk HLA allele mismatch combinations responsible for severe acute graft-versus-host disease and implication for its molecular mechanism

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In allogenic hematopoietic stem-cell transplantation, an effect of HLA locus mismatch in allele level on clinical outcome has been clarified. However, the effect of each HLA allele mismatch combination is little known, and its molecular mechanism to induce acute graft-versus-host disease (aGVHD) remains to be elucidated. A total of 5210 donor-patient pairs who underwent transplantation through Japan Marrow Donor Program were analyzed. All HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 alleles were retrospectively typed in all pairs. The impacts of the HLA allele mismatch combinations and amino acid substitution positions in 6 HLA loci on severe aGVHD were analyzed. A total of 15 significant high-risk HLA allele mismatch combinations and 1 HLA-DRB1-DQB1 linked mismatch combinations (high-risk mismatch) for severe aGVHD were identified, and the number of high-risk mismatches was highly associated with the occurrence of severe aGVHD regardless of the presence of mismatch combinations other than high-risk mismatch. Furthermore, 6 specific amino acid substitution positions in HLA class I were identified as those responsible for severe aGVHD. These findings provide evidence to elucidate the mechanism of aGVHD on the basis of HLA molecule. Furthermore, the identification of high-risk mismatch, that is, nonpermissive mismatch, would be beneficial for the selection of a suitable donor. (Blood. 2007;110:2235-2241)

Introduction

Allogenic hematopoietic stem-cell transplantation (HSCT) from an HLA-matched unrelated (UR) donor has been established as a treatment for hematologic malignancies, when an HLA-identical sibling donor is unavailable.[1,2] When a matched unrelated donor was not found in the donor registry, a partially HLA-matched unrelated donor was one of the candidates for alternative donor. But the higher risk of immunologic events, especially graft-versus-host disease (GVHD), was an important drawback. Extensive recent research has accumulated evidence of the role of each HLA locus mismatch on clinical outcome for UR-HSCT,[3-9] which has made it easy to search and select a partially matched donor. To further expand options for donor selection, our next challenge is to identify permissive and nonpermissive mismatch combinations of each HLA allele. Although there were some divisional trials with small populations,[10,11] a large-scale cohort is essential for comprehensive analysis to identify nonpermissive mismatch combinations that are significant risk factors for severe acute graft-versus-host disease (aGVHD).

In this study, we identified nonpermissive HLA mismatch allele combinations of all major 6 HLA loci, and their responsible positions of amino acid substitution for aGVHD.

Patients, materials, and methods

Patients

A total of 5210 donor-patient pairs who underwent transplantation through the Japan Marrow Donor Program (JMDP) with T-cell–replete marrow from a serologically HLA-A, -B, and -DR antigen-matched donor between January 1993 and January 2006 were analyzed in this cohort study. Patients who received a transplant of harvested marrow outside Japan (n = 51) or were unavailable for blood sample (n = 428) were not eligible for this study of a total of 5689 consecutively registered patients.

Patient characteristics are shown in Table S1, available on the *Blood* website (see the Supplemental Materials link at the top of the online article). The final clinical survey of these patients was completed by June 1, 2006. Informed consent was obtained from patients and donors in accordance with the Declaration of Helsinki, and approval of the study was obtained from the Institutional Review Board of Aichi Cancer Center and JMDP.

HLA typing of patients and donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were identified by the methods described previously.[4,5] Six HLA locus alleles were typed in all 5210 pairs. HLA genotypes of HLA-A, -B, -C, -DQB1, and -DPB1 allele of patient and donor were reconfirmed by the Luminex microbead method (Luminex 100 System; Luminex, Austin, TX). For convenience, we showed the frequency of HLA alleles that existed with

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more than a 5% allele frequency in the current Japanese data set and less than a 1% allele frequency in white populations[12] in Table S2.

Matching of HLA allele between patient and donor

For the analysis of aGVHD, HLA allele mismatch among the donor-recipient pair was scored when the recipient's alleles were not shared by the donor (GVH vector). We also used GVH vectors for the analysis of overall survival (OS) to indicate OS of aGVHD high-risk or low-risk group.

Evaluation of acute GVHD

Occurrences of aGVHD were graded with grade 0, I, II, III, and IV according to established criteria.[13] Grades III and IV were defined as severe aGVHD.

Definitions of amino acid substitution

Amino acid sequences of HLA-A, -B, -C, -DR, -DQ, and -DP molecules were obtained from IMGT/HLA sequence database.[14] For example, Tyr9A-Phe9A indicated amino acid substitutions of position 9 in HLA-A molecule at which the donor had tyrosine and the patient phenylalanine. Substituted amino acids in HLA class I were summarized in Tables S3-S5.

Definition of nonpermissive HLA combinations

We defined the nonpermissive HLA allele combination as a significant risk factor for severe aGVHD, because severe aGVHD was a solid marker for alloreactivity in HSCT and was the main contributor to transplantation-related mortality.[15,16]

Definition of hydropathy scale

The hydropathy scale proposed by Kyte and Doolittle[17] evaluates the hydrophilicity and hydrophobicity of 20 amino acids to estimate the protein structure. Hydrophobic amino acid has a plus value and hydrophilic amino acid a minus value, and their absolute value indicates the grade of each property.

Statistical analysis

Cumulative incidences of aGVHD were assessed by the method described elsewhere to eliminate the effect of competing risk.[18,19] The competing event regarding aGVHD was defined as death without aGVHD. A log-rank test was applied to assess the impact by the factor of interest. Multivariable Cox regression analyses[20] were conducted to evaluate the impact of HLA allele mismatch combination, and the positions and types of amino acid substitution (for example, alanine, arginine, asparagines) of HLA molecules.

The HLA mismatch combination was evaluated for each locus separately, and the HLA match and HLA one-locus mismatch in every locus were analyzed. For example, A0206-A0201 mismatch combination meant that the donor has HLA-A*0206, recipient has HLA-A*0201, and another HLA-A allele of each donor and recipient was identical. This mismatch was compared with the HLA-A allele match. The mismatch combination of which the number of pairs was less than 10 was lumped together as "other mismatch." This is because, according to the computer simulation by Peduzzi et al,[21] it is generally accepted that regression analysis for a variable having fewer than 10 events might give an unreliable estimation. The model was constructed with mismatch combinations, mismatch status in other loci (match, 1 locus mismatch, and 2 locus mismatches as ordinal variable), and potential confounders. Confounders considered were sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high, and diseases other than leukemia), GVHD prophylaxis (cyclosporine [CSP] vs FK 506 [FK]), ATG (ATG vs no ATG), and preconditioning (total body irradiation [TBI] vs non-TBI). We used these confounders in all analyses in this paper to keep results comparable.

The impact of positions and types of amino acid substitution in HLA molecules was evaluated in pairs with HLA one-locus mismatch in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 separately. The amino acid positions we analyzed were all those at which amino acid was substituted in each locus. We analyzed the impact of each amino acid substitution on each position separately. Multivariable Cox models including positions and types of amino acid substitution, mismatch status in other loci (match, 1 locus mismatch, and 2 locus mismatches as ordinal variable), and confounders described in "Statistical analysis" were constructed.

We applied a P value of less than .005 as statistically significant to eliminate false-positive associations. All the analyses were conducted by STATA version 9.2 (Stata, College Station, TX).

Validation of statistical analysis

We validated the statistical analysis using 2 methods, traditional training-and-test method and bootstrap resampling method, in HLA-A analysis to confirm the usability of bootstrap resampling. In the traditional training-and-test method, donor-recipient pairs were divided at random in 2 equally scaled groups, group A and group B. When consistent results were obtained in both analyses, we considered the results as validated. In the bootstrap resampling method,[22] we estimated the measure of association with the resampled data repeatedly drawn from the original data. Although around 100 to 200 bootstrapped samplings are generally sufficient,[23] we explored 500, 1000, 5000, 10 000, and 50 000 bootstrappings in analysis of HLA-A mismatch combinations. We confirmed that an analysis using more than 5000 bootstrappings made the results stable. Because there was high concordance between these 2 methods (Table S6), we adopted bootstrap resampling using 10 000 bootstrap samples for all analyses in this paper as the method for validation. This is because traditional training-and-test methods do not work efficiently when small subgroups are considered as in this paper. Only when the results of base analysis and validating analysis using bootstrap resampling were significant concurrently were the results of the analysis judged to be statistically significant. When the result of base analysis was significant but the result of validating analysis using bootstrap resampling was not, we indicated this by adding an asterisk next to the P value of the base analysis.

Results

Impact of HLA allele mismatch combinations on severe aGVHD

Hazard ratios (HRs) of HLA allele mismatch combinations in HLA-A and -C on severe aGVHD are shown in Table 1 (HLA-B, -DR, -DQ, and -DP are available in Table S7).

In HLA-A locus mismatch combinations, A*0206-A*0201 (HR: 1.78; CI, 1.32-2.41), A*0206-A*0207 (HR: 3.45; CI: 2.09-5.70), A*2602-A*2601 (HR: 3.35; CI: 1.89-5.91), and A*2603-A*2601 (HR: 2.17; CI: 1.29-3.64), were significant risk factors for severe aGVHD.

In HLA-C locus mismatch combinations, 7 combinations were significant risk factors for severe aGVHD; those were as follows: Cw*0401-Cw*0303 (HR: 2.81; CI: 1.72-4.60), Cw*0801-Cw*0303 (HR: 2.32; CI: 1.58-3.40), Cw*0303-Cw*1502 (HR: 3.22; CI: 1.75-5.89), Cw*0304-Cw*0801 (HR: 2.34; CI: 1.55-3.52), Cw*1402-Cw*0304 (HR: 3.66; CI: 2.00-6.68), Cw*1502-Cw*0,304 (HR: 3.77; CI: 2.20-6.47), and Cw*1502-Cw*1402 (HR: 4.97; CI: 3.41-7.25). To summarize, high-risk HLA allele mismatch combinations for severe aGVHD, that is, nonpermissive mismatch combinations, of all major 6 HLA loci were listed in Table 2. A total of 15 nonpermissive HLA allele mismatch combinations (4 in HLA-A, 1 in HLA-B, 7 in HLA-C, 1 in HLA-DRB1, and 2 in HLA-DPB1) and 1 HLA-DRB1-DQB1 linked mismatch combination (Table 2 legend) were identified.

We divided donor-recipient pairs into 4 groups according to the number of nonpermissive mismatches: (1) full match (in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) group; (2) zero nonpermissive mismatch (with mismatches other than nonpermissive mismatches)

Table 1. Multivariable analysis of impact of mismatch pairs for sever aGVHD in HLA-A and -C

Mismatch combination, donor-patient	N	HR (95% CI)	*P*
A locus match	4510	1	NA
A0201-A0206	138	1.23 (0.87-1.73)	.223
A0206-A0201	131	1.78 (1.32-2.41)	< .001
A0201-A0207	28	0.83 (0.34-2.03)	.699
A0207-A0201	20	1.12 (0.42-3.02)	.809
A0201-A0210	11	1.57 (0.58-4.23)	.367
A0206-A0207	27	3.45 (2.09-5.70)	< .001
A0207-A0206	22	0.71 (0.23-2.24)	.571
A2402-A2420	60	0.64 (0.32-1.30)	.225
A2420-A2402	30	1.18 (0.56-2.49)	.66
A2601-A2602	24	0.64 (0.26-1.58)	.34
A2602-A2601	21	3.35 (1.89-5.91)	< .001
A2601-A2603	34	1.37 (0.73-2.57)	.326
A2603-A2601	35	2.17 (1.29-3.64)	.003
A2602-A2603	10	1.23 (0.30-4.98)	.763
A2603-A2602	12	1.50 (0.48-4.68)	.485
A other mismatch	97	1.47 (1.00-2.15)	.047
C locus match	3685	1	NA
C0102-C0303	30	2.83 (1.50-5.32)	.001*
C0303-C0102	38	1.05 (0.47-2.36)	.899
C0102-C0304	12	1.85 (0.59-5.81)	.287
C0304-C0102	19	0.89 (0.28-2.79)	.854
C0102-C0401	14	1.87 (0.77-4.55)	.164
C0102-C0803	24	1.97 (0.87-4.42)	.099
C0803-C0102	10	1.66 (0.53-5.19)	.383
C0102-C1402	16	3.86 (1.98-7.51)	< .001*
C1402-C0102	13	0.46 (0.06-3.33)	.45
C0303-C0304	83	1.08 (0.63-1.85)	.761
C0304-C0303	62	0.83 (0.41-1.68)	.614
C0303-C0401	31	1.73 (0.89-3.36)	.103
C0401-C0303	42	2.81 (1.72-4.60)	< .001
C0303-C0702	25	1.16 (0.52-2.62)	.706
C0702-C0303	18	2.16 (0.96-4.85)	.062
C0303-C0801	76	1.07 (0.63-1.84)	.782
C0801-C0303	80	2.32 (1.58-3.40)	< .001
C0303-C1502	25	3.22 (1.75-5.89)	< .001
C0304-C0401	15	3.02 (1.34-6.79)	.007
C0401-C0304	12	6.22 (3.07-12.5)	< .001*
C0304-C0702	26	2.35 (1.16-4.76)	.017
C0702-C0304	33	1.22 (0.58-2.59)	.59
C0304-C0801	69	2.34 (1.55-3.52)	< .001
C0801-C0304	47	1.64 (0.98-2.76)	.057
C0304-C1402	28	3.06 (1.68-5.60)	< .001*
C1402-C0304	23	3.66 (2.00-6.68)	< .001
C0304-C1502	53	1.82 (1.08-3.05)	.023
C1502-C0304	27	3.77 (2.20-6.47)	< .001
C0801-C0102	10	2.88 (0.92-9.03)	.068
C0801-C0803	27	1.55 (0.69-3.48)	.284
C0803-C0801	26	2.04 (1.04-3.99)	.037
C0801-C1502	36	1.59 (0.79-3.21)	.19
C1502-C0801	23	2.28 (1.07-4.85)	.031
C1402-C1502	55	1.67 (1.01-2.77)	.043
C1502-C1402	50	4.97 (3.41-7.25)	< .001
C other mismatch	347	1.69 (1.34-2.14)	< .001

A0206-A0201 mismatch combination meant that the donor has HLA-A*0206, recipient has HLA-A*0201 and another HLA-A allele of each donor and recipient was identical. Each mismatch pair in HLA-A was compared with the HLA-A allele match, and each mismatch pair in HLA-C was compared with the HLA-C allele match. Confounders considered were sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, high and diseases other than leukemia), GVHD prophylaxis, (CSP vs. FK), ATG (ATG vs. no ATG) and preconditioning (TBI vs non-TBI).

HR denotes hazard ratio; CI, confidence interval; NA, not applicable.

*The result of base analysis was significant, but the result of validating analysis using bootstrap resampling was not. The results of the analysis were thus judged not to be statistically significant.

Table 2. Nonpermissive allele mismatch combinations for severe aGVHD

Mismatch combination, donor-patient	N	HR (95% CI)	*P*
A0206-A0201	131	1.78 (1.32-2.41)	< .001
A0206-A0207	27	3.45 (2.09-5.70)	< .001
A2602-A2601	21	3.35 (1.89-5.91)	< .001
A2603-A2601	35	2.17 (1.29-3.64)	.003
B1501-B1507	19	3.34 (1.85-5.99)	< .001
C0303-C1502	25	3.22 (1.75-5.89)	< .001
C0304-C0801	69	2.34 (1.55-3.52)	< .001
C0401-C0303	42	2.81 (1.72-4.60)	< .001
C0801-C0303	80	2.32 (1.58-3.40)	< .001
C1402-C0304	23	3.66 (2.00-6.68)	< .001
C1502-C0304	27	3.77 (2.20-6.47)	< .001
C1502-C1402	50	4.97 (3.41-7.25)	< .001
DR0405-DR0403	53	2.13 (1.28-3.53)	.003
(DR1403-DQ0301)-(DR1401-DQ0502)	19	2.81 (1.44-5.51)	.002
DP0301-DP0501	49	2.41 (1.49-3.89)	< .001
DP0501-DP0901	71	2.03 (1.30-3.16)	.002

Analysis method is the same as in Table 1. We surveyed specific linked mismatches between nonpermissive mismatches elucidated. As a result, obvious specific linked mismatches exist only between DRB1*1403- DRB1*1401 and DQB1*0301- DQB1*0502. Therefore, we could not evaluate which mismatch combination impacted aGVHD, and we considered this linked mismatch did so. On the other hand, because other nonpermissive mismatch combinations had no specific link with the others, we judged other than DRB1*1403- DRB1*1401 and DQB1*0301- DQB1*0502 nonpermissive mismatches solely impacted aGVHD. (DR1403-DQ0301)-(DR1401-DQ0502) linked mismatch meant that the donor has HLA-DRB1*1403-HLADQB1*0301 and the recipient has HLA-DRB1*1401-HLADQB1*0502.

HR indicates hazard ratio; CI, confidence interval.

group; (3) 1 nonpermissive mismatch (with or without mismatches other than nonpermissive mismatches) group; and (4) 2 or more nonpermissive mismatches (with or without mismatches other than nonpermissive mismatches) group, and analyzed for association with severe aGVHD. This analysis excluded pairs with 2 locus mismatches in the same locus. Patient characteristics according to the number of nonpermissive mismatches are shown in Table 3. The curve of cumulative incidence of severe aGVHD is shown in Figure 1A. Multivariable analysis revealed that severe aGVHD occurred with almost equal frequency between the full match group and zero nonpermissive mismatch group, and was significantly associated with the number of nonpermissive mismatches (Table 4). Relative risk of significant factor for aGVHD and OS is shown in Table S8. In terms of the mortality due to aGVHD according to the number of nonpermissive mismatches, one nonpermissive mismatch group and 2 or more nonpermissive mismatch groups showed higher mortality (19.7% and 15.8%, respectively) than full match group and zero nonpermissive mismatch group (8.5% and 11.4%, respectively).

Impact of positions and types of amino acid substitutions of HLA molecules for severe aGVHD

One specific amino acid substitution at position 9 in HLA-A molecule and 6 specific amino acid substitutions at positions 9, 77, 80, 99, 116, and 156 in HLA-C molecule were significant risk factors for severe aGVHD: Tyr9A-Phe9A (HR: 1.66; CI: 1.19-3.32), Tyr9C-Ser9C (HR: 1.66; CI: 1.23-2.25), Asn77C-Ser77C (HR: 1.87; CI: 1.46-2.39), Lys80C-Asn80C (HR: 1.87; CI: 1.46-2.39), Tyr99C-Phe99C (HR: 1.64; CI: 1.21-2.22), Leu116C-Ser116C (HR: 3.40; CI: 2.20-5.25), and Arg156C-Leu156C (HR: 1.48; CI: 1.15-1.90) (Table 5). The amplitude of hydropathy scales were 4.1, 0.5, 2.7, 0.4, 4.1, 4.6, and 8.3, respectively. Although all

Table 3. Patient characteristics according to number of nonpermissive mismatches

Group	Total	Full match	Zero nonpermissive mismatch	One nonpermissive mismatch	Two or more nonpermissive mismatches
Total	4050	712	2670	602	66
Patient age, median y	30	32	30	29	29
Sex, donor/patient, no. patients					
Male/male	1673	312	1096	237	28
Male/female	785	134	518	119	14
Female/male	769	115	524	117	13
Female/female	823	151	532	129	11
Disease, no. patients					
ALL	981	162	668	139	12
ANLL	1075	196	698	158	23
CML	703	119	453	115	16
Hereditary disease	85	14	56	15	0
MDS	476	91	304	72	9
Malignant lymnphoma	349	69	229	48	3
Multiple myeloma	42	8	29	4	1
Severe aplastic anemia	247	33	175	37	2
Other disease	92	20	58	14	0
Risk of leukemia relapse,* no. patients					
Standard risk	1308	249	857	181	21
High risk	1451	228	962	231	30
Diseases other than leukemia	1291	235	851	190	15
GVHD prophylaxis, no. patients					
Cyclosporin-based	2198	402	1444	319	33
Tacrolimus-based	1852	310	1226	283	33
ATG, no. patients					
ATG	323	48	215	53	7
Non-ATG	3727	664	2455	549	59
Preconditioning, no. patients					
TBI regimen	3117	539	2071	449	58
Non-TBI regimen	933	173	599	153	8

ALL indicates acute lymphoblastic leukemia; ANLL, acute non-lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; and TBI, total body irradiation.

*Standard risk for leukemia relapse was defined as the status of the 1st CR of AML and ALL and the 1st CP of CML at transplant, while high risk was defined as a more advanced status than standard risk in AML, ALL, and CML, and diseases other than leukemia was defined as other than ALL, ANLL, and CML.

amino acid positions substituted in each HLA locus were analyzed, amino acid substitutions of any other HLA-A and -C positions were not significant risk factors. As for HLA-B, DRB1, DQB1, and DPB1, there was no significant association between the positions of amino acid substitution and severe aGVHD. Impact for OS about positions and types of amino acid substitutions that were significant risk factors for aGVHD was shown in Table S9.

Discussion

Extensive recent research has accumulated evidence of the role of each HLA locus mismatch on clinical outcome for UR-HSCT.[3-9] Our next concern is identifying the combinations of HLA allele mismatch and the positions of amino acid substitution of the HLA molecules responsible for aGVHD. In the present study, multivariable analysis revealed that 15 combinations of HLA allele mismatch and 1 HLA-DRB1-DQB1 haplotype mismatch significantly increase the occurrence of severe aGVHD (Table 2), and most of them increased the mortality rate after transplantation (data not shown). Thus, these mismatch combinations of HLA allele might be called nonpermissive clinically. We speculated that the effect of HLA locus mismatch was a reflection and summation of these HLA allele mismatch combinations. Discrepancies of responsible HLA locus for aGVHD between ethnically diverse transplantations might be explained by the proportions of nonpermissive mismatch combinations in each HLA locus. The same study in other populations would be needed to clarify this question as well as the severity of aGVHD. Interestingly, the full match group and zero nonpermissive mismatch group showed an almost equal occurrence of severe aGVHD, though pairs in zero nonpermissive mismatch group had one or more mismatches other than nonpermissive mismatches. And HR was elevated with the increase in the number of nonpermissive mismatches (Figure 1A; Table 4), while the number of nonpermissive mismatches also had a significant effect on OS after transplantation (Figure 1B; Table 4). These findings indicated at least that nonpermissive mismatches should be avoided in donor selection for UR-HSCT, and that the order of donor selection based on this nonpermissive mismatch would be useful, instead of that based on HLA locus mismatch. We also speculated that there are permissive mismatches in mismatches other than nonpermissive mismatches. It is therefore an important task in the future to identify permissive mismatches for partially HLA-matched donor selection. On the other hand, we do not deny the possibility that some mismatch combinations not classified as nonpermissive may actually be potential nonpermissive ones. Misclassification might happen because of insufficient statistical power due to the relatively small number of subjects in subcategories.

At present, there have been only a few reports indicating that the transplant-related immunologic reactions and clinical outcomes were caused by the HLA allele mismatch combinations. Macdonald

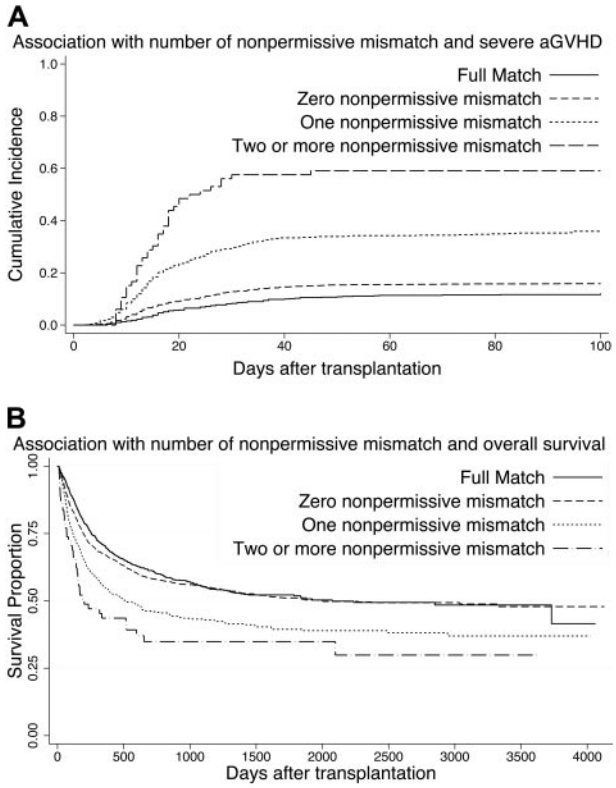


Figure 1. Impact of number of nonpermissive mismatches on severe aGVHD and overall survival. (A) Cumulative incidence of severe aGVHD according to number of nonpermissive mismatches. — indicates full match (in HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1) group; ----, zero nonpermissive mismatch (with mismatches other than nonpermissive mismatches) group; ····, one nonpermissive mismatch (with or without mismatches other than nonpermissive mismatches) group; and – – –, 2 or more nonpermissive mismatches (with or without mismatches other than nonpermissive mismatches) group. (B) Kaplan-Meier estimates of survival according to number of nonpermissive mismatches. Each group was divided as described for panel A.

et al[24] reported that cytotoxic T lymphocytes (CTLs) discriminate between HLA-B*4402 and HLA-B*4403, and induce strong alloresponses, but the stronger T-cell alloreactivity is observed toward HLA-B*4403 compared with HLA-B*4402 in vitro. Zino et al[10] and Fleischhauer et al[11] attempted to develop an algorithm for prediction of nonpermissive HLA-DPB1 mismatches. The present report is the first to provide far more precise and detailed evidence for numerous HLA allele mismatch combinations for severe aGVHD.

Table 5. Multivariable analysis of impact of amino acid substitution on HLA class I molecules for severe aGVHD

Posisiton and kind of amino acid substitution, donor-recipient	HS	N	Event†	HR (95% CI)	*P*
HLA-A locus					
Tyr9A-Phe9A	4.1	163	64	1.66 (1.19-2.32)	.003
Asn116A-Asp116A	0	32	15	2.25 (1.26-4.01)	.005*
HLA-C locus					
Tyr9C-Ser9C	0.5	146	59	1.66 (1.23-2.25)	.001
Asn77C-Ser77C	2.7	205	90	1.87 (1.46-2.39)	< .001
Lys80C-Asn80C	0.4	205	90	1.87 (1.46-2.39)	< .001
Tyr99C-Phe99C	4.1	146	59	1.64 (1.21-2.22)	.001
Leu116C-Ser116C	4.6	53	30	3.40 (2.20-5.25)	< .001
Arg156C-Leu156C	8.3	251	88	1.48 (1.15-1.90)	.002

HLA-B. -DRB1, -DQB1 -DPB1 locus had no significant substitutions. The impact of positions and types of amino acid substitution in HLA molecules was evaluated in pairs with HLA one-locus mismatch in HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1 separately. For example, Tyr9A-Phe9A indicated amino acid substitutions of position 9 in HLA-A molecule at which donor had tyrosine and patient phenylalanine. The impacts of positions and kinds of amino acid substitutions in each HLA molecule were evaluated in pairs with HLA one locus mismatch in each HLA locus separately. Pairs which substituted specific amino acid at each position were compared with amino acid matched pairs at that position.

HS indicates hydropathy scale; HR, hazard ratio; CI, confidence interval; Tyr, tyrosine; Phe, phenylalanine; Asn, asparagine; Asp, asparatic acid; Ser, serine; Lys, lysine; Leu, leucine; and Arg, arginine.

*Result of base analysis was significant but result of validating analysis using bootstrap resampling was not. Results of analysis were thus judged not to be statistically significant.

†Measured in number of occurrences of severe acute GVHD.

In this study, substitutions of specific amino acids at positions 9, 77, 80, 99, 116, and 156 were elucidated as a significant risk factor for severe aGVHD. We speculated that the responsibility of positions 77 and 80 in HLA-C for severe aGVHD was associated with ligand matching of NK-cell receptor (KIR2DL). Although the role of KIR2DL in acute GVHD has been controversial,[25] a recent JMDP analysis demonstrated that KIR2DL ligand mismatched pairs in GVH vector induced severe aGVHD in UR-HSCT with T-cell–replete marrow.[9] The ligand of KIR2DL is located at positions 77 and 80, which are completely linked in HLA-C molecule. And almost all pairs in this study with Asn77C-Ser77C and Lys80C-Asn80C substitutions have a KIR2DL mismatch in GVH vector.

Except for positions 77 and 80, which are associated with KIR2DL ligand in HLA-C, positions 9, 99, 116, and 156 were elucidated. Positions 9, 99, and 116 are located in the beta-plated

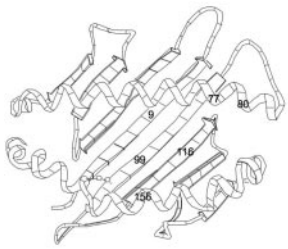
Table 4. Multivariable analysis of impact of number of nonpermissive mismatches on severe aGVHD and overall survival

			Univariate analysis		Multivariate analysis		Bootstrap (10000)	
	N	Event*	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
For severe aGVHD								
Full match group	972	129	1.00	NA	1.00	NA	1.00	NA
Zero nonpermissive mismatch group	2446	411	1.21 (0.95-1.54)	.111	1.00 (0.75-1.32)	.996	1.00 (0.74-1.33)	.996
One nonpermissive mismatch group	571	211	2.88 (2.20-3.78)	< .001	2.22 (1.62-3.04)	< .001	2.22 (1.63-3.02)	< .001
Two or more nonpermissive mismatch group	61	36	5.62 (3.77-8.39)	< .001	3.68 (2.33-5.80)	< .001	3.68 (2.33-5.80)	< .001
For overall survival								
Full match group	972	400	1.00	NA	1.00	NA	1.00	NA
Zero nonpermissive mismatch group	2446	1021	1.10 (0.98-1.23)	.091	1.06 (0.94-1.20)	.315	1.06 (0.94-1.20)	.299
One nonpermissive mismatch group	571	309	1.55 (1.34-1.78)	< .001	1.51 (1.30-1.76)	< .001	1.51 (1.29-1.77)	< .001
Two or more nonpermissive mismatch group	61	39	2.12 (1.54-2.90)	< .001	2.25 (1.65-3.08)	< .001	2.25 (1.65-3.08)	< .001

Each group was compared with Full match group. Confounders considered were sex (donor-recipient pairs), patient age (linear), donor age (linear), type of disease, risk of leukemia relapse (standard, highand diseases other than leukemia), GVHD prophylaxis, (CSP vs. FK), ATG (ATG vs. no ATG) and preconditioning (TBI vs. non-TBI).

HR indicates hazard ratio; CI, confidence interval; Boot strap (10000), bootstrap resampling using 10000 bootstrapping.

*For severe aGVHD, "Event" refers to number of occurrences; for overall survival, number of deaths.



	beta-plate sheet			alpha helix
Position of HLA class I	9	99	116	156
Peptide-binding pocket	B C	A B D	F	D E
Amino acid substitution				
HLA-A	Tyr-Phe		Asn-Asp*	
HLA-C	Tyr-Ser	Tyr-Phe	Leu-Ser	Arg-Leu

Figure 2. Schematic presentation of HLA class I molecule and summary of features of significant amino acid substituted positions. Numbers in schema of HLA molecule indicate substituted amino acid positions that were elucidated as significant risk factor for severe aGVHD. Positions 9, 99, and 116 are located in the beta-plated sheet and positions 77, 80, and 156 in the alpha helix of HLA class I molecule (left). Positions 77 and 80 are associated with KIR2DL ligand in HLA-C molecule. Position 9 constitutes peptide-binding pockets B and C; position 99 constitutes A, B, and D pockets; position 116 constitutes F pocket; and position 156 constitutes D and E pockets (right). For example, Tyr-Phe indicated amino acid substitution at indicated position in HLA molecule at which donor had tyrosine and patient phenylalanine. Tyr indicates tyrosine; Phe, phenylalanine; Asn, asparagine; Asp, asparatic acid; Ser, serine; Lys, lysine; Leu, leucine; and Arg, arginine. *Result of base analysis was significant but result of validating analysis using bootstrap resampling was not. Results of analysis were thus judged not to be statistically significant.

sheet, and position 156 is in the alpha helix of HLA class I molecule (Figure 2).[26,27] Position 9 constitutes peptide-binding pockets B and C, position 99 constitutes A, B, and D pockets, position 116 constitutes F pocket, and position 156 constitutes D and E pockets.[28] As a result, all amino acid positions elucidated in this study were important positions for peptide binding and T-cell recognition, although all substituted positions including positions at which residues are not accessible in the vicinity of peptide binding sites were analyzed.

To our knowledge, amino acid substitutions at position 9 (Tyr9A-Phe9A and Tyr9C-Ser9C) and position 99 (Tyr99C-Phe99C) were newly identified in the present study as responsible for severe aGVHD.

Ferrara et al reported that the amino acid substitution at position 116 in HLA class I molecule increased the risk for aGVHD, although the substituted amino acid was not taken into consideration.[29] In our study, specific amino acid substitution at position 116 had a significant effect in HLA-C (Leu116C-Ser116C) and a marginal effect in HLA-A (Asn116A-Asp116A) for severe aGVHD (Table 5).

Position 156 of HLA molecule was certified to modify T-cell alloreactivity in vitro in HLA-A2,[30-32] HLA-B35,[33] and HLA-B44.[24] For example, in contrast to Asp156B in HLA-B*4402, the nonpolar nature of substituted Leu156B in HLA-B*4403 lost many interactions such as hydrogen bonds and van der Waals interactions with the other amino acid residues that constructed binding pockets. As a result, this substitution made the significant conformation change for alloreactivity.[24] In the HLA-B*3501 and HLA-B*3508 combination, Leu156B in HLA-B*3501 with nonpolar residue was substituted for Asp156B in HLA-B*3508 with polar residue, and induced strong alloreactivity.[33] In our study, the magnitude of the polar change of each substituted amino acid was calculated by "hydropathy scale,"[17] because the influence of this scale on the amino acid interaction was much greater than the influence of the isoelectric point.[34] Specific amino acid substitutions at position 9, 99, 116, and 156, which were not associated with KIR2DL ligand, were found to induce great polar changes except for Tyr9C-Ser9C. Generally speaking, the 3 major physicochemical properties of amino acids that play important roles in protein structure are the hydropathy scale, isoelectric point, and molecular weight, and molecular weight is reflected in the size of amino acids.[34] Indeed, although tyrosine and serine in Tyr9C-Ser9C show few differences in hydropathy scale and isoelectric point, their molecular weights are quite different and may well induce an important conformation change in the HLA molecule. Thus, the change in the conformation by the polar change of the HLA molecule might be one of the mechanisms inducing alloreactivity. These data serve to clarify the mechanisms of aGVHD based on the HLA molecule.

The analysis of HLA-B, -DRB1, -DPB1, and -DQB1 mismatch for the substitution of amino acid elucidated no responsible position for severe aGVHD, and the analysis of HLA-A elucidated only one position. We speculate that the reason for the above result in HLA class I was that in this population there were fewer HLA-mismatched pairs in HLA-A and -B than in HLA-C. Although the findings are due mainly to the HLA-C molecule, specific amino acid substitution at positions 9, 99, 116, and 156 on the HLA class I molecule may induce strong alloreactivity because the structures of HLA class I molecules are quite similar.[29] Indeed, position 9 is selected in HLA-A and -C concurrently, and position 116 had a significant effect on HLA-C and a marginal effect on HLA-A (Figure 2). In HLA class II, we speculated that the molecular base of aGVHD caused by the HLA class II mismatch might be different from that in HLA class I.

In conclusion, we clarified nonpermissive mismatch combinations of all major 6 HLA loci. These data would be beneficial for the selection of suitable donors and international donor exchange for UR-HSCT. Furthermore, we identified the positions and types of amino acid substitutions responsible for severe aGVHD and presented speculations for alloreactivity on the basis of the conformation change of the HLA molecule. These findings provide evidence to elucidate the mechanism of aGVHD on the basis of the HLA molecule.

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Authorship

Contribution: T.S., Y.M., T.K., T.J., and Y.K. participated in the conception of this study; K.K., H.I., and H.S. performed the execution for histocompatibility; Y.M. and S.K. performed the execution for transplantation; T.K. and K.M. performed statistical

data analysis; T.K. and Y.M. wrote the paper; all authors checked the final version of the paper.

A complete list of the institutions participating and registering patients through the Japan Marrow Donor Program for the present study is available on the *Blood* website; see the Supplemental Appendix link at the top of the online article.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. N Engl J Med. 1993;328:593-602.
2. Kodera Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. Bone Marrow Transplant. 1999; 24:995-1003.
3. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by comprehensive matching of HLA class I and II alleles in the donor and recipient. Blood. 1998;92:3515-3520.
4. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor: Japan Marrow Donor Program. N Engl J Med. 1998;339:1177-1185.
5. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A, HLA-B, and HLA-DR matched unrelated donors. Blood. 2002;99:4200-4206.
6. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004;104:1923-1930.
7. Petersdorf EW, Kollman C, Hurley CK, et al. Effect of HLA class II gene disparity on clinical outcome in unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia: the US National Marrow Donor Program Experience. Blood. 2001;98:2922-2929.
8. Davies SM, Kollman C, Anasetti C, et al. Engraftment and survival after unrelated-donor bone marrow transplantation: a report from the national marrow donor program. Blood. 2000;96:4096-4102.
9. Morishima Y, Yabe T, Matsuo K, et al. Effects of HLA allele and killer immunoglobulin-like receptor ligand matching on clinical outcome in leukemia patients undergoing transplantation with T-cell-replete marrow from an unrelated donor. Biol Blood Marrow Transplant. 2007;13:315-328.
10. Zino E, Frumento G, Marktel S, et al. A T-cell epitope encoded by a subset of HLA-DPB1 alleles determines nonpermissive mismatches for hematologic stem cell transplantation. Blood. 2004;103:1417-1424.
11. Fleischhauer K, Locatelli F, Zecca M, et al. Graft rejection after unrelated donor hematopoietic stem cell transplantation for thalassemia is associated with nonpermissive HLA-DPB1 disparity in host-versus-graft direction. Blood. 2006;107: 2984-2992.
12. Allele Frequencies Database: USA Caucasian Bethesda, USA Olmstead County Minesota. Available at: http://www.allelefrequencies.net. Accessed May 1, 2007.
13. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15:825-828.
14. IMGT/HLA Sequence Database. Available at: http://www.ebi.ac.uk/imgt/hla/. Accessed February 1, 2007.
15. Socie G. Graft-versus-host disease–from the bench to the bedside? N Engl J Med. 2005;353: 1396-1397.
16. Hansen JA, Gooley TA, Martin PJ, et al. Bone marrow transplants from unrelated donors for patients with chronic myeloid leukemia. N Engl J Med. 1998;338:962-968.
17. Kyte J, Doolittle RF. A simple method for displaying the hydropathic character of a protein. J Mol Biol. 1982;157:105-132.
18. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695-706.
19. Coviello V, Boffess M. Cumulative incidence estimation in the presence of compiting risks. Stata J. 2004;4:103-112.
20. Cox DR. Regression models and life-tables. J R Stat Soc (B). 1972;34:187-220.
21. Peduzzi P, Concato J, Kemper E, Holford TR, Feinstein AR. A simulation study of the number of events per variable in logistic regression analysis. J Clin Epidemiol. 1996;49:1373-1379.
22. Efron B. Bootstrap methods: another look at the jackknife. Ann Stat. 1979;7:1-26.
23. Manly BFJ. Randomization, Bootstrap and Monte Carlo Methods in Biology. London, United Kingdom: Chapman and Hall; 1997.
24. Macdonald WA, Purcell AW, Mifsud NA, et al. A naturally selected dimorphism within the HLA-B44 supertype alters class I structure, peptide repertoire, and T cell recognition. J Exp Med. 2003;198:679-691.
25. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol. 2005;5:201-214.
26. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human class I histocompatibility antigen, HLA-A2. Nature. 1987;329:506-512.
27. Petersdorf EW, Hansen JA, Martin PJ, et al. Major-histocompatibility-complex class I alleles and antigens in hematopoietic-cell transplantation. N Engl J Med. 2001;345:1794-1800.
28. Steven GE, Peter P, Linda DB. The HLA Facts Book. London, United Kingdom: Academic Press; 2000.
29. Ferrara GB, Bacigalupo A, Lamparelli T, et al. Bone marrow transplantation from unrelated donors: the impact of mismatches with substitutions at position 116 of the human leukocyte antigen class I heavy chain. Blood. 2001;98:3150-3155.
30. Hogan KT, Clayberger C, Bernhard EJ, et al. Identification by site-directed mutagenesis of amino acid residues contributing to serologic and CTL-defined epitope differences between HLA-A2.1 and HLA-A2.3. J Immunol. 1988;141:2519-2525.
31. Mattson DH, Shimojo N, Cowan EP, et al. Differential effects of amino acid substitutions in the beta-sheet floor and alpha-2 helix of HLA-A2 on recognition by alloreactive viral peptide-specific cytotoxic T lymphocytes. J Immunol. 1989;143: 1101-1107.
32. Shimojo N, Cowan EP, Engelhard VH, Maloy WL, Coligan JE, Biddison WE. A single amino acid substitution in HLA-A2 can alter the selection of the cytotoxic T lymphocyte repertoire that responds to influenza virus matrix peptide 55-73. J Immunol. 1989;143:558-564.
33. Tynan FE, Elhassen D, Purcell AW, et al. The immunogenicity of a viral cytotoxic T cell epitope is controlled by its MHC-bound conformation. J Exp Med. 2005;202:1249-1260.
34. Biro JC. Amino acid size, charge, hydropathy indices and matrices for protein structure analysis. http://www.tbiomed.com/content/pdf/1742-4682-3-15.pdf. Accessed February 1, 2007.

P.28～41 の日本語版抄録

「日本骨髄バンクを介した非血縁者間骨髄移植の成績報告書（2006 年度集計）」より抜粋

【T 細胞非除去非血縁者間骨髄移植を受けた白血病患者における HLA 型と KIR リガンド適合度の臨床成績への影響について】

森島泰雄、屋部登志雄、松尾恵太郎、柏瀬貢一、猪子英俊、佐治博夫、山本健、丸屋悦子、赤塚美樹、鬼塚真、坂巻壽、佐尾浩、小川誠司、加藤俊一、十字猛夫、笹月健彦、小寺良尚、日本骨髄バンク

日本骨髄バンクを介した白血病移植1790症例におけるHLA座とkiller immunoglobulin-like receptor (KIR)の影響を同時に多変量解析にて調べた。

移植片対白血病効果（GVL）は、白血病の病型により異なっていた。急性リンパ性白血病（ALL）では、HLA-C不適合が、hazard ratio (HR) 0.47, p=0.003)で慢性骨髄性白血病では、HLA-DPB1不適合がHR 0.35, p<0.001で、白血病の再発は有意に低下していた。一方、NK細胞受容体の一つであるKIR２DLのリガンドの不適合（GVH方向）は、ALLにおいて、HR 2.55 p=0.017と白血病の再発率が高まっていた。

移植片の拒絶（2次的正着不全）率は、KIR2DLリガンドの拒絶方向不適合で、HR 4.39 P=0.012と高くなっていた。

急性移植片対宿主病（GVHD）は、HLA-A, B, C, DPB1の各座不適合、ならびにKIR2DLリガンド不適合（GVH方向）で有意に高率であった。

これらの結果として、HLA-A, B, DQB1およびKIR2DLリガンド（GVHD方向）不適合において有意に移植後の生存率が低下していた。

まとめとして、HLA-C, HLA－DPB1およびKIR2DLリガンド不適合が、白血病の再発に関与していたが、白血病の病型により異なっていた。さらに、KIR2DLリガンド不適合は、急性GVHDや拒絶を増加させており、T細胞非除去非血縁者間骨髄移植における生存に対して何ら良い効果を示さなかった。

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Effects of HLA Allele and Killer Immunoglobulin-Like Receptor Ligand Matching on Clinical Outcome in Leukemia Patients Undergoing Transplantation With T-cell–Replete Marrow From an Unrelated Donor

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ABSTRACT

The responsible human leukocyte antigen (HLA) locus and the role of killer immunoglobulin-like receptor (KIR) ligand matching on transplantation outcome were simultaneously identified by multivariate analysis in 1790 patients with leukemia who underwent transplantation with T-cell–replete marrow from an unrelated donor (UR-BMT) through the Japan Marrow Donor Program. The graft-versus-leukemia (GVL) effect depended on leukemia cell type. HLA-C mismatch reduced the relapse rate in acute lymphoblastic leukemia (ALL) (hazard ratio [HR] = 0.47; $P = .003$), and HLA-DPB1 mismatch reduced it in chronic myeloid leukemia (CML) (HR = 0.35; $P < .001$). In contrast, KIR2DL ligand mismatch in the graft-versus-host (GVH) direction (KIR-L-MM-G) increased in ALL (HR = 2.55; $P = .017$). An increased rejection rate was observed in KIR2DL ligand mismatch in the host-versus-graft direction (HR = 4.39; $P = .012$). Acute GVH disease (GVHD) was increased not only in the mismatch of HLA-A, -B, -C, and -DPB1, but also in KIR-L-MM-G. As a whole, the mismatch of HLA-A, -B, and -DQB1 locus and KIR-L-MM-G resulted in increased mortality. In conclusion, not only the mismatch of HLA-C and -DPB1, but also KIR-L-MM-G affected leukemia relapse, which should be considered based on leukemia cell type. Furthermore, KIR-L-MM induced adverse effects on acute GVHD (aGVHD) and rejection, and brought no survival benefits to patients with T-cell–replete UR-BMT.

KEY WORDS

KIR ligand incompatibility • HLA • Leukemia • Unrelated bone marrow transplantation

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) from a human leukocyte antigen (HLA)-matched unrelated (UR) donor has been established as one mode of curative therapy for hematologic malignancies and other hematologic or immunologic disorders [1,2]. Extensive research on genetic factors such

as HLA has produced mounting evidence of the presence of HLA alleles that drastically affect HSCT outcome through T cells. Induction of the graft-versus-leukemia (GVL) effect to reduce relapse of leukemia is considered an advantage of allogeneic HSCT [3]. There have been several large-scale analyses of UR-HSCT. The Japan Marrow Donor Program (JMDP) demonstrated the effect of matching of HLA class I alleles (HLA-A, -B, and -C) on the development of severe acute graft-versus-host disease (aGVHD) and the importance of HLA-A and -B allele matching for better survival in T-cell–replete UR-HSCT [4,5]. The Fred Hutchinson Cancer Research Center and the US National Marrow Donor Program (NMDP) reported the importance of HLA class II matching in GVHD and survival [6,7]. Updated analysis of the NMDP indicated that HLA-A allele-level mismatching, HLA-B serologic mismatching, and HLA-DRB1 mismatching are significant risk factors for severe aGVHD, and that disparity in HLA class I (HLA-A, -B, or -C) and/or HLA-DRB1 increased the mortality [8]. Furthermore, the role of HLA-DPB1 matching has been elucidated for aGVHD [9-11] and leukemia relapse [12]. However, the aforementioned reports have produced considerable conflicting results.

It has become evident that natural killer (NK) cells and the subpopulation of T cells express NK cell receptors, and that the activity of NK cells is controlled by the recognition of HLA class I molecules on the target cells by NK cell inhibitory and activating receptors [13,14]. The genotype and haplotype of the killer immunoglobulin-like receptors (KIRs) have been identified, and ligand specificities of KIRs have been characterized. C1 specificity of the HLA-C epitope (Asp80) is the ligand of inhibitory KIR2DL2/3, C2 specificity (Lys80) is the ligand of inhibitory KIR2DL1, and HLA-Bw4 is the ligand of KIR3DL1. With allogeneic HSCT, the disparities of these receptors between donor and recipient are suspected to induce transplant-related immunologic events through activation of NK cells, and evidence of the clinical outcome of HSCT in relation to KIR disparities has been accumulated [15]. However, reports of KIR ligand matching in UR-HSCT have shown contradictory results [16]. Limited patient numbers, different diseases, and various GVHD prophylaxes make it difficult to draw definite conclusions from these studies.

In the present study, the effects of HLA locus and KIR ligand matching were simultaneously analyzed in leukemia patients receiving T-cell–replete marrow from unrelated donors through the JMDP after a myeloablative conditioning regimen, focusing in particular on the influence of leukemia cell type on the GVL effect.

PATIENTS AND METHODS

Patients

A total of 1790 consecutive leukemia patients who underwent transplantation with marrow from a serologically HLA-A, -B, and -DR antigen–matched donor in Japan between January 1993 and March 2000 through the JMDP were analyzed. No patients receiving T-cell–depleted marrow and/or antithymocyte globulin (ATG) as GVHD prophylaxis were eligible for this study. Partial HLA-A and -B alleles and complete HLA-DRB1 alleles were identified as confirmatory HLA typing during the coordination process, and HLA-A, -B, -C, -DQB1, and -DPB1 alleles were retrospectively reconfirmed or identified after transplantation. The final clinical survey of these patients was completed as of June 1, 2005. Informed consent was obtained from patient and donor according to the Declaration of Helsinki, and approval was obtained from the JMDP and the Institutional Review Board of the Aichi Cancer Center.

Characteristics of patients and donors are listed in Table 1. The patients' age ranged from 0 to 59 years (median, 27 years), and donors' age ranged from 20 to 51 years (median, 35 years). There were 577 patients with acute myeloblastic leukemia (AML), of whom 186 underwent transplantation while in first complete remission (CR), 191 who did so while in second or further CR, and 200 who did so while in non-CR; 617 patients with acute lymphoblastic leukemia (ALL), of whom 236 underwent transplantation while in first CR, 207 who did so while in second or further CR, and 174 who did so while in non-CR; and 596 patients with chronic myeloid leukemia (CML), of whom 417 were in the first chronic phase (CP), 34 were in the second or further CP, 90 were in the accelerated phase, and 55 were in the blastic phase. Standard risk for leukemia relapse was defined as the status of the first CR of AML and ALL and the first CP of CML at transplantation, whereas high risk was defined as a more advanced status than standard risk in AML, ALL, and CML.

HLA Typing of Patients and Donors

Alleles at the HLA-A, -B, -C, -DRB1, -DQB1, and -DPB1 loci were identified as described previously [4,5]. HLA 6 locus alleles were typed in 1773 pairs, and HLA 5 locus alleles except HLA-DPB1 were typed in 17 pairs. HLA genotypes of HLA-A, -B, -C, -DQB1, and -DPB1 alleles of patient and donor were reconfirmed by the Luminex microbead method (100 System; Luminex, Austin, TX) adjusted for the JMDP [17] and in part by the sequencing-based typing method in 2004 and 2005. As a result, all HLA alleles that were observed with $> 0.1\%$ frequency among Japanese were identified. The numbers of

Table 1. *Patient characteristics and matching status of HLA allele and KIR2DL ligand*

	Patient Number (%) M/MM*	Patient Age Median (years) M/MM*	Patient Sex Female (%) M/MM*	Donor Age Median (years) M/MM*	Donor Sex Female (%) M/MM*	Sex Match (%) M/MM*	Stage at Transplant High (%) M/MM*	GVHD Prophylaxis Cyclosporine (%) M/MM*	Total Body Irradiation (%) M/MM*
All leukemia (n = 1790)									
HLA-A	1484/306	27/26	39/37	34/33	38/40	57/55	52/57	73/73	83/72
HLA-B	1645/145	27/26	40/34	34/35	39/36	56/63	52/51	72/76	83/84
HLA-C	1256/534	27/26	39/41	34/33	38/40	56/58	52/55	74/70	83/82
HLA-DRB1	1434/356	27/26	40/38	34/34	38/41	57/57	51/60	74/66	83/82
HLA-DQB1	1391/399	27/26	40/38	34/33	38/41	57/57	52/56	74/67	83/83
HLA-DPB1	612/1163	26/27	42/39	34/34	39/39	60/56	50/55	75/71	81/84
KIR2DL-G†	1693/97	26/27	39/35	34/34	39/43	57/74	53/63	73/64	83/84
KIR2DL-R‡	1679/111	27/25	39/40	34/32	39/60	57/51	53/59	73/67	83/84
Acute myeloblastic leukemia (n = 577)									
HLA-A	486/91	28/27	44/44	33/33	38/39	58/55	67/71	72/60	81/89
HLA-B	537/40	27/31	45/33	33/35	39/30	56/73	67/83	71/68	83/80
HLA-C	405/172	28/28	43/45	33/34	39/37	56/61	66/73	74/63	82/83
HLA-DRB1	474/103	28/27	44/43	33/33	37/47	58/55	66/77	72/63	82/86
HLA-DQB1	469/108	27/29	45/40	33/33	38/43	57/56	67/72	72/64	83/81
HLA-DPB1	206/366	27/28	48/42	34/33	40/38	58/57	65/70	71/70	81/84
KIR2DL-G†	546/31	28/28	43/55	33/33	38/39	57/65	67/71	72/52	82/83
KIR2DL-R‡	546/31	28/28	43/55	33/35	38/39	59/32	68/68	71/58	82/83
Acute lymphoblastic leukemia (n = 617)									
HLA-A	515/102	20/19	41/40	34/32	42/42	55/50	60/69	73/74	91/88
HLA-B	567/50	19/20	41/42	33/36	42/38	54/60	61/70	72/80	91/86
HLA-C	437/180	19/19	41/41	34/32	41/42	54/57	61/63	73/72	91/89
HLA-DRB1	485/132	19/19	41/42	33/33	43/36	55/52	61/64	74/70	90/90
HLA-DQB1	467/150	19/20	41/41	34/33	42/41	55/51	61/63	75/68	90/92
HLA-DPB1	190/425	19/29	43/40	34/33	38/43	61/52	61/62	77/71	89/91
KIR2DL-G†	587/30	20/17	42/20	33/35	42/40	55/53	61/73	73/73	91/83
KIR2DL-R‡	577/40	19/19	39/40	34/30	42/43	54/53	61/73	73/70	90/93
Chronic myelocytic leukemia (n = 596)									
HLA-A	483/113	32/31	33/35	34/34	35/40	59/60	29/35	73/81	76/72
HLA-B	541/55	32/29	34/27	34/37	36/38	56/60	29/36	74/78	74/85
HLA-C	414/182	32/31	33/36	35/34	35/39	60/58	30/31	74/76	75/74
HLA-DRB1	475/121	32/33	34/31	34/36	35/40	58/63	27/41	77/64	76/70
HLA-DQB1	455/141	32/31	34/33	35/33	35/39	57/65	28/35	76/69	75/74
HLA-DPB1	216/372	31/33	35/33	34/35	38/34	60/59	28/31	76/73	73/76
KIR2DL-G†	560/36	32/32	34/31	35/32	35/50	59/53	29/44	75/67	71/83
KIR2DL-R‡	556/40	32/27	34/28	35/31	36/38	59/65	29/38	75/68	75/75

Standard-first complete remission or first chronic phase; high more advanced stage than standard.
*M/MM match/mismatch in GVH direction for HLA matching.
†KIR2DL ligand mismatching in GVH direction.
‡KIR2DL ligand mismatching in HVG direction.

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identified alleles in this study were 25 in HLA-A, 43 in HLA-B, 20 in HLA-C, 33 in HLA-DRB1, 14 in HLA-DQB1, and 21 in HLA-DPB1.

Matching of HLA Allele and KIR2DL Ligand

For the analysis of GVHD and leukemia relapse, HLA allele mismatch among the donor–recipient pair was scored when the recipient's alleles were not shared by the donor (graft-versus-host [GVH] direction). For graft rejection, HLA allele mismatch among the donor–recipient pair was scored when the donor's alleles were not shared by the patient (host-versus-graft [HVG] direction). For survival, the mismatch was defined as that of either the GVH direction or the HVG direction.

KIR2DL ligand specificity of HLA-C antigen was determined according to the HLA-C allele. The epitope of HLA-Cw3 group (C1 specificity) consists of Asn80, and that of the HLA-Cw4 group (C2 specificity) consists of Lys80.

KIR ligand mismatch in the GVH direction (KIR-L-MM-G) was scored when the donor's KIR2DL epitope of HLA-C was not shared by the patient epitope. This mismatch occurred when KIR2DL2/3- or KIR2DL1-positive effector cells were activated without the expression of corresponding HLA-C ligand (C1 or C2, respectively) on the patient's target cells. KIR ligand mismatch in HVG direction (KIR-L-MM-R) was scored when the patient's KIR2DL epitope of HLA-C was not shared by the donor. This mismatch occurred when patient KIR2DL2/3- or KIR2DL1-positive effector cells were activated without the expression of corresponding HLA-C ligand (C1 or C2, respectively) on donor cells.

Matching Status of HLA Locus in Allele Level and KIR2DL Ligand

The matching status of HLA allele matching in the GVH direction in each HLA locus and KIR ligand matching in both directions are given in Table 1. The HLA-C epitope of KIR2DL was estimated from HLA-C allele type, with 92.4% of the HLA-C allele belonging to the Cw3 group (C1 specificity) and 7.6% belonging to the Cw4 group (C2 specificity). KIR2DL ligand match in both directions occurred in 1583 pairs (88.4%). KIR-L-MM-G, which occurred in the combination of KIR2DL ligand in patient–donor pairs, was found in 97 pairs (5.4%): C1/C1 and C1/C2 in 92 pairs, C2/C2 and C1/C2 in 4 pairs, and C1/C1 and C2/C2 in 1 pair. KIR-L-MM-R, which occurred in the combination of KIR2DL ligand in patient and donor pairs, was found in 111 pairs (6.2%): C1/C2 and C1/C1 in 105 pairs, C1/C2 and C2/C2 in 5 pairs, and C1/C1 and C2/C2 in 1 pair. Mismatches in both directions were found in only 1 pair. Because all pairs were a serologic HLA-B match in this study, the combination of KIR3DL1 and its ligand of Bw4 matched in all pairs.

Definition of Transplantation-Related Events

The occurrence of aGVHD was evaluated according to grading criteria in patients who survived more than 8 days after transplantation, and chronic GVHD (cGVHD) according to the criteria in patients who survived more than 100 days after transplantation as described previously [5]. Rejection was defined as when the peripheral granulocyte count became $< 500/\mu L$ with the finding of severe hypoplastic marrow in engrafted patients. Engraftment was defined as a peripheral granulocyte count of $> 500/\mu L$ for 3 successive days in patients surviving > 21 days after transplantation.

GVHD Prophylaxis

Among the 1790 patients transplanted with T-cell–replete marrow, 1302 received a cyclosporine-based regimen and 488 received a tacrolimus-based regimen for GVHD prophylaxis. Anti-thymocyte globuline (ATG) was not given for GVHD prophylaxis.

Preconditioning Regimen

All patients were preconditioned with a myeloablative regimen, with 1480 receiving total body irradiation (TBI)-containing regimens and 310 receiving non-TBI regimens.

Statistical Analysis

All of the analyses were conducted using STATA version 8.2 (STATA Corp, College Station, TX). Overall survival rate was assessed by the Kaplan-Meier product limit method [18]. Cumulative incidences of aGVHD, cGVHD, rejection, and leukemia relapse were assessed as described previously to eliminate the effect of competing risk [19,20]. The competing events regarding aGVHD, cGVHD, rejection, and relapse were defined as death without aGVHD, cGVHD, rejection, and relapse, respectively. For each endpoint, a log-rank test was applied to assess the impact of the factor of interest.

Cox proportional hazard models [21] were applied to assess the impact of HLA allele matching (mismatch vs match [hazard risk = 1.0] as a reference group) as well as KIR ligand matching (mismatch vs match in the GVH direction and mismatch vs match in the HVG direction) including the following confounders. The confounders considered were sex (donor–recipient pairs), patient age (older: linear), donor age (older: linear), type of disease (AML, CML, or ALL), risk of leukemia relapse (high vs standard),

Table 2. *Effects of HLA and KIR ligand matching for leukemia relapse*

	All Leukemia Cell Types			Acute Myeloblastic Leukemia			Acute Lymphoblastic Leukemia			Chronic Myeloid Leukemia		
	HR*	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P	HR	(95% CI)	P
HLA-A	1.19	(0.89-1.59)	.251	0.92	(0.54-1.58)	.761	1.18	(0.76-1.86)	.462	1.63	(0.89-2.97)	.114
HLA-B	1.01	(0.65-1.59)	.953	1.36	(0.65-2.88)	.416	0.98	(0.48-1.98)	.952	0.62	(0.22-1.76)	.367
HLA-C	0.71	(0.53-0.96)	.025	0.8	(0.49-1.30)	.366	0.47	(0.28-0.78)	.003	1.2	(0.62-2.29)	.591
HLA-DRB1	1.05	(0.73-1.53)	.789	0.78	(0.40-1.52)	.466	0.91	(0.51-1.61)	.737	1.25	(0.55-2.85)	.59
HLA-DQB1	1.10	(0.77-1.58)	.579	1.55	(0.82-2.95)	.178	1.11	(0.63-1.95)	.71	0.86	(0.39-1.93)	.72
HLA-DPB1	0.68	(0.55-0.85)	.001	0.76	(0.52-1.09)	.137	0.92	(0.65-1.28)	.604	0.35	(0.21-0.58)	<.001
KIR2DL-G†	1.55	(0.92-2.63)	.103	1.05	(0.37-3.02)	.926	2.55	(1.18-5.52)	.017	1.23	(0.38-3.94)	.727
KIR2DL-R‡	0.73	(0.40-1.34)	.313	0.53	(0.15-1.78)	.305	1.30	(0.53-3.19)	.569	0.5	(0.14-1.80)	.292

HLA matching in GVH direction.

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.

†KIR2DL ligand mismatching in GVH direction.

‡KIR2DL ligand mismatching in HVG direction.

GVHD prophylaxis (tacrolimus-based vs cyclosporine-based and ATG vs cyclosporine-based), numbers of transplanted cells (linear), and preconditioning (non-TBI vs TBI). The numbers of nucleated cells before manipulation of bone marrow were replaced with the numbers of transplanted cells.

Multivariate analysis for clinical outcomes, including KIR ligand matching and HLA-C matching in all pairs (not restricted to HLA-C mismatch), made it possible to evaluate whether these factors are independent. The results of all pairs by multivariate analysis are presented in the Results section and in Tables 2, 3, and 4. HLA-C–mismatched pairs were selected for the analysis of cumulative incidence in KIR ligand matching.

RESULTS

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Leukemia Relapse

When all leukemia patients (AML, ALL, and CML) were analyzed together, HLA-C mismatch was found to be a factor reducing the relapse rate (HR = 0.71; P = .025) (Table 2). This GVL effect was remarkable in ALL patients (HR = 0.47; P = .003), especially in high risk (HR = 0.40; P = .004) but not in standard risk (HR = 0.85; P = .728). No such effect was observed in AML patients (HR = 0.80; P = .366) or CML patients (HR = 1.20; P = .591).

Cumulative incidence curves of relapse by leukemia cell type are shown in Figure 1. The relapse rate 5 years after transplantation was 16.7% (95% confidence interval [CI] = 11.6%-30.9%) for HLA-C mismatch and 29.8% (95% CI = 25.5%-34.3%) for HLA-C match in ALL patients (P = .012); 17.6% (95% CI = 12.2%-23.8%) and 25.9% (95% CI = 21.1%-30.9%), respectively, in AML patients (P = .342); and 11.7% (955 CI = 12.2%-23.8%) and 12.0% (95% CI = 9.0%-15.4%), respectively, in CML patients (P = .485).

HLA-DPB1 mismatch was shown to reduce the overall leukemia relapse rate (HR = 0.68; P = .001) (Table 2). This effect was significant in CML (HR =

Table 3. *Effects of HLA and KIR ligand matching for acute GVHD, chronic GVHD, and rejection in all leukemia cell types*

	Acute GVHD (Grade 2-4) (n = 1751)			Acute GVHD (Grade 3-4) (n = 1751)			Chronic GVHD (n = 1109)			Rejection (n = 1664)		
	HR*	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
HLA-A	1.22	(1.02-1.46)	.034	1.44	(1.11-1.86)	.006	1.41	(1.08-1.85)	.013	0.72	(0.24-2.14)	.555
HLA-B	1.43	(1.28-1.82)	.003	1.40	(1.00-1.95)	.05	1.00	(0.65-1.52)	.991	1.16	(0.32-4.16)	.82
HLA-C	1.29	(1.08-1.55)	.006	1.39	(1.06-1.83)	.017	1.38	(1.07-1.78)	.014	1.87	(0.72-4.86)	.201
HLA-DRB1	1.15	(0.90-1.47)	.254	1.09	(0.77-1.54)	.644	0.91	(0.63-1.31)	.607	0.49	(0.10-2.33)	.366
HLA-DQB1	1.02	(0.81-1.29)	.871	1.13	(0.81-1.59)	.465	1.20	(0.85-1.69)	.288	0.62	(0.07-5.16)	.536
HLA-DPB1	1.39	(1.19-1.63)	<.001	1.26	(1.00-1.60)	.053	0.86	(0.70-1.05)	.138	1.08	(0.59-2.41)	.843
KIR2DL-G†	1.70	(1.28-2.26)	<.001	2.35	(1.62-3.40)	<.001	1.13	(0.68-1.87)	.64	0.62	(0.07-5.16)	.655
KIR2DL-R‡	1.04	(0.77-1.42)	.78	1.33	(0.88-2.02)	.18	0.88	(0.55-1.42)	.603	4.39	(1.38-13.96)	.012

HLA matching in GVH direction for acute GVHD and chronic GVHD, and HLA matching in HVG direction for rejection.

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.

†KIR2DL ligand mismatching in GVH direction.

‡KIR2DL ligand mismatching in HVG direction.

Table 4. *Effects of HLA and KIR ligand matching for mortality*

	All Leukemia Cell Types			Acute Myeloblastic Leukemia			Acute Lymphoblastic Leukemia			Chronic Myeloid Leukemia		
	HR*	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
HLA-A	1.36	(1.16-1.59)	<.001	1	(0.75-1.34)	.978	1.46	(1.11-1.90)	.006	1.77	(1.35-2.33)	<.001
HLA-B	1.40	(1.13-1.73)	.002	1.43	(0.96-2.12)	.079	1.47	(1.03-2.09)	.036	1.18	(0.80-1.72)	.402
HLA-C	1.17	(0.99-1.37)	.067	1.18	(0.89-1.55)	.246	0.99	(0.74-1.31)	.928	1.42	(1.04-1.93)	.025
HLA-DRB1	0.92	(0.74-1.15)	.463	0.74	(0.50-1.10)	.136	1.04	(0.72-1.49)	.849	0.99	(0.65-1.50)	.951
HLA-DQB1	1.28	(1.04-1.58)	.018	1.29	(0.89-1.87)	.184	1.33	(0.93-1.90)	.108	1.18	(0.79-1.75)	.422
HLA-DPB1	1.06	(0.91-1.23)	.474	0.96	(0.75-1.24)	.772	1.33	(1.02-1.75)	.038	0.97	(0.74-1.27)	.827
KIR2DL-G†	1.80	(1.39-2.34)	<.001	1.93	(1.22-3.05)	.005	1.57	(0.96-2.56)	.069	2.23	(1.42-3.50)	<.001
KIR2DL-R‡	1.07	(0.81-1.41)	.612	1.08	(0.66-1.75)	.769	0.98	(0.59-1.61)	.934	1.07	(0.66-1.72)	.787

*Hazard ratio of mismatch with match as a reference adjusted for patient age, donor age, sex-matching disease, GVHD prophylaxis, total body irradiation, transplanted cell dose, risk status, and other matching status of HLA and KIR ligand.
†KIR2DL ligand mismatching in GVH direction.
‡KIR2DL ligand matching in HVG direction.

0.35; $P < .001$), and both high-risk and standard-risk CML had a significantly lower relapse rate of HLA-DPB1 mismatch (HR = 0.35; $P < .001$ and HR = 0.39; $P = .012$, respectively). No significant effect was observed in AML (HR = 0.76; $P = .137$) or ALL (HR = 0.92; $P = .604$).

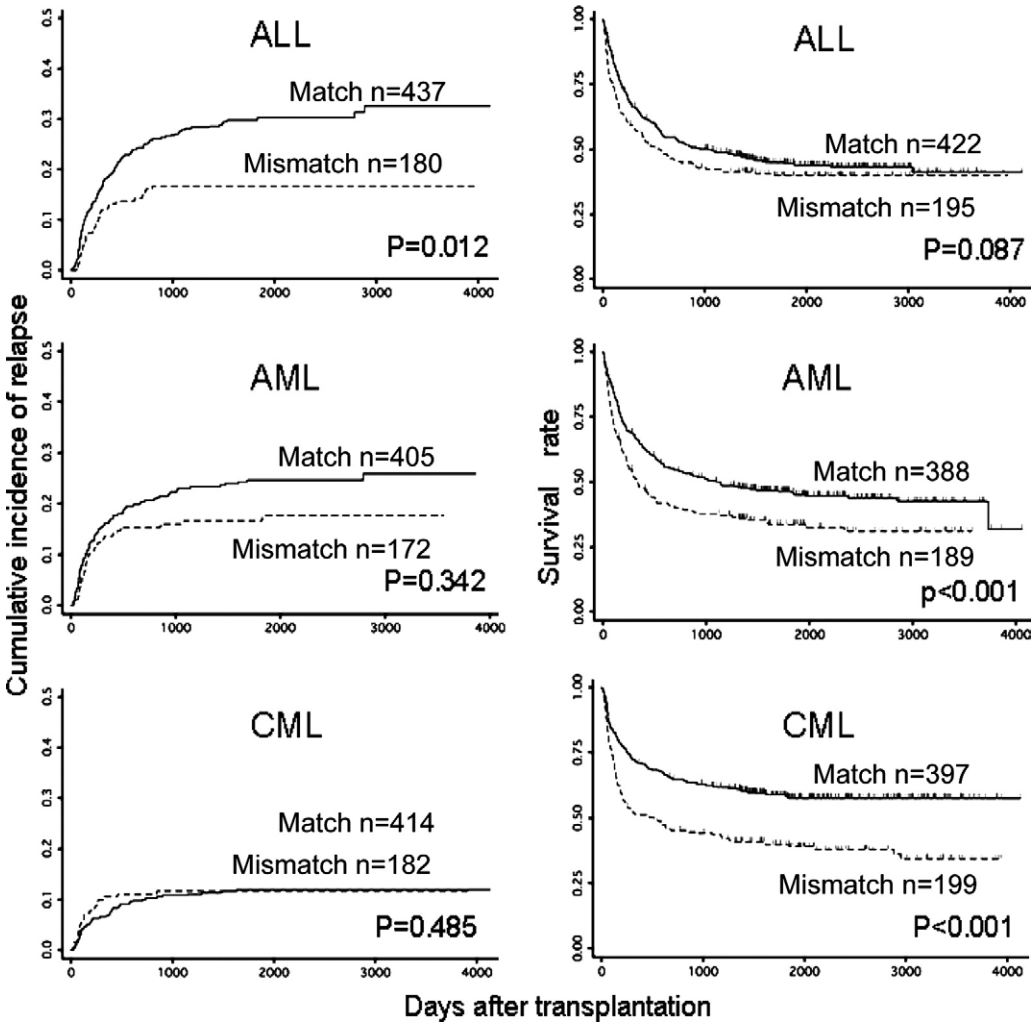

Figure 1. Cumulative incidence of relapse and survival by matching of HLA-C in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-C for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. The solid line represents match; the dotted line, mismatch.

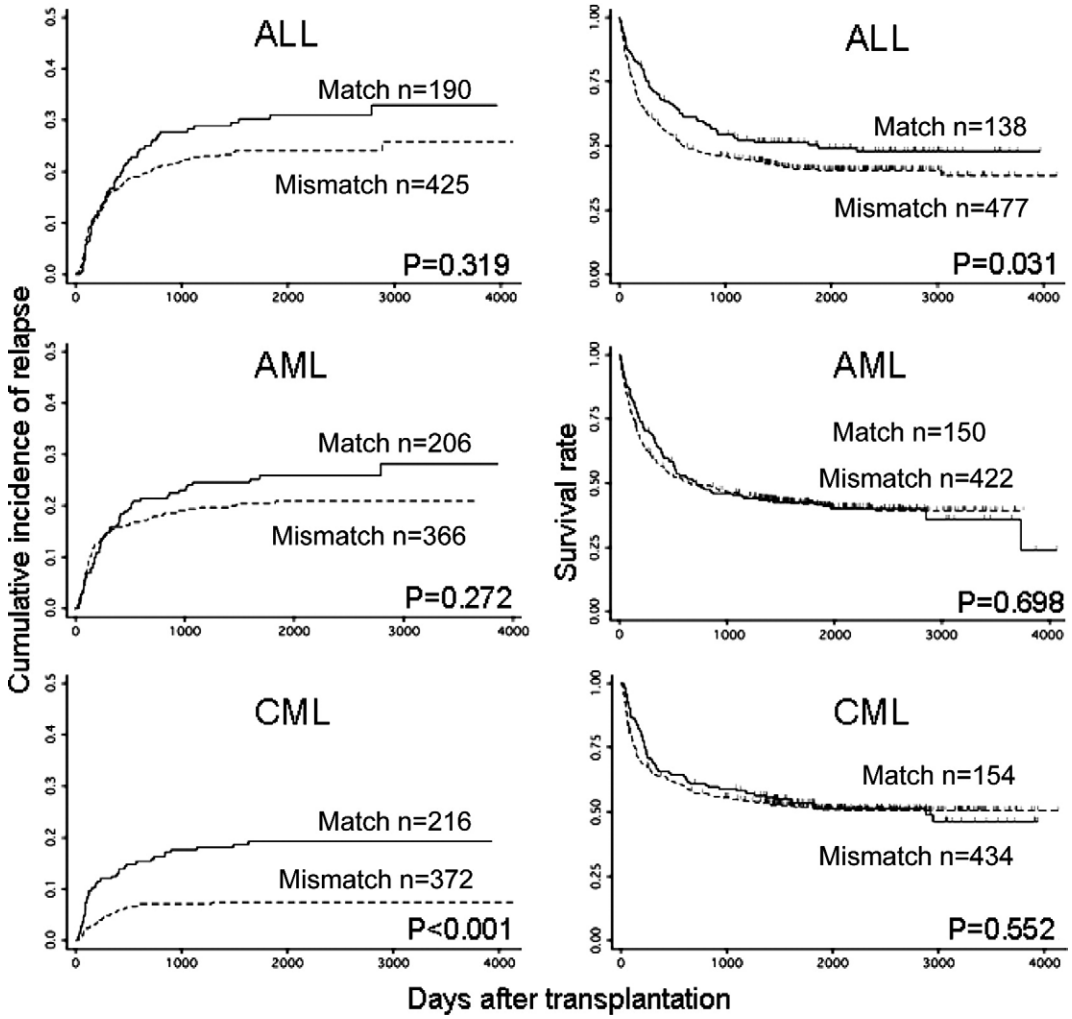

Figure 2. Cumulative incidence of relapse and survival by matching of HLA-DPB1 in patients with ALL, AML, and CML. All patients were analyzed. The direction of mismatching of HLA-DPB1 for relapse is GVH for relapse, and the direction for survival is GVH and/or HVG. Solid line, match; dotted line, mismatch.

As shown in Figure 2, the relapse rate 5 years after transplantation was 7.1% (95% CI = 5.0%-10.4%) for HLA-DPB1 mismatch and 19.3% (95% CI = 14.3%-24.9%) for HLA-DPB1 match in CML patients ($P < .001$); 20.4% (95% CI = 16.4%-24.8%) and 25.9% (95% CI = 19.9%-32.2%), respectively, in AML patients ($P = .272$); and 24.0% (95% CI = 19.9%-28.3%) and 30.2% (95% CI = 23.7%-37.0%), respectively, in ALL patients ($P = .319$).

Mismatch of HLA-A, -B, -DRB1, and -DQB1 was not a significant risk factor for leukemia relapse by multivariate analysis (Table 2).

Patients with KIR-L-MM-G had a higher relapse rate than those with KIR2DL ligand match in ALL (HR = 2.55; $P = .017$) (Table 2). This adverse effect on leukemia relapse was remarkable in high-risk ALL (HR = 3.03; $P = .013$), but not in standard-risk ALL (HR = 1.11; $P = .921$). In AML and CML, KIR-L-MM-G had no effect on leukemia relapse (HR = 1.05; $P = .926$ and HR = 1.23; $P = .727$, respectively).

Because KIR-L-MM occurs in HLA-C mismatch pairs, the cumulative incidence of leukemia relapse was analyzed in HLA-C mismatch patients in either direction by leukemia cell type (Figure 3). The relapse rate 5 years after transplantation was 31.0% (95% CI = 5.6%-47.9%) for KIR-L-MM-G and 16.3% (95% CI = 11.0%-22.4%) for match in ALL patients ($P = .026$); 11.1% (95% CI = 3.5%-23.6%) and 11.8% (95% CI = 7.4%-17.3%), respectively, in CML patients ($P = .634$); and 12.9% (95% CI = 4.1%-27.0%) and 16.3% (95% CI = 11.0%-22.6%), respectively, in AML patients ($P = .757$).

Significant clinical risk factors for leukemia relapse by multivariate analysis included status at transplantation (standard vs high, HR = 3.00; $P < .001$) and disease (HR = 0.75; $P < .001$) in all leukemia patients.

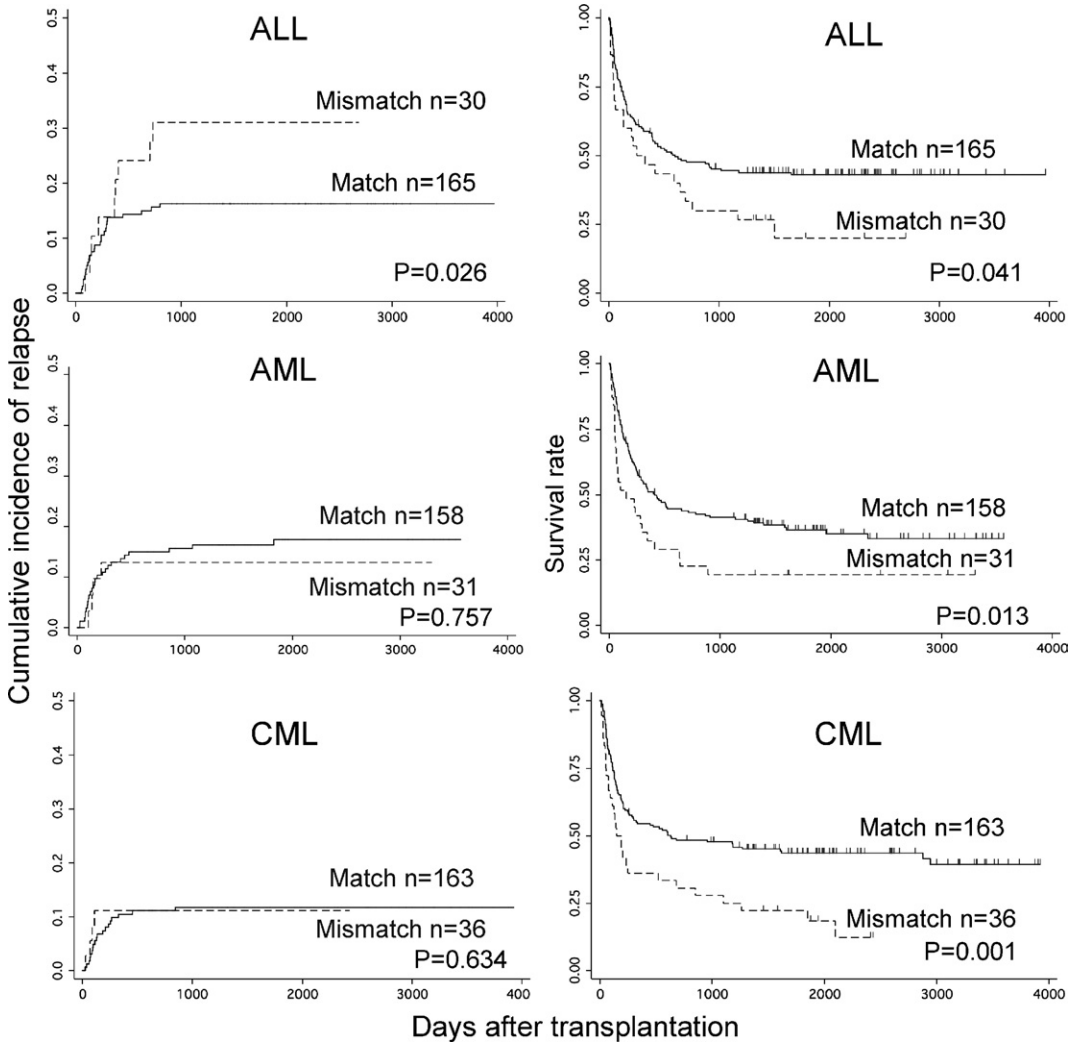

Figure 3. Cumulative incidence of relapse and survival by matching of KIR2DL ligand in the GVH direction in HLA-C–mismatched patients with ALL, AML, and CML. HLA-C–mismatched patients were selected for this analysis. The directions of HLA-C mismatching were GVH and/or HVG. The solid line represents KIR2DL ligand match in the GVH direction; the dotted line, KIR2DL mismatch in the GVH direction.

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Rejection

Rejection rates in patients who engrafted marrow and survived more than 21 days were analyzed. KIR-L-MM-R was found to be a significantly higher risk factor for rejection compared with match (HR = 4.39; P = .012), and no HLA mismatch was considered significant by multivariate analysis (Table 3). Older donor age was a significant clinical risk factor for rejection (HR = 1.08; P = .002); other clinical factors were not significant.

The cumulative incidence of graft rejection was 5.7% (95% CI = 2.3%-11.3%) in KIR-L-MM-R (n = 106) and 1.8% (95% CI = 0.8%-3.3%) in match (n = 447) (P = .019) 1 year after transplantation in HLA-C–mismatched patients in either direction. Engraftment rate was not influenced by HLA and KIR ligand matching (data not shown).

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Acute GVHD

HLA allele mismatch of each HLA-A, -B, and -C locus was found to be an independent risk factor for grade 3-4 aGVHD and grade 2-4 aGVHD, and the mismatch of each HLA-DRB1 and -DQB1 locus was not a significant risk factor. HLA-DPB1 mismatch was a significant risk factor for grade 2-4 aGVHD and a marginal risk factor for grade 3-4 aGVHD (Table 3). When analyzed by leukemia cell type, AML showed no significant HLA mismatch locus for aGVHD (data not shown).

KIR-L-MM-G was associated with a significantly higher risk of grade 2-4 aGVHD (HR = 1.70; $P < .001$) and grade 3-4 aGVHD (HR = 2.35; $P < .001$) compared with KIR ligand match (Table 3). By leukemia cell type, the HR of KIR-L-MM-G in grade 3-4 aGVHD was 2.76 for AML ($P = .005$), 1.75 for ALL ($P = .111$), and 2.79 for CML ($P < .001$).

In HLA-C mismatch patients, the incidence of 40.3% in KIR-L-MM-G (95% CI = 29.3%-50.9%) was significantly higher than the 25.8% in match (95% CI = 21.9%-30.0%) ($P = .011$) for grade 3-4 aGVHD.

Significant clinical risk factors for grade 3-4 GVHD by multivariate analysis were GVHD prophylaxis (tacrolimus vs cyclosporine, HR = 0.72; $P = .016$), patient age (HR = 0.99; $P = .019$), donor age (HR = 1.02; $P = .001$), and disease (HR = 1.28; $P = .001$) in all leukemia patients.

Effects of HLA Locus Mismatch and KIR Ligand Mismatch on Chronic GVHD

The occurrence of cGVHD was analyzed in patients who survived more than 100 days after transplantation. HLA-A mismatch and HLA-C mismatch were found to be significant factors (HR = 1.41; $P = .013$ and HR = 1.38; $P = .014$, respectively). KIR-L-MM-G was not significant (HR = 1.13; $P = .640$) (Table 3).

In HLA-C mismatch patients, the cumulative incidence of cGVHD 3 years after transplantation was 43.2% in KIR-L-MM-G (95% CI = 27.2%-58.3%) and 40.4% in KIR2DL ligand match (95% CI = 35.4%-46.1%) ($P = .727$). Significant clinical risk factors for cGVHD by multivariate analysis were patient age (HR = 1.01; $P = .0004$), disease (HR = 1.23; $P = .003$), and TBI (HR = 1.54; $P = .004$).

Effects of HLA Allele Mismatch and KIR Ligand Mismatch on Survival

In all leukemia patients, HLA allele mismatch of each HLA-A, -B, and -DQB1 locus was found to be an independent risk factor for mortality after transplantation, and the mismatch of HLA-C was of marginal risk. HLA mismatch in each HLA-DRB1 and -DPB1 locus was not a significant factor. By leukemia cell type, mismatch of HLA-A, -B, and -DPB1 was a significant risk factor in ALL, and mismatch of HLA-A and -C was a significant risk factor in CML (Table 4).

Survival 5 years after transplantation was 39.8% in HLA-C mismatch (95% CI = 32.8%-46.7%) and 44.5% in HLA-C match (95% CI = 39.6%-49.3%) in ALL ($P = .088$); 33.7% (95% CI = 26.9%-40.6%) and 46.3% (95% CI = 41.2%-51.2%), respectively, in AML ($P < .001$); and 39.7% (95% CI = 32.8%-46.5%) and 58.3% (95% CI = 53.2%-63.1%), respectively, in CML ($P < .001$) (Figure 1).

Survival 5 years after transplantation was 40.9% in HLA-DPB1 mismatch (95% CI = 36.3%-45.4%) and 50.3% in HLA-DPB1 match (95% CI = 41.5%-58.4%) in ALL ($P = .031$); 41.8% (95% CI = 37.0%-46.6%) and 42.6% (95% CI = 34.5%-50.4%), respectively, in AML ($P = .698$); and 51.4% (95% CI = 46.5%-56.1%) and 53.4% (95% CI = 45.1%-61.0%), respectively, in CML ($P = .522$) (Figure 2).

KIR-L-MM-G was also found to be a significant risk factor for mortality (HR = 1.80; $P < .001$). Particularly in AML and CML patients, KIR-L-MM-G had a significantly higher adverse effect than match (HR = 1.93; $P = .005$ and HR = 2.23; $P < .001$, respectively); its effect was moderate in ALL patients (HR = 1.57; $P = .069$) (Table 4).

In HLA-C mismatch patients in either direction, the survival rate 5 years after transplantation was 20.0% for KIR-L-MM-G (95% CI = 6.9%-38.0%) and 43.0% in match (95% CI = 35.3%-50.5%) in ALL ($P = .041$); 19.4% (95% CI = 7.9%-34.6%) and 36.5% (95% CI = 28.8%-44.2%), respectively, in AML ($P = .013$); and 22.2 (95% CI = 10.5%-36.7%) and 43.6% (95% CI = 35.8%-51.1%), respectively, in CML ($P = .001$) (Figure 3).

Significant clinical factors for mortality by multivariate analysis were patient age (HR = 1.02; $P < .001$), donor age (HR = 1.01; $P = .037$), disease (HR = 0.88; $P = .006$), and the status at transplantation (high vs standard, HR = 2.14; $P < .001$).

DISCUSSION

In the present study, we attempted to elucidate how disparities of HLA and KIR affect leukemia relapse and the other transplantation-related immunologic events and to explore how these findings can be applied to induce a GVL effect and improve patient survival in the unrelated setting. Simultaneous analysis of HLA and KIR ligand matching by multivariate analysis made it possible to clarify the role of these antigens in UR-HSCT.

To the best of our knowledge, this is the first report to elucidate the HLA locus responsible for the GVL effect by leukemia cell type in T-cell–replete UR-HSCT. The sequentially registered 577 AML, 617 ALL, and 596 CML patients sufficed to analyze the effects of HLA and KIR ligand matching in the 3 major leukemia cell types.

HLA-C mismatch reduced the relapse rate overall, as reported previously [4]. The GVL effect of HLA-C mismatch depended on the leukemia cell type. ALL patients with HLA-C mismatch showed a significantly lower leukemia relapse risk than those with match, whereas AML and CML patients did not. Furthermore, CML patients with HLA-DPB1 mismatch

showed a significantly lower leukemia relapse rate than those with match, whereas AML and ALL patients did not. Although the reason why the HLA locus responsible for the GVL effect differs with leukemia cell type remains unknown, the different expression of HLA antigens, such as HLA-C, HLA-DPB1, or co-stimulatory molecules on leukemia cells, might modify the immune response of effector cells to leukemia cells. The finding of HLA-DPB1 is in line with a previous report in CML and ALL patients treated with T cell–depleted UR-HSCT [12].

In contrast, an impact of HLA-A and -B allele mismatch on leukemia relapse was not observed. Because HLA-A and/or -B allele mismatch induces severe aGVHD, no GVL effect of HLA-A and /or -B allele mismatch might imply that the target antigenic peptide recognized by effector T cells responsible for aGVHD is not expressed on leukemia cells.

Unexpectedly, KIR-L-MM-G increased the leukemia relapse rate overall. A significantly increased relapse rate in the mismatched group was observed in ALL, but not in AML and CML. Simultaneous multivariate analysis of HLA-C mismatch and KIR-L-MM-G revealed that contrary reactions of these mismatches occurred independently. Although the mechanism involved in this detrimental effect of KIR-L-MM-G on leukemia relapse is not known, the activation of KIR-positive NK cells or T cells might cause immune dysfunction, which abrogates the GVL effect.

The GVL effect of donor-derived KIR-positive NK cells transplanted purified $CD34^+$ stem cells with HLA haploidentical donor was reported in AML patients, but not in ALL patients [22]. In T-cell–replete UR-HSCT, published reports show contradictory effects of KIR ligand mismatch on leukemia relapse. A GVL effect in myeloid malignancies [23-25], a higher leukemia relapse rate [26], and no significant effect [27-29] all have been reported. The use of ATG for GVHD prophylaxis might be a key to understanding these diverse results. Our analysis of T-cell–replete UR-BMT with no use of ATG provided reliable evidence for the adverse effect of KIR-L-MM-G on relapse of ALL relapse. No effect on relapse of AML or CML was reported in a recent large-scale study of myeloid malignancy from the Center for International Blood and Marrow Transplant Research, the European Blood and Marrow Transplant Registry, and the Dutch Registry [30]. Whether KIR ligand match affects leukemia relapse adversely or beneficially is a critical issue for clinical transplantation and immunotherapy using NK cells, and further large-scale comparative studies considering GVHD prophylaxis are warranted.

A higher rejection rate (HR = 4.39; $P = .012$) was found for KIR-L-MM-R; that is, in this mismatch combination, patient KIR2DL-positive effector cells lacking donor KIR ligand are reconstituted and activated after transplantation, which induces the rejection of engrafted donor-derived hematopoietic stem cells. “Hybrid resistance” has been extensively analyzed in mice to induce graft rejection by NK cells [31]. The same mechanism of rejection induced by NK cells might be considered in humans, although 88% of KIR ligand mismatch pairs and 86% of match pairs were given cyclophosphamide as a preconditioning. The effects of HLA class I mismatch for graft rejection were reported [5,32,33]; our data suggest that the effect of HLA-C mismatch were mainly because of KIR2DL ligand mismatch in the HVG direction, and may not result from the HLA-C allele mismatch itself. Our findings are in agreement with a report showing the effect of rejection but not engraftment by KIR2DL ligand mismatch in UR-HSCT [29].

Since the first JMDP report [4], HLA-class I mismatch has been known to significantly increase aGVHD, whereas HLA-DRB1 mismatch has only a marginal effect on aGVHD. The present study has confirmed those earlier findings. We could add the new data on HLA-DPB1 matching showing that HLA-DPB1 mismatch induces moderate aGVHD. Our finding of the effect of HLA-DPB1 on aGVHD concurs with other reports [9-11], although there we found no difference in aGVHD between 2 allele mismatches and 1 allele mismatch of HLA-DPB1.

The international collaborative study is expected to reconcile discrepancies of allele matching in ethnically diverse transplantation populations. Furthermore, the identification of nonpermissive HLA allele mismatch and amino acid substitution responsible for aGVHD, leukemia relapse, and survival might explain these discrepancies in diverse ethnic populations.

Interestingly, KIR-L-MM-G had a higher HR of severe aGVHD than did match. Because these values were adjusted by HLA allele matching and clinical factors, this finding demonstrates that KIR-L-MM-G is a factor independent of HLA allele matching. In fact, among HLA-C mismatch patients, KIR-L-MM-G was associated with a higher rate of grade 3-4 aGVHD than match. In KIR-L-MM-G, the donor-derived KIR2DL2/3- or KIR2DL1-positive effector cells are suspected to react with patient cells that lack the corresponding KIR2DL epitope of HLA-C. These effector cells induce aGVHD through several possible mechanisms. First, NK cells derived from donor graft might directly attack the patient target cells. This is unlikely, however, because in vivo infusion of alloreactive NK cells were found to not cause aGVHD [34], and NK cells were seen to play mainly a protective role for GVHD in a murine experimental model [35]. Alternatively, activated NK cells might

affect donor-derived T cells that induce aGVHD. Third, KIR2DL-positive T cells might induce aGVHD directly. The presence of KIR2DL-positive T cells was reconstituted after UR-HSCT [36].

Conflicting findings have been reported in terms of the effect of KIR-L-MM-G on aGVHD in T-cell–replete UR-HSCT. Some studies have found a trend toward less aGVHD [23], whereas others have reported an increased risk of aGVHD [27,29]. The variety of GVHD prophylaxis, HLA matching, and other clinical factors, and limited patient numbers in each study makes it difficult to determine the role of KIR ligand matching in clinical outcomes. The use of ATG and/or the T-cell depletion method for GVHD prophylaxis will be a key strategy in resolving the discrepancy regarding aGVHD in UR-HSCT [35,37] and in HLA haplotype–identical related HSCT with T-cell depletion [38]. That is, T cell and NK-cell reconstitution after transplantation might affect immunologic events induced by the interaction of KIR and HLA-C epitopes. In addition, genotyping of KIR genes, especially for activating KIR such as KIR2DS, is required to understand the mechanism of KIR involved in aGVHD and the GVL effect [39]. The East Asian population, including Japanese, is known to have several characteristic HLA types. Similarly, the frequencies of both the KIR ligand epitope and the KIR genotype are distinctive in the Japanese population. For example, a higher frequency of C1 epitope and dominance of the KIR "A" haplotype were reported [40]. Those features might contribute considerably to our results. The combination of KIR2DL1 and C2 epitope has been reported to show higher affinity and a stronger inhibitory signal compared with the combination of KIR2DL2/3 and C1 epitope [14].

HLA-A and HLA-C mismatch have been identified as significant independent factors inducing cGVHD, underscoring our previous finding of the importance of HLA class I matching. No influence of KIR-L-MM-G on cGVHD (in contrast to aGVHD) indicates that the KIR-related immunologic reaction has no relation to cGVHD.

There is another model regarding the KIR ligand effect in HSCT, the so-called "missing KIR ligand theory." Hsu et al reported this effect on survival and relapse of AML and myelodysplastic syndrome in T-cell–depleted HLA-matched related HSCT [41] and on relapse in AML, ALL, and CML in UR-HSCT in non-JMDP populations [42]. Lack of either KIR2DL ligand in a patient should activate the corresponding donor NK cells and induce the GVL effect.

In the analysis of KIR matching including HLA mismatch pairs, the mismatch pairs in the "missing KIR ligand theory" with either C1C1 or C2C2 patient epitope were divided into match and mismatch in the "KIR ligand matching theory" by donor epitope. When the donor has either C1C1 or C2C2, the KIR ligand matching theory indicates match, and when the donor has C1C2, the theory indicates mismatch. In this combination, donors with C1C2 (n = 92) had a significantly higher rate of severe aGVHD (44.4%) than donors with either C1C1 or C2C2 (19.2%) (n = 1413; $P < .001$). Therefore, we considered the "ligand matching model" to be applied in this JMDP study.

Finally, because survival after transplantation is influenced not only by leukemia relapse, but also by transplantation-related mortality resulting from aGVHD, cGVHD, fatal infections, or graft failure, the effect of HLA matching and KIR ligand matching should be discussed in light of these events.

The present study has more precisely elucidated the impact of HLA matching on leukemia patient survival. The mismatch of HLA-A and -B alleles resulted in significantly higher mortality. HLA-C and HLA-DQB1 mismatch emerged as a risk factor for poorer survival for the first time in the JMDP study. Increased survival in ALL with HLA-C mismatch cannot be linked to the compensation from a lower leukemia relapse rate. HLA-DPB1 mismatch did not significantly affect overall mortality despite the increase in moderately aGVHD. These observations of HLA-C and -DQB1 mismatch in the JMDP are in line with those of other recent reports. The NMDP reported an adverse effect of HLA-C mismatch [8], and another study reported that not only HLA-C mismatch in early-stage CML, but also HLA-DQB1 mismatched CML patients with multiple mismatch posed increased risk for mortality [43].

It should be noted that KIR-L-MM-G resulted in higher mortality in UR-HSCT with T-cell–replete marrow regardless of leukemia cell type. KIR-L-MM-G might induce an immunodeficient state that would result in a higher risk for opportunistic infections [44,45]. Thus, infectious complications by cytomegalovirus and the like should be explored in relation to KIR.

We estimate that about 30% of patients in the Japanese population have HLA-C mismatch donors, of whom 15.0% are KIR-L-MM in the GVH direction, 20.8% are KIR-L-MM in the HVG direction, and 35.6% are KIR-L-MM in either direction, when HLA-A, -B, and -DRB1 genotyping is used as the donor confirmatory typing. Because both KIR2DL ligand matching and/or HLA matching itself affect aGVHD, cGVHD, rejection, ALL relapse, and survival, as described earlier, HLA-C typing is essential in selecting a suitable donor to reduce the risk of aGVHD and improve survival in practice.

In conclusion, our analysis has produced important findings for transplantation immunology and the selection of donors in UR-HSCT. First, HLA-C and HLA-DPB1 mismatches are expected to induce a ben-

eficial GVL effect, which should be considered in terms of the leukemia cell type of individual patients. Second, KIR-L-MM should be avoided, because it induces only adverse effects on transplantation outcome and provides no benefits for patients undergoing T-cell–replete UR-HSCT.

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REFERENCES

1. Kernan NA, Bartsch G, Ash RC, et al. Analysis of 462 transplantations from unrelated donors facilitated by the National Marrow Donor Program. *N Engl J Med*. 1993;328: 593-602.
2. Kodera Y, Morishima Y, Kato S, et al. Analysis of 500 bone marrow transplants from unrelated donors (UR-BMT) facilitated by the Japan Marrow Donor Program: confirmation of UR-BMT as a standard therapy for patients with leukemia and aplastic anemia. *Bone Marrow Transplant*. 1999;24:995-1003.
3. Weiden PI, Floutney N, Thomas ED, et al. Antileukemia effect of graft-versus-host disease in human recipients of allogeneic marrow grafts. *N Engl J Med*. 1997;300:1068-1073.
4. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. *N Engl J Med*. 1998;339:1177-1185.
5. Morishima Y, Sasazuki T, Inoko H, et al. The clinical significance of human leukocyte antigen (HLA) allele compatibility in patients receiving a marrow transplant from serologically HLA-A–, HLA-B–, and HLA-DR–matched unrelated donors. *Blood*. 2002; 99:4200-4206.
6. Petersdorf EW, Gooley TA, Anasetti C, et al. Optimizing outcome after unrelated marrow transplantation by compre-

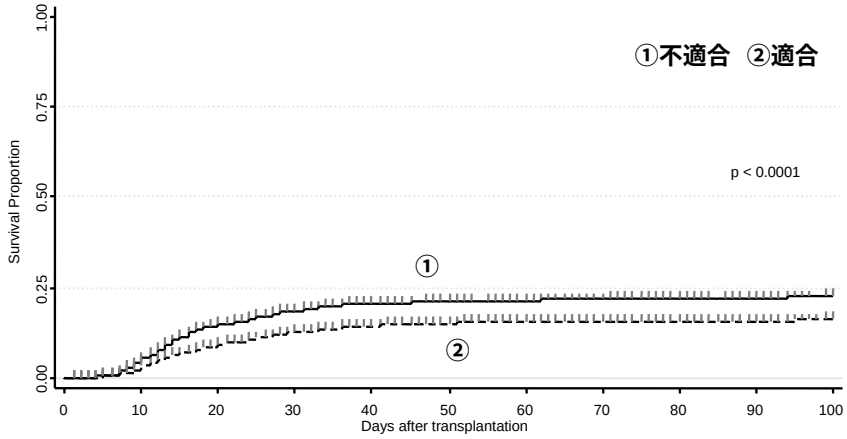
hensive matching of HLA class I and II alleles in the donor and recipient. *Blood*. 1998;92:3515-3520.
7. Petersdorf EW, Kollman C, Hurley CK, et al. Effect of HLA class II gene disparity on clinical outcome in unrelated donor hematopoietic cell transplantation for chronic myeloid leukemia: the US National Marrow Donor Program Experience. *Blood*. 2001;98:2922-2929.
8. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. *Blood*. 2004;104:1923-1930.
9. Petersdorf EW, Gooly T, Malkki M, et al. The biological significance of HLA-DP gene variation in haematopoietic cell transplantation. *Br J Haematol*. 2001;112:988-994.
10. Loiseau P, Esperou H, Busson M, et al. DPB1 disparities contribute to severe GVHD and reduced patient survival after unrelated donor bone marrow transplantation. *Bone Marrow Transplant*. 2002;30:497-502.
11. Shaw BE, Potter MN, Mayor NP, et al. The degree of matching at HLA-DPB1 predicts for acute graft-versus-host disease relapse following haematopoietic stem cell transplantation. *Bone Marrow Transplant*. 2003;31:1001-1008.
12. Shaw BE, Marsh SGE, Mayer NP, et al. HLA-DPB1 matching status has significant implications for recipients of unrelated donor stem cell transplants. *Blood*. 2006;107:1220-1226.
13. Moretta L, Moretta A. Killer immunoglobulin-like receptors. *Curr Opin Immunol*. 2004;16:626-633.
14. Parham P. MHC class I molecules and KIRs in human history, health and survival. *Nat Rev Immunol*. 2005;5:201-214.
15. Dupont B, Hsu KC. Inhibitory killer Ig-like receptor genes and human leukocyte antigen class I ligand in hematopoietic stem cell transplantation. *Curr Opin Immunol*. 2004;16:634-643.
16. Witt CS, Christiansen FT. The relevance of natural killer cell human leukocyte antigen epitopes and killer cell immunoglobulin-like receptors in bone marrow transplantation. *Vox Sang*. 2006;90:10-20.
17. Itoh Y, Mizuki N, Shimada T, et al. High-throughput DNA typing of HLA-A, -B, -C, and -DRB1 loci by a PCR-SSOP-Luminex method in the Japanese population. *Immunogenetics*. 2005;57:1-13.
18. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
19. Gooley TA, Leisenring W, Crowley J, et al. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med*. 1999;18:695-706.
20. Coviello V, Boggess M. Cumulative incidence estimation in the presence of competing risks. *Stata J*. 2004;4:103-112.
21. Cox DR. Regression models and life-tables. *J R Stat Soc B*. 1972;34:187-220.
22. Ruggeri L, Capanni M, Urbani E, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. *Science*. 2002;295:2097-2100.
23. Giebel S, Locatelli FW, Lamparelli T et al. Survival advantage with KIR ligand incompatibility in hematopoietic stem cell transplantation from unrelated donors. *Blood*. 2003;102:814-819.
24. Beelen DW, Ottinger HD, Ferencik S, et al. Genotypic inhibitory killer immunoglobulin-like receptor ligand incompatibility enhances the long-term antileukemic effect of unmodified allogeneic hematopoietic stem cell transplantation in patients with myeloid leukemias. *Blood*. 2005;105:2594-2600.
25. Elmaagacli AH, Ottinger H, Koldehoff M, et al. Reduced risk for molecular disease in patients with chronic myeloid leukemia after transplantation from a KIR-mismatched donor. *Transplantation*. 2005;79:1741-1747.
26. Bomhauser M, Schwerdtfeger R, Martin H, et al. Role of KIR ligand incompatibility in hematopoetic stem cell transplantation using unrelated donors. *Blood*. 2004;103:2860-2861.
27. Davies SM, Ruggieri L, DeFor T, et al. Evaluation of KIR ligand incompatibility in mismatched unrelated donor hematopoietic transplants. *Blood*. 2002;100:3825-3827.
28. Lowe EJ, Turner V, Handgretinger R, et al. T-cell alloreactivity dominates natural killer cell alloreactivity in minimally T-cell–depleted HLA-nonidentical pediatric bone marrow transplantation. *Br J Haematol*. 2003,123:3232-3236.
29. De Santis D, Bishara A, Witt CS, et al. Natural killer cell HLA-C epitopes and killer cell immunoglobulin-like receptors both influence outcome of mismatched unrelated donor bone marrow transplants. *Tissue Antigens*. 2005;65:519-528.
30. Farag SS, Bacigargo A, Eapen M, et al. The effect of KIR liand incompatibility on the outcome of unrelated donor transplantation: A report from the Center for International Blood and Marrow Transplant Research, the European Blood and Marrow Transplant Registry, and the Dutch Registry. *Biology Blood Marrow Transplant*. 2006;12:876-884.
31. Cudkowicz G, Bennett M. Peculiar immunobiology of bone marrow allografts. II. Rejection of parental grafts by resistant F1 hybrid mice. *J Exp Med*. 1971;134:1513-1528.
32. Petersdorf EW, Longton GM, Anasetti C, et al. Association of HLA-C disparity with graft failure after marrow transplantation from unrelated donor. *Blood*. 1997;89:1818-1823.
33. Petersdorf EW, Hansen JA, Martin PJ, et al. Major histcompatibility complex class I alleles and antigens in hematopoietic cell transplantation. *N Engl J Med*. 2001;345:1794-1800.
34. Miller JS, Soignier Y, Panoskaltsis-Mortari A, et al. Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer. *Blood*. 2005;105:3051-3057.
35. Asai O, Longo LL, Tian ZG, et al. Suppression of graft-versus-host disease and amplification of graft-versus-tumor effects by activated natural killer cells after allogeneic bone marrow transplantation. *J Clin Invest*. 1998;101:1835-1842.
36. Tanaka J, Mori A, Ohta S, et al. Expression of HLA-C specific natural killer cell receptors (CD158a and CD158b) on peripheral blood mononuclear cells after allogeneic bone marrow transplantation. *Br J Haematol*. 2001;105:89-91.
37. Cooley S, McCullar V, Wangen R, et al. KIR reconstitution is altered by T cells in the graft and correlates with clinical outcomes after unrelated donor transplantation. *Blood*. 2005; 106:4370-4376.
38. Bishara A, Santis DD, Witt CC, et al. The beneficial role of inhibitory KIR genes of HLA class I NK epitopes in haploidentically mismatched stem cell allografts may be masked by residual donor-alloreactive T cells causing GVHD. *Tissue Antigens*. 2004;63:204-211.
39. Verheyden S, Schots R, Duquet W, et al. A defined donor activating natural killer cell receptor genotype protects against leukemic relapse after related HLA-identical hematopoietic stem cell transplantation. *Leukemia*. 2002;19:1446-1451.
40. Yawata M, Yawata N, McQueen KL, et al. Predominance of group A KIR haplotypes in Japanese associated with diverse NK

cell repertoires of KIR expression. *Immunogenetics*. 2002;54: 543-550.
41. Hsu KC, Keeve-Taylor CA, Wilton A, et al. Improved outcome in HLA-identical sibling hematopoietic stem-cell transplantation for acute myelogeneous leukemia predicted by KIR and HLA genotypes. *Blood*. 2005;106:4878-4884.
42. Hsu KC, Gooley T, Malkki M, et al. KIR ligand and prediction after unrelated donor hematopoitic cell transplantation for hematologic malignancy. *Biology Blood Marrow Transplant*. 2006;12: 876-884.
43. Petersdorf EW, Anasetti C, Martin P, et al. Limit of HLA mismatching in unrelated hematopoietic cell transplantation. *Blood*. 2004;104:2976-2980.
44. Schaffer M, Malmberg KJ, Ringden O, et al. Increased infection-related mortality in KIR-ligand–mismatched unrelated allogeneic hematopoietic stem cell transplantation. *Transplantation*. 2004;78:1081-1085.
45. Cook M, Briggs D, Craddok C, et al. Donor KIR genotype has a major influence on the rate of cytomegalovirus reactivation following T-cell–replete stem cell transplantation. *Blood*. 2006;107:1230-1232.

「日本骨髄バンクを介した非血縁者間骨髄移植の成績報告書
（2006年度集計）」から抜粋

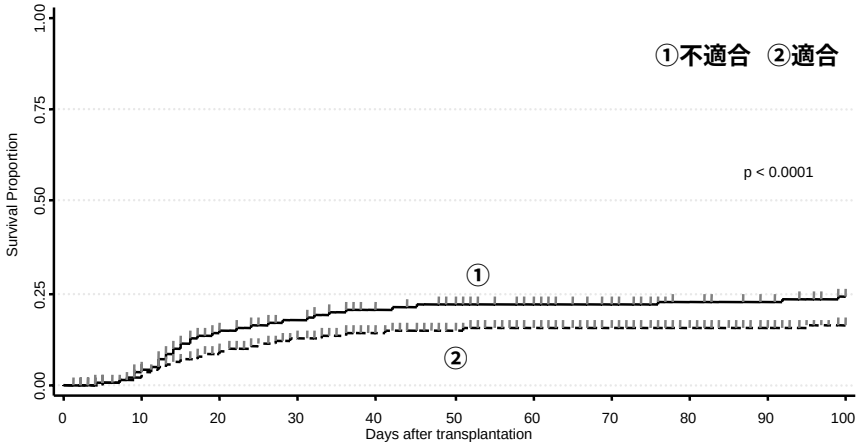
- スライド1～4は、DNAレベルでの適合/不適合について解析したものです。
- また、各ローカス毎のマッチングについての解析では他のローカスが一致した症例のみについて解析されています（例えばHLA-Aの一致、不一致で生存率を比較する場合にはBとDRが一致した症例のみについて解析）。
- ここでは、普段、先生方から多く寄せられる質問に関するスライドを掲載しましたが、疾患別等、他の解析結果については、上記報告書をご覧ください。

1．HLAミスマッチ症例における急性GVHD Grade3-4発症率



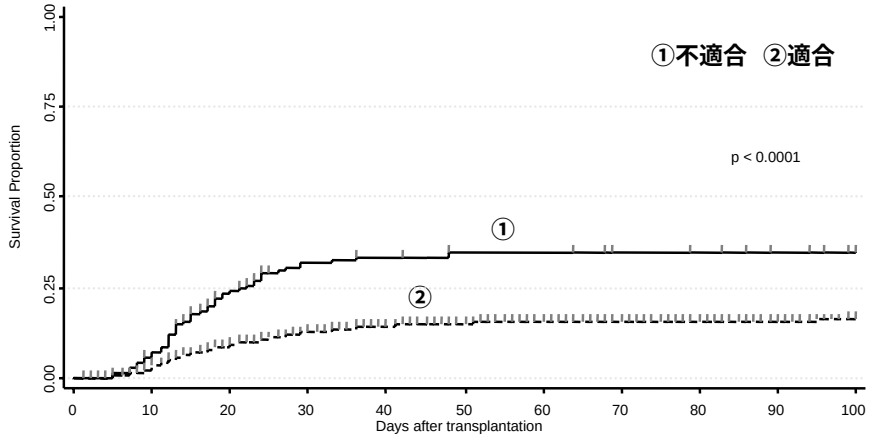
群	症例数	発症数	発症率
不適合	2995	677	23±2%
適合	3488	561	16±1%

2. HLA-A locusミスマッチ症例における急性GVHD Grade3-4発症率



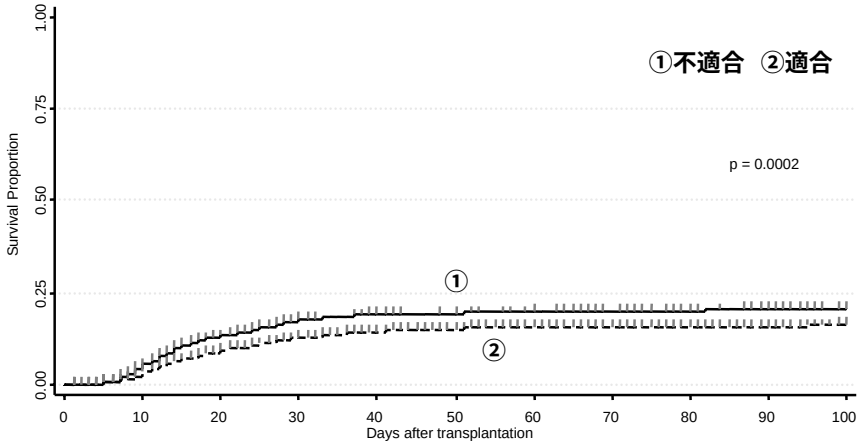
群	症例数	発症数	発症率
不適合	572	136	24±4%
適合	3488	561	16±1%

3. HLA-B locusミスマッチ症例における急性GVHD Grade3-4発症率



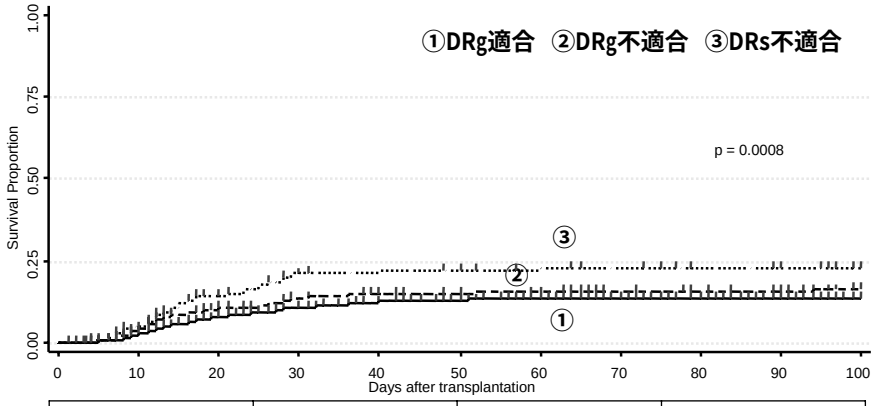
群	症例数	発症数	発症率
不適合	203	71	35±7%
適合	3488	561	16±1%

4. HLA-DRB1 locusミスマッチ症例における急性GVHD Grade3-4発症率



群	症例数	発症数	発症率
不適合	1145	236	21±2%
適合	3488	561	16±1%

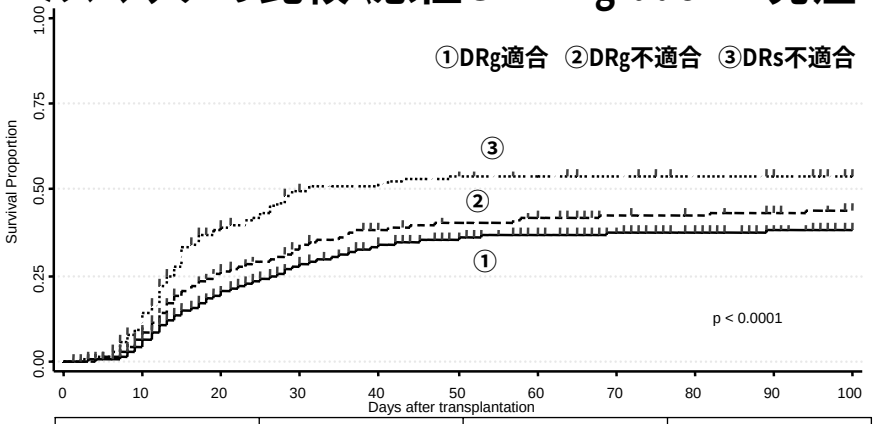
9. HLA-DR血清学的ミスマッチと遺伝学的ミスマッチの比較（急性GVHD grade3-4発症率）



群	症例数	発症数	発症率
DRg適合	2709	388	14±1%
DRg不適合	502	84	16±3%
DRs不適合	195	45	23±6%

（HLA-A,B,C遺伝子型適合症例）

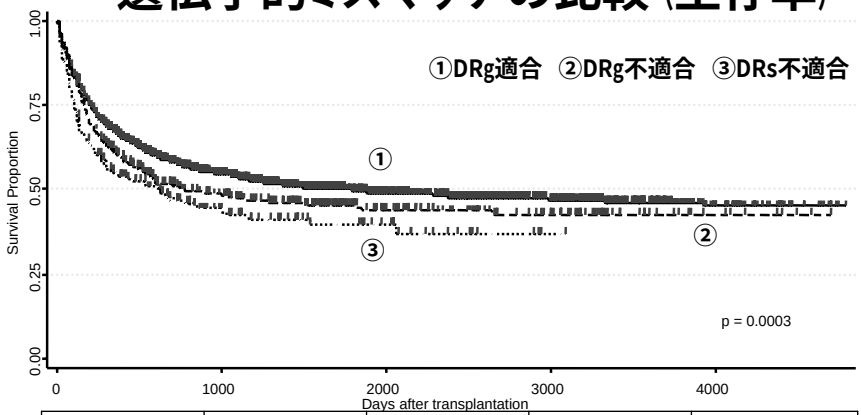
10. HLA-DR血清学的ミスマッチと遺伝学的ミスマッチの比較（急性GVHD grade2-4発症率）



群	症例数	発症数	発症率
DRg適合	2709	1063	38±2%
DRg不適合	502	223	44±4%
DRs不適合	195	106	54±7%

（HLA-A,B,C遺伝子型適合症例）

11. HLA-DR血清学的ミスマッチと遺伝学的ミスマッチの比較（生存率）



群	症例数	発症数	3年生存率	5年生存率
DRg適合	2721	1297	70±3%	65±3%
DRg不適合	514	270	65±8%	60±9%
DRs不適合	197	109	46±16%	40±17%

（HLA-A,B,C遺伝子型適合症例）