抗HLA抗体モニタリング
（カテゴリー2：生体腎移植）

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腎移植認定医第18回集中教育セミナー
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日本臨床腎移植学会
COI開示

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発表に関連し，開示すべきCOI 関係にある企業などはありません。

Department of Renal Transplant Surgery　Aichi Medical University School of Medicine

本日の内容

- モニタリングの意義？
- どのように実施、臨床に役立てる？
- 課題（問題点）
- 今後について（将来展望）

平成30年度診療報酬改定

平成30年度診療報酬改定の概要－医科

Ⅱ　新しいニーズにも対応でき、安心・安全で納得できる質の高い医療の実現・充実

1.　重点的な対応が求められる医療分野の充実

1～5）（略）（医科Ⅰ参照）

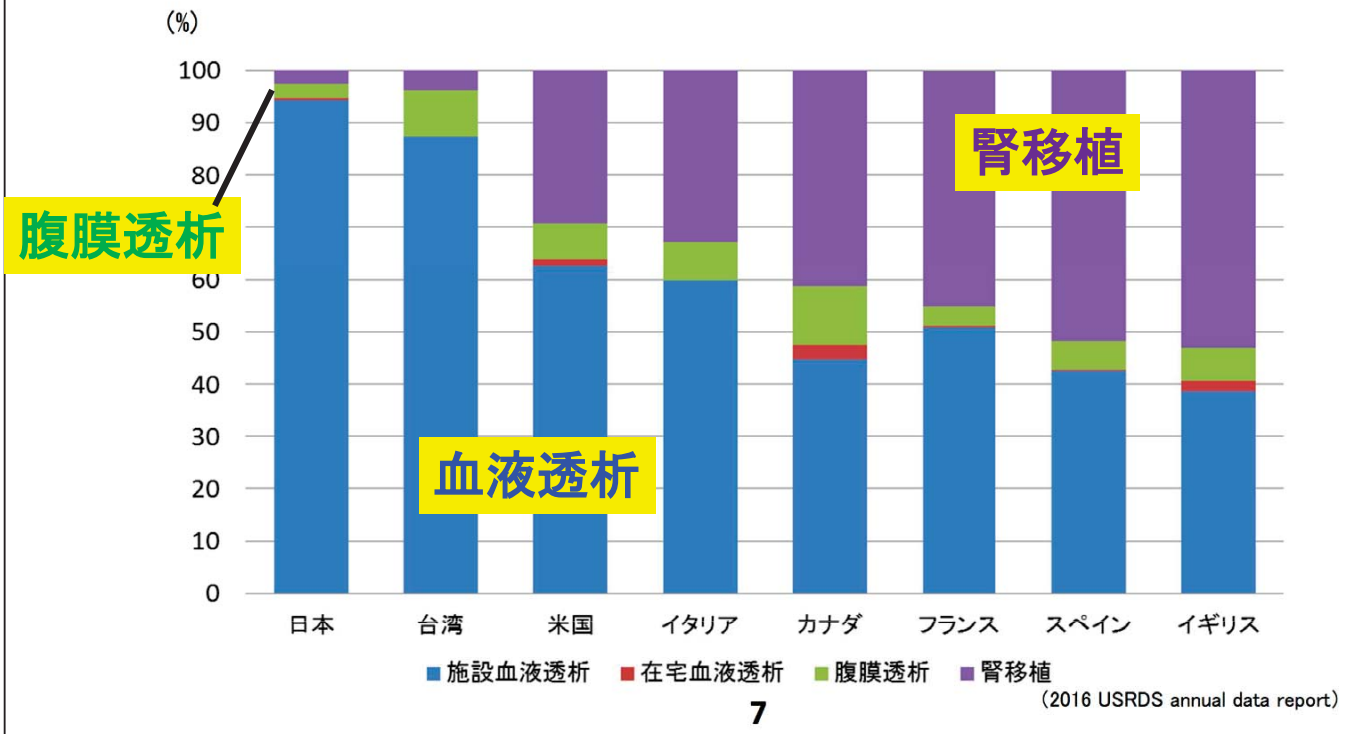
6）適切な腎代替療法の推進

2.　先進的な医療技術の適切な評価と着実な導入

1）遠隔診療の評価　（医科Ⅰ参照）
2）遺伝学的検査の評価の充実
3）麻酔科の診療に係る評価の見直し
4）高度な放射線治療機器の効率的な利用の推進
5）デジタル病理画像を用いた病理診断の評価及び保険医療機関間の連携による病理診断の要件の見直し
6）移植医療の評価の充実
7）性別適合手術の保険適用
8）手術等医療技術の適切な評価

我が国及び諸外国における末期腎不全に対する腎代替療法の割合

○ 我が国では、血液透析を行う患者の割合が、その他の国に比べて、多い。



(2016 USRDS annual data report)

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（平成30年度診療報酬改定資料）

平成30年度診療報酬改定　Ⅱ－2－6）移植医療の評価の充実①

移植医療の評価の充実①

【課題】
- 臓器移植後に抗HLA抗体が出現した症例に対して治療を行うことにより、予後が改善するとの報告があるが、移植術後の経過中における抗HLA抗体検査の費用については、算定対象としていない。
- 造血幹細胞移植について、移植登録をした患者の約30%の患者が待機中に移植中止となっており、コーディネート体制の充実を含めた、実施体制の整備が必要である。

➢ 臓器移植患者の予後改善のため、移植後の経過中に実施される抗HLA抗体検査の評価を行う。

（新）　抗HLA抗体（スクリーニング検査）　1,000点（1月につき）

［算定要件］
(1) 肺移植、心移植、肝移植、膵移植、小腸移植又は腎移植後の患者に対して実施した場合に、原則として1年に1回に限り算定する。
(2) ただし、抗体関連拒絶反応を強く疑う場合等、医学的必要性がある場合には、1年に1回に限り別に算定できる。

（新）　抗HLA抗体（抗体特異性同定検査）　5,000点（1月につき）

［算定要件］
(1) 抗HLA抗体（スクリーニング検査）によって陽性が確認された症例について、抗体関連拒絶反応の確定診断目的に行われた場合に算定する。
(2) ただし、抗体関連拒絶反応と診断された患者の経過観察時に行った場合には、1年に2回に限り別に算定できる。

➢ 造血幹細胞移植の成績向上の観点から、移植のコーディネート期間の短縮に資するような体制や、専門的な医師・看護師の配置か

造血幹細胞移植

（新）　非血縁者間移植

［算定要件］　骨髄移植又は末

（新）　コーディネート体

［算定要件］　別に厚生労働大臣が定める施設基準に適合しているものとして地方厚生局長等に届け出た保険医療機関において、非血縁者間末梢血幹細胞移植を実施した場合に、所定点

［施設基準］
(1) 当該手術に係る10年以上の経験を有する専任の常勤医師が配置されていること。
(2) 同種移植のコーディネートの十分な体制が整備されていること。
(3) 当該療養を担当する診療科が、関係学会による認定を受けていること。

臓器移植後　抗HLA抗体
年1回　スクリーニング検査（1000点）
陽性の場合には
→　特異性同定検査（5000点）

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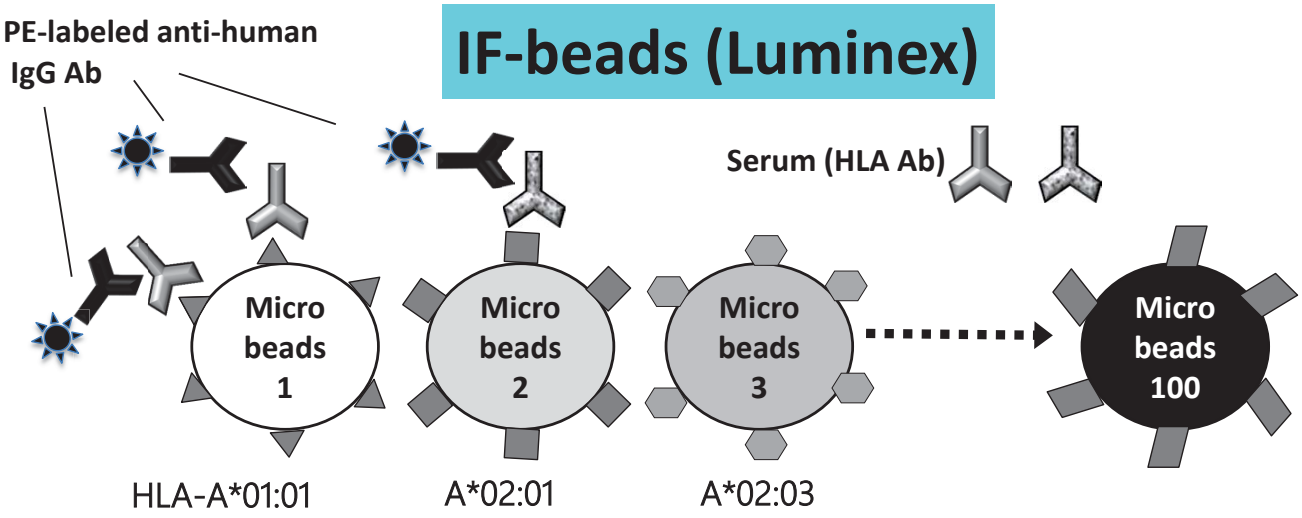
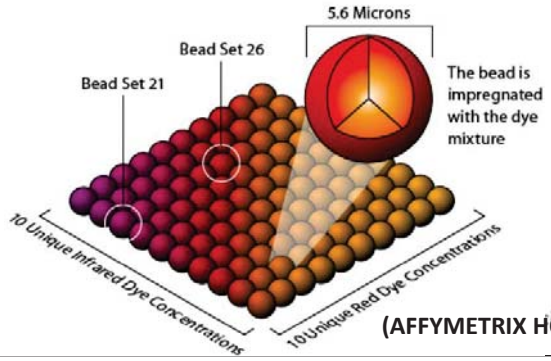
IF-beads (Luminex)



Recombinant HLA molecule (Single antigen beads)

(LUMINEX HOME PAGE)

The Luminex 200 System with xPONENT 3.1 Software



(AFFYMETRIX HOME PAGE)

HLA抗体検査　意義

✓ 移植前検査　→移植適応

→脱感作療法

不可欠！

まるめ（包括）

✓ 移植後検査（モニタリング）

保険収載

情報がない！

→ABMR診断？治療？

→治療効果判定？

→Minimization指標？

Acute　ABMR

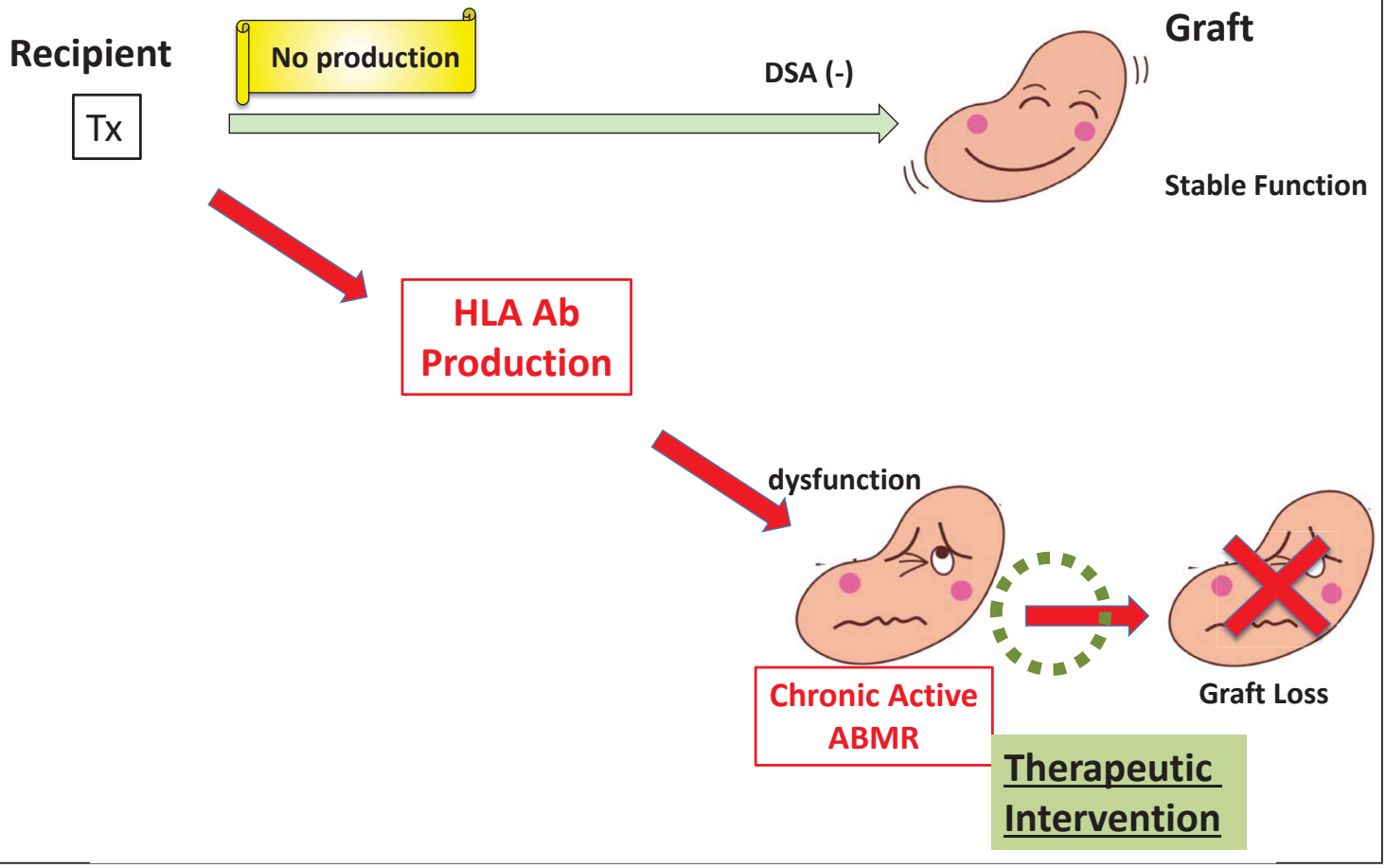
- ✓ 先に臨床所見（急激に）
- ✓ 抗HLA抗体は後付け（確定診断は病理）
- ✓ DSA-negative ABMR
- ✓ Reversible

モニタリングの意義？

Chronic　ABMR

- ✓ 維持期
- ✓ 緩徐に
- ✓ Irreversible

Strategy to control DSA-induced (chronic) ABMR



The Treatment of Antibody-Mediated Rejection in Kidney Transplantation: An Updated Systematic Review and Meta-Analysis

Susan S. Wan, MMed (Clin Epi), FRACP,[1,2] Tracey D. Ying, MMed (Clin Epi), FRACP,[1,2] Kate Wyburn, FRACP, PhD,[1,2] Darren M. Roberts, FRACP, PhD,[3,4] Melanie Wyld, MBA, MPH,[1,5] and Steven J. Chadban, FRACP, PhD[1,2]

Transplantation 2018, 102: 557-568.

Background. Current treatments for antibody-mediated rejection (AMR) in kidney transplantation are based on low-quality data from a sm…ents targeting B cells, plasma cells, and the complement system have featured in recent st…ed a systematic review and meta-analysis of controlled trials in kidney transplant recipients using Medline, EMBASE, and CENTRAL from inception to February 2017. **Results.** Of 14,380 citations, we identified 21 studie…10 randomized controlled… 2011, we… additional studies evaluat… bortezom… inhibitor (two), and eculizumab (one). Risk of bias was serious or unclear overall and evidence quality was low for the majority of treatment strategies. Sufficient RCTs for pooled analysis were available only for antibody removal, and here… difference between groups for graft survival (HR 0.76; 95% CI 0.35-1.63; $P = 0.475$). Studies show… in treatments, definition of AMR, quality, and follow-up. Plasmapheresis and IVIG were used as standard-of-care in recent studies, and to this combination, rituximab seemed to add little or no benefit. Insufficient data are available to assess the efficacy of bortezomib and complement inhibitors. **Conclusion.** Newer studies evaluating rituximab showed … ment inhibitors for the treatment of AMR … standard-of-care for the treatment of acute AMR.

(*Transplantation* 2018;102: 557–568)

Plasmapheresis

IVIG

Rituximab

Bortezomib

Complement Inhibitor

Standard-of-care for acute ABMR

Transplant International

REVIEW

Transpl Int 2019, 32: 775-788.

The therapeutic challenge of late antibody-mediated kidney allograft rejection

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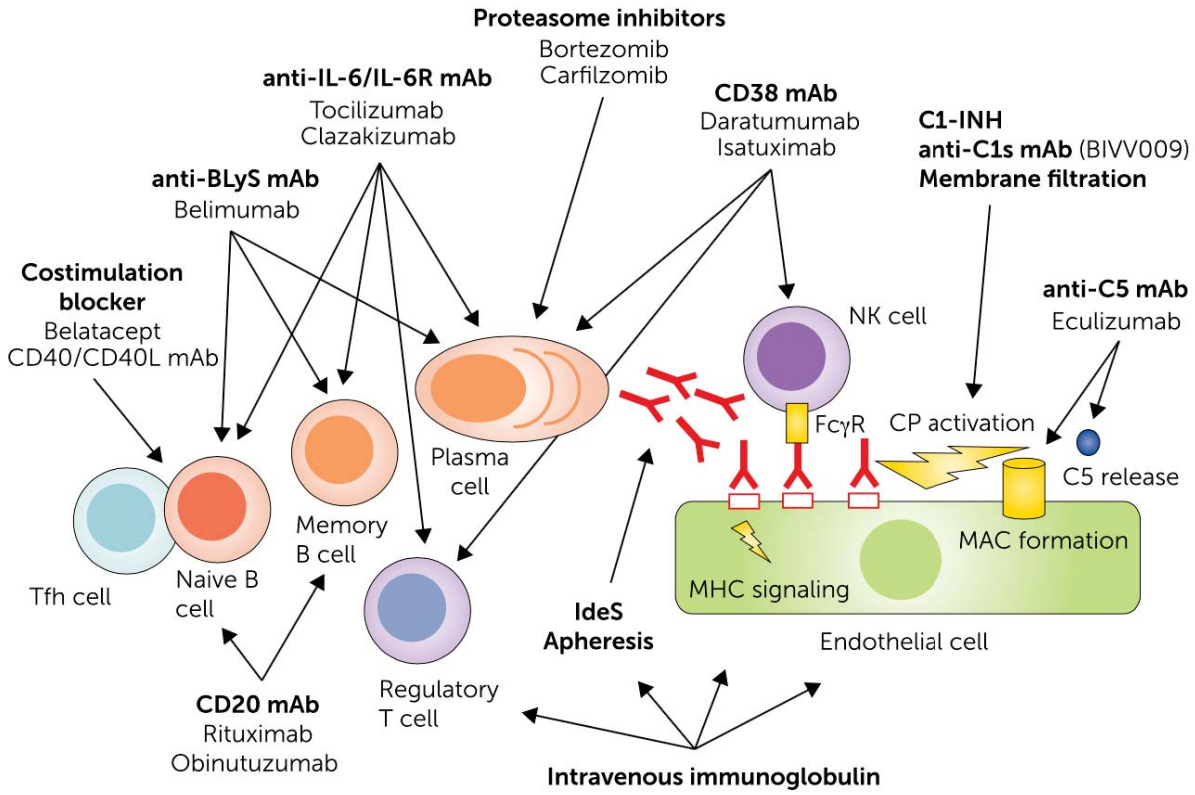
SUMMARY

Late antibody-mediated rejection (ABMR) is a cardinal cause of kidney allograft failure, manifesting as a continuous and, in contrast with early rejection, often clinically silent alloimmune process. While significant progress has been made towards an improved understanding of its molecular mechanisms and the definition of diagnostic criteria, there is still no approved effective treatment. In recent small randomized controlled trials, therapeutic strategies with promising results in observational studies, such as proteasome inhibitor bortezomib, anti-C5 antibody eculizumab, or high dose intravenous immunoglobulin plus rituximab, had no significant impact in late and/or chronic ABMR. Such disappointing results reinforce a need of new innovative treatment strategies. Potential candidates may be the interference with interleukin-6 to modulate B cell alloimmunity, or innovative compounds that specifically target antibody-producing plasma cells, such as antibodies against CD38. Given the phenotypic heterogeneity of ABMR, the design of adequate systematic trials to assess the safety and efficiency of such therapies, however, is challenging. Several trials are currently being conducted, and new developments will hopefully provide us with effective ways to counteract the deleterious impact of antibody-mediated graft injury. Meanwhile, the weight of evidence would suggest that, when approaching using existing treatments for established antibody-mediated rejection, "less may be more".

Depo … Iedicine



Transpl Int 2019, 32: 775-788.

Strategy to control DSA-induced (chronic) ABMR

Recipient

Tx

No production

DSA (-)

Graft

Stable Function

Too late after graft dysfunction !

HLA Ab Production

dysfunction

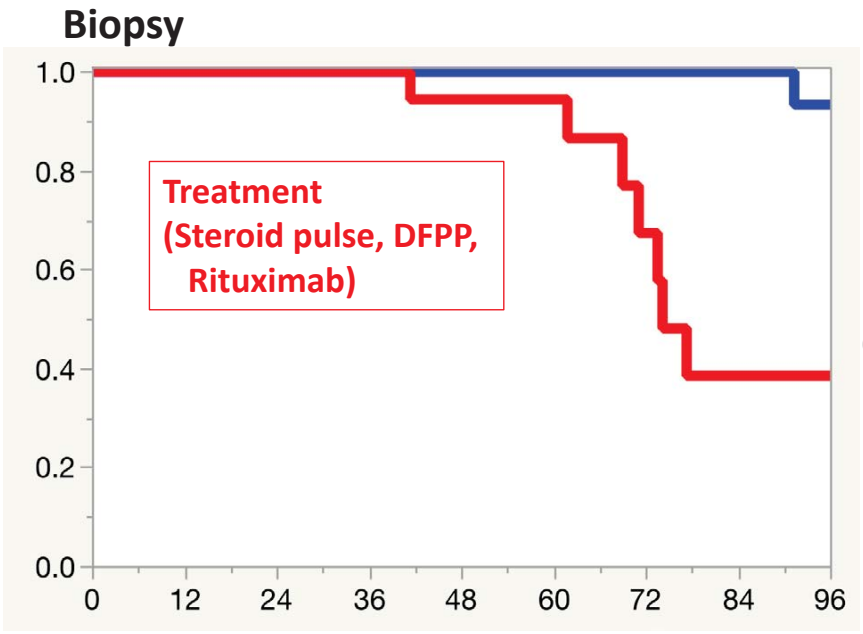
Preemptive Medicine

Chronic Active ABMR

Graft Loss

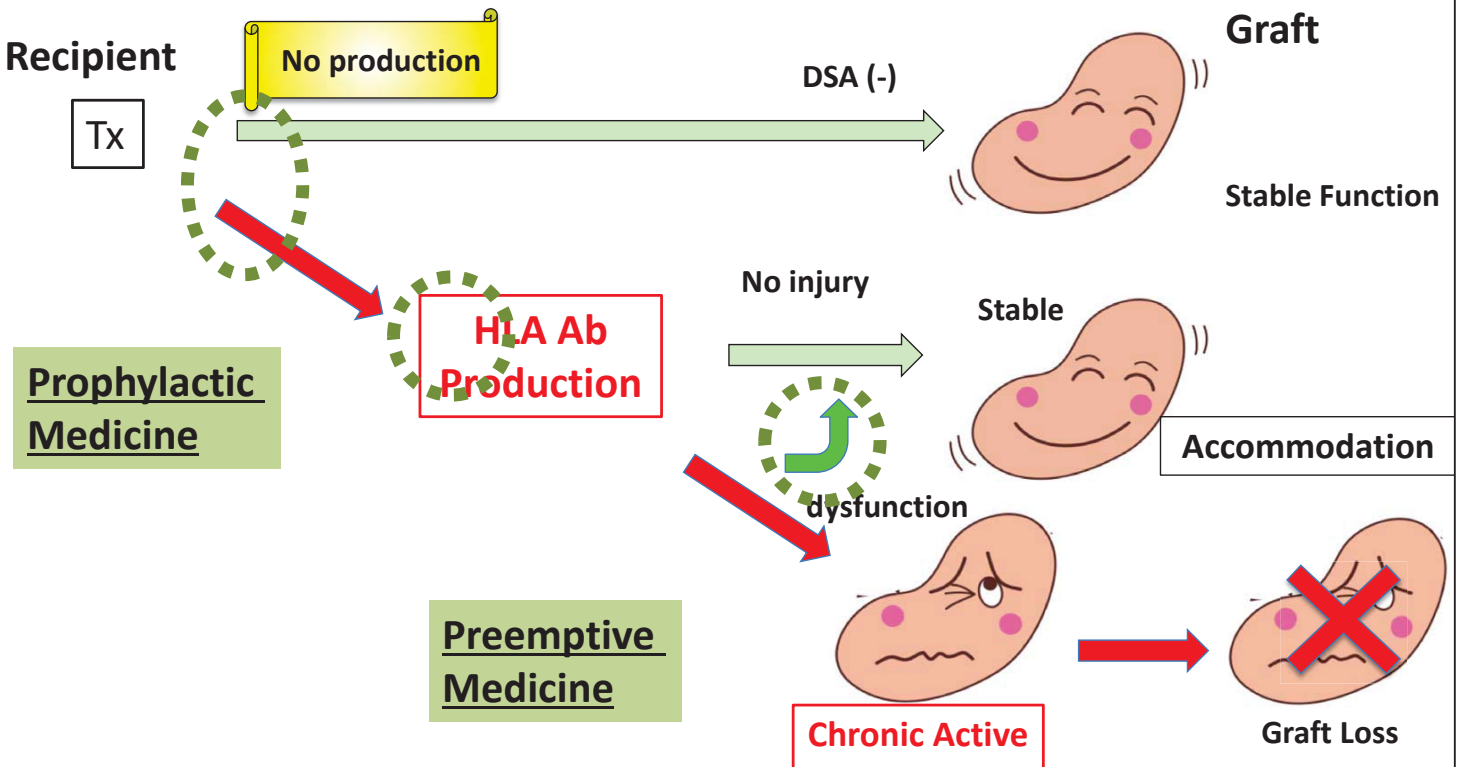
Therapeutic Intervention

Graft survival in patients with de novo DSA



Yamamoto T, et al. Transplantation 2016; 100: 2194-202, 4-year follow-up

Strategy to control DSA-induced (chronic) ABMR



モニタリングの意義

- ✓ Subclinical chronic ABMRをみつけることができるか？（Cr上昇、タンパク尿の前に）
- ✓ 治療法は確立されていないが、可能性あり

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- どのように実施、臨床に役立てる？
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- 自施設（関連施設）での検査

(High volume center)

→ 検査技師が大変！

- 外注（検査施設）での検査

→ 移植医が大変！

■ 移植前（移植手術　まるめ）

HLA-DNAタイピング　(A, B, DRB1) 2桁、4桁

(DRB3,4,5)

(+ DQB1) (+ DQA1, C, DPB1, DPA1)

移植後必要時？

NGS利用？

■ 移植後（H30年度　保険収載）

✓保険の範囲内（精度の高い　検査）

✓移植医の負担が少ない（オーダーの手間、準備）

✓理解しやすい結果（結果の解釈）

✓データの管理

外注検査は大変！

愛知医大

✓ 自施設で可能 （病院の承認おりず→外注）
FCXM，ICFA， FlowPRA，LABScreen SAB

■ 生体移植前
FCXM，FlowPRA（研究費）
Neg --- > 移植日（外注にて）HLA typing (NGS)
Pos --- > 事前（外注にて）HLA typing (NGS)
＋ LABScreenSAB

■ 移植後（抗体検査）
(2016-2017) 自施設 FlowPRA --- > LABScreen SAB（研究費）
(2018-) 外注 LABScreen PRA --- > LABScreen SAB（保険）

外注の実際、管理

✓ 保険の範囲内 （病院事務と交渉、契約すみ）
スクリーニング(LABScreen PRA) --- 10,000円
特異性検査 (LABScreen SAB) --- 50,000円

✓ 年1回、血漿保存 → まとめて送付 (100 + 50 + α)

✓ HLA情報の送付 (Excel)

✓ 結果受領 e-mail → DISCUSSION

✓ 最終結果 郵送 (PDF: 電子カルテ用，保険請求用）
e-mail (Excel: データ管理用)

✓ 外来担当医に連絡、電子カルテ（掲示板）記載

●HLAタイピング結果

報告書ＩＤ	名前	遺伝子名	Allele1	Allele2
AI166	[redacted]（患者）	A	A*02:01:01:01	A*02:06:01:01
		B	B*15:11:01	B*48:01:01:01
		C	C*03:03:01:01	C*03:04:01:02
		DRB1	DRB1*09:01:02/09:31	DRB1*14:54:01
		DRB345	DRB3*02:02:01	DRB4*01:03:02
		DQA1	DQA1*01:04:01:01	DQA1*03:02:01:01
		DQB1	DQB1*03:03:02	DQB1*05:02:01
		DPA1	DPA1*01:03:01	DPA1*02:02:02
		DPB1	DPB1*05:01:01	DPB1*41:01:01
AI167	[redacted]（ドナー）	A	A*02:01:01:01	A*33:03:01:01
		B	B*40:02:01:01	B*44:03:01:10
		C	C*03:04:01:02	C*14:03
		DRB1	DRB1*09:01:02/09:31	DRB1*13:02:01
		DRB345	DRB3*03:01:01	DRB4*01:03:02
		DQA1	DQA1*01:02:01:09	DQA1*03:02:01:02
		DQB1	DQB1*03:03:02	DQB1*06:04:01
		DPA1	DPA1*01:03:01:05	DPA1*02:01:01:02
		DPB1	DPB1*04:02:01	DPB1*05:01:01

Depart of Medicine

抗HLA抗体シングル同定検査(ClassⅡ)結果報告書 2/3

● MFI（Normal)とHLAタイプ

MFI
>1000
>5000
>10000
＊カットオフ値は1000とした。

Cntl	PC	NC	PC/NC
Single	4,848	63	77
Supplement			

Single Antigen

抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele	抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele
DR1	DRB1*01:01	1076					DQ6	DQA1*01:02,DQB1*06:02	1971			○	○
DR1	DRB1*01:02	1448					DQ6	DQA1*01:01,DQB1*06:02	2674			○	○
DR103	DRB1*01:03	1050					DQ6	DQA1*01:03,DQB1*06:03	6535			○	
DR17	DRB1*03:01	1445					DQ6	DQA1*01:02,DQB1*06:04	1488			○	
DR18	DRB1*03:02	1891					DQ6	DQA1*01:02,DQB1*06:09	3716			○	
DR4	DRB1*04:01	1147			○		DQ7	DQA1*03:01,DQB1*03:01	171	○	○		
DR4	DRB1*04:02	1203			○		DQ7	DQA1*02:01,DQB1*03:01	159	○	○		
DR4	DRB1*04:04	763			○		DQ7	DQA1*05:03,DQB1*03:01	0	○	○		
DR4	DRB1*04:05	1251			○	○	DQ7	DQA1*05:05,DQB1*03:01	14	○	○		
DR7	DRB1*07:01	512					DQ7	DQA1*06:01,DQB1*03:01	7	○	○		
DR4	DRB1*04:03	1911			○		DQ8	DQA1*02:01,DQB1*03:02	284				
DR8	DRB1*08:01	1248					DQ8	DQA1*03:01,DQB1*03:02	549				
DR9	DRB1*09:01	2157	○	○			DQ8	DQA1*03:02,DQB1*03:02	1				
DR9	DRB1*09:02	551	○				DQ9	DQA1*02:01,DQB1*03:03	182	○	○		
DR10	DRB1*10:01	1068					DQ9	DQA1*03:01,DQB1*03:03	90	○	○		
DR11	DRB1*11:01	275					DQ9	DQA1*03:02,DQB1*03:03	0	○	○		
DR11	DRB1*11:04	106					DP1	DPA1*01:03,DPB1*01:01	553				
DR12	DRB1*12:01	529	○	○			DP1	DPA1*02:01,DPB1*01:01	150				
DR12	DRB1*12:02	96	○				DP2	DPA1*01:03,DPB1*02:01	499				
DR13	DRB1*13:01	1072					DP5	DPA1*02:02,DPB1*05:01	858				
DR13	DRB1*13:03	770					DP3	DPA1*01:03,DPB1*03:01	682				
DR14	DRB1*14:01	1076					DP3	DPA1*01:05,DPB1*03:01	378				
DR14	DRB1*14:02	339					DP3	DPA1*02:01,DPB1*03:01	581				
DR14	DRB1*14:54	840					DP4	DPA1*01:03,DPB1*04:01	302				
DR15	DRB1*15:01	1700			○	○	DP4	DPA1*01:03,DPB1*04:02	553				
DR15	DRB1*15:02	1643			○		DP5	DPA1*02:01,DPB1*05:01	598				
DR15	DRB1*15:03	872			○		DP6	DPA1*02:01,DPB1*06:01	1088				
DR16	DRB1*16:01	987					DP6	DPA1*01:03,DPB1*06:01	2244				
DR16	DRB1*16:02	712					DP9	DPA1*02:01,DPB1*09:01	296				
DR52	DRB3*01:01	348	○	○			DP10	DPA1*02:02,DPB1*10:01	1855				
DR52	DRB3*02:02	694	○				DP11	DPA1*01:03,DPB1*11:01	743				
DR52	DRB3*03:01	603	○				DP28	DPA1*01:03,DPB1*28:01	1148				
DR53	DRB4*01:01	359	○		○		DP13	DPA1*02:01,DPB1*13:01	821				
DR53	DRB4*01:03	138	○	○	○	○	DP13	DPA1*02:02,DPB1*13:01	1456				
DR51	DRB5*01:01	1452			○	○	DP13	DPA1*03:01,DPB1*13:01	2112				
DR51	DRB5*02:02	1183			○		DP14	DPA1*02:01,DPB1*14:01	1619				
DO2	DOA1*02:01,DOB1*02:01	138					DP15	DPA1*02:01,DPB1*15:01	1127				
DO2	DOA1*03:01,DOB1*02:01	529					DP17	DPA1*02:01,DPB1*17:01	696				
DO2	DOA1*04:01,DOB1*02:01	1116					DP18	DPA1*02:01,DPB1*18:01	181				
DO2	DOA1*05:01,DOB1*02:01	55					DP18	DPA1*01:05,DPB1*18:01	206				
DO2	DOA1*02:01,DOB1*02:02	182					DP18	DPA1*01:04,DPB1*18:01	543				
DO4	DOA1*02:01,DOB1*04:01	2501			○	○	DP19	DPA1*01:03,DPB1*19:01	1190				
DO4	DOA1*03:03,DOB1*04:01	11437			○	○	DP20	DPA1*03:01,DPB1*20:01	1536				
DO4	DOA1*02:01,DOB1*04:02	5246			○		DP23	DPA1*01:03,DPB1*23:01	1866				
DO4	DOA1*04:01,DOB1*04:02	1698			○		DP28	DPA1*01:05,DPB1*28:01	743				
DO5	DOA1*01:01,DOB1*05:01	4057					DP28	DPA1*04:01,DPB1*28:01	1166				
DO5	DOA1*01:02,DOB1*05:02	2291					DP11	DPA1*02:02,DPB1*11:01	836				
DO6	DOA1*01:03,DOB1*06:01	1486			○								

Depart of Medicine

報告書作成日： 2018年9月3日　　検査担当者：
報告書確認者：

抗HLA抗体シングル同定検査(ClassⅡ)結果報告書 1/3

報告書ＩＤ	AI077	患者氏名	
採血日	2018/6/28	ドナー氏名	

●スクリーニング検査結果

ClassⅡ判定	陽性

●同定検査結果 (LABScreen Single Beadsを使用)

ClassⅡ陽性抗体	DR1	DR103	DR17	DR18	DR4		DR8	DR9	DR10			DR13
	DR14	DR15		DR51								
	DQ2	DQ4	DQ5	DQ6								
						DP6		DP10		DP13	DP14	DP15
			DP19	DP20	DP23		DP28					

●HLAタイピングデータ

遺伝子名	患者		ドナー	
	Allele1	Allele2	Allele1	Allele2
HLA-DRB1	DRB1*09:01	DRB1*12:01	DRB1*04:05	DRB1*15:01
	DR9	DR12	DR4	DR15
HLA-DRB345	DRB4*01:03	DRB3*01:01	DRB4*01:03	DRB5*01:01
	DR53	DR52	DR53	DR51
HLA-DQB1	DQB1*03:01	DQB1*03:03	DQB1*04:01	DQB1*06:02
	DQ7	DQ9	DQ4	DQ6
HLA-DPB1				

●クロス反応が予想される抗HLA抗体

DSA	DR4,DR15,DR51,DQ4,DQ6

コメント	DSA DRB1*04:05 (MFI 1251)　DSA DRB1*15:01 (MFI 1700) DSA DRB5*01:01 (MFI 1452)　DSA DQB1*04:01 (MFI 11437) DSA DQB1*06:02 (MFI 2674)

Department of I　　School of Medicine

抗HLA抗体シングル同定検査(ClassⅠ)結果報告書 2/3

● MFI（Normal)とHLAタイプ

MFI　>1000　>5000　>10000

＊カットオフ値は1000とした。

Cnt	PC	NC	PC/NC
Single	6,243	122	51
Supplement	7,353	25	292

Single Antigen

抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele	抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele
A1	A*01:01	3847					B37	B*37:01	0				
A2	A*02:01	358			○	○	B38	B*38:01	19				
A2	A*02:03	78			○		B39	B*39:01	0				
A2	A*02:06	223			○		B60	B*40:01	0				
A3	A*03:01	2887					B61	B*40:02	0				
A11	A*11:01	2660					B61	B*40:06	0				
A11	A*11:02	3429					B41	B*41:01	0				
A23	A*23:01	9080					B42	B*42:01	0				
A24	A*24:02	11836					B44	B*44:03	0	○	○		
A24	A*24:03	9730					B44	B*44:02	0	○			
A25	A*25:01	69					B45	B*45:01	0				
A26	A*26:01	0			○	○	B46	B*46:01	0				
A29	A*29:01	0					B47	B*47:01	0				
A29	A*29:02	0					B48	B*48:01	0				
A30	A*30:01	0					B49	B*49:01	732				
A30	A*30:02	0					B50	B*50:01	0				
A31	A*31:01	0					B51	B*51:01	597				
A32	A*32:01	106					B51	B*51:02	548				
A33	A*33:01	0	○				B52	B*52:01	350				
A33	A*33:03	0	○	○			B53	B*53:01	0				
A34	A*34:01	0					B54	B*54:01	0				
A34	A*34:02	0					B55	B*55:01	16			○	
A36	A*36:01	2054					B56	B*56:01	0				
A43	A*43:01	0					B57	B*57:01	52				
A66	A*66:01	0					B57	B*57:03	156				
A66	A*66:02	0					B58	B*58:01	42				
A68	A*68:01	331					B59	B*59:01	522				
A68	A*68:02	248					B67	B*67:01	0				
A69	A*69:01	84					B73	B*73:01	0				
A74	A*74:01	0					B78	B*78:01	0				
A80	A*80:01	5574					B81	B*81:01	0				
B7	B*07:02	0					B82	B*82:01	0				
B8	B*08:01	0					Cw1	C*01:02	0				
B13	B*13:01	0					Cw2	C*02:02	0				
B13	B*13:02	0					Cw10	C*03:02	0				
B64	B*14:01	0					Cw10	C*03:04	0				
B65	B*14:02	0					Cw9	C*03:03	0				
B62	B*15:01	0					Cw4	C*04:01	0				
B75	B*15:02	0					Cw5	C*05:01	0				
B75	B*15:11	0					Cw6	C*06:02	0				
B72	B*15:03	0					Cw7	C*07:02	0				
B71	B*15:10	0					Cw8	C*08:01	0				
B76	B*15:12	2098					Cw12	C*12:03	0				
B77	B*15:13	0					Cw14	C*14:02	0				
B63	B*15:16	453					Cw15	C*15:02	0				
B18	B*18:01	0					Cw16	C*16:01	0				
B27	B*27:05	0					Cw17	C*17:01	30				
B27	B*27:08	0					Cw18	C*18:02	0				
B35	B*35:01	0	○	○	○	○							

Supplement

抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele	抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele
A1	A*01:02	1916					B39	B*39:05	0				
A2	A*02:05	875			○		B39	B*39:06	0				
A2	A*02:07	37			○		B39	B*39:13	0				
A2	A*02:10	123			○		B61	B*40:03	0				
A2	A*02:18	46			○		B61	B*40:04	0				
A3	A*03:02	3017					B4005	B*40:05	0				
A26	A*26:02	0			○		B41	B*41:02	0				
A26	A*26:03	0			○		B42	B*42:02	0				
B7	B*07:14	0					B48	B*48:02	0				

Department　　ool of Medicine

報告書作成日： 2018年8月28日　　検査担当者：
報告書確認者：

抗HLA抗体シングル同定検査(Class Ⅰ)結果報告書 1/3

報告書ＩＤ	AI009	患者氏名	
採血日	2018/4/11	ドナー氏名	

●スクリーニング検査結果

Class Ⅰ 判定	陽性

●同定検査結果 (LABScreen Single Beadsを使用)

Class Ⅰ 陽性抗体	A1		A3	A11	A23	A24			A80			
			A36							B76		

●HLAタイピングデータ

遺伝子名	患者		ドナー	
	Allele1	Allele2	Allele1	Allele2
HLA-A	A*33:03	-	A*02:01	A*26:01
	A33		A2	A26
HLA-B	B*35:01	B*44:03	B*35:01	B*55:02
	B35	B44	B35	B55
	Bw6	Bw4	Bw6	Bw6
HLA-C				

●クロス反応が予想される抗HLA抗体

DSA	陰性

コメント	

Department o　　:hool of Medicine

運用の課題（自施設 vs 外注）

- ✓どのタイミングで検査？（年1回　スクリーニング）--- 自、外
- ✓電子カルテ　オーダー？ --- 自、外
- ✓採血、発送？ --- 外
- ✓HLAデータ（提供）？ --- 外
- ✓結果の取り込み（自動）？ --- 自、外
- ✓オーダーから結果参照まで連動？ --- 自、外

臨床で役立てるか？

- ✓結果解釈 --- double checkが望ましい
  （Technical issue は、検査側に問い合わせる）
- ✓方針決定 --- discussion
  （経過観察、血中濃度チェック、再検、腎生検、治療）
- ✓患者説明、実施 --- > 電子カルテに記載
- ✓評価、解析 --- > 次の対策

Department of Renal Transplant Surgery
Aichi Medical University School of Medicine

患者名　ID
移植日
HLA （R/D）

MFI 毎年の結果をならべる

前　1Y　2Y　3Y

				前	1Y	2Y	3Y
		LD/CD		KY	KY	KY	KY
				P1894	P2497	P2881	P3717
	Re Typing	DRB1		13:02	13:02	13:02	13:02
				15:01	15:01	15:01	15:01
		DQB1		06:02	06:02	06:02	06:02
				06:04	06:04	06:04	06:04
	Do Typing	DRB1		11:01	11:01	11:01	11:01
				15:01	15:01	15:01	15:01
		DQB1		03:01	03:01	03:01	03:01
				06:02	06:02	06:02	06:02
		HLA Ab		POS		POS	POS
		DSA		NDSA DRB1*0404(2780)	NDSA DRB1*0404(2463)	DSA DQB1*03:01 (13442) NDSA DRB1*0404(2734)	DSA DQB1*03:01 (1406)
		採血日		PreTx	2016/10/12	2017/4/12	2018/4/11
		検査日		2016/1/29	2017/1/19	2017/12/25	2018/8/11
			MAX	2780	2463	13455	1706
Lot 11			NC	22	5	36	136
			PC	9738	10607	9372	6422
DRB1*01:01	X	DR1		96	62	187	0
DRB1*01:02	X	DR1		105	73	217	0
DRB1*01:03	X	DR103		125	54	142	0
DRB1*03:01	X	DR17		432	394	326	0
DRB1*03:02	X	DR18		1085	1054	499	0
DRB1*04:01	X	DR4		2098	1787	2510	1380
DRB1*04:02	X	DR4		2070	1671	2197	1168
DRB1*04:04		DR4		2780	2463	2734	1706
DRB1*04:05		DR4		2016	1730	1849	1080
DRB1*07:01		DR7		207	140	257	0
DRB1*04:03		DR4		2167	1723	2332	1145
DRB1*08:01	X	DR8		201	115	252	0
DRB1*09:01	X	DR9		360	220	263	0
DRB1*09:02	X	DR9		335	239	262	0
	X	DR10		114	84	240	0
			1	265	49	106	0
			1	244	53	86	0
			2	109	52	149	0
			2	143	68	93	0
			3	182	88	127	0
			3	133	58	226	0
			4	160	80	139	0
			4	191	112	81	0
			4	187	105	112	0
			5	122	58	123	0
			5	160	64	150	0
			5	139	54	94	0
			6	193	278	368	0
			6	154	200	481	86
X	DRB3*01:01	DR52		187	74	94	0
X	DRB3*02:02	DR52		151	77	147	0
X	DRB3*03:01	DR52		534	206	165	0
X	DRB4*01:01	DR53		197	97	163	0

DR

			前	1Y	2Y	3Y
		LD/CD	101	101	101	101
			2016/04/12	2016/04/12	2016/04/12	2016/04/12
			KY	KY	KY	KY
			P1894	P2497	P2881	P3717
	Re Typing	DRB1	13:02	13:02	13:02	13:02
			15:01	15:01	15:01	15:01
		DQB1	06:02	06:02	06:02	06:02
			06:04	06:04	06:04	06:04
	Do Typing	DRB1	11:01	11:01	11:01	11:01
			15:01	15:01	15:01	15:01
		DQB1	03:01	03:01	03:01	03:01
			06:02	06:02	06:02	06:02
		HLA Ab	POS		POS	POS
		DSA	NDSA DRB1*0404(2780)	NDSA DRB1*0404(2463)	DSA DQB1*03:01 (13442) NDSA DRB1*0404(2734)	DSA DQB1*03:01 (1406)
		採血日	PreTx	2016/10/12	2017/4/12	2018/4/11
		検査日	2016/1/29	2017/1/19	2017/12/25	2018/8/11
		MAX	2780	2463	13455	1706
Lot 11		NC	22	5	36	136
		PC	9738	10607	9372	6422
DQA1*02:01	DQB1*04:02	DQ4	119	50	219	0
DQA1*04:01	DQB1*04:02	DQ4	191	75	351	0
DQA1*01:01	DQB1*05:01	DQ5	740	1580	1085	724
DQA1*01:02	DQB1*05:02	DQ5	141	76	62	0
DQA1*01:03	DQB1*06:01	DQ6	61	28	95	0
DQA1*01:02	DQB1*06:02	DQ6	209	85	158	0
DQA1*01:01	DQB1*06:02	DQ6	163	69	182	0
DQA1*01:03	DQB1*06:03	DQ6	48	21	64	0
DQA1	1*06:04	DQ6	206	117	188	0
DQA1	1*06:09	DQ6	60	23	71	0
DQA1	1*03:01	DQ7	217	81	6060	107
DQA1*02:01	DQB1*03:01	DQ7	176	89	8551	412
DQA1*05:03	DQB1*03:01	DQ7	153	75	12433	1429
DQA1*05:05	DQB1*03:01	DQ7	156	69	13442	1406
DQA1*06:01	DQB1*03:01	DQ7	138	49	12376	1322
DQA1*02:01	DQB1*03:02	DQ8	176	69	7150	187
DQA1*03:01	DQB1*03:02	DQ8	212	80	8328	376
DQA1*03:02	DQB1*03:02	DQ8	199	71	12228	943
DQA1*02:01	DQB1*03:03	DQ9	215	87	9828	423
DQA1*03:01	DQB1*03:03	DQ9	203	87	6868	208
DQA1*03:02	DQB1*03:03	DQ9	161	57	13455	1086
DPA1*01:03	DPB1*01:01	DP1	186	74	135	0
DPA1*02:01	DPB1*01:01	DP1	126	70	103	0
DPA1*01:03	DPB1*02:01	DP2	269	63	102	0
DPA1*02:02	DPB1*05:01	DP5	103	71	196	0
DPA1*01:03	DPB1*03:01	DP3	268	76	157	0
DPA1*01:05	DPB1*03:01	DP3	209	49	119	0
DPA1*02:01	DPB1*03:01	DP3	293	65	96	0
DPA1*01:03	DPB1*04:01	DP4	92	41	104	0
DPA1*01:03	DPB1*04:02	DP4	281	49	94	0
DPA1*02:01	DPB1*05:01	DP5	105	40	88	0

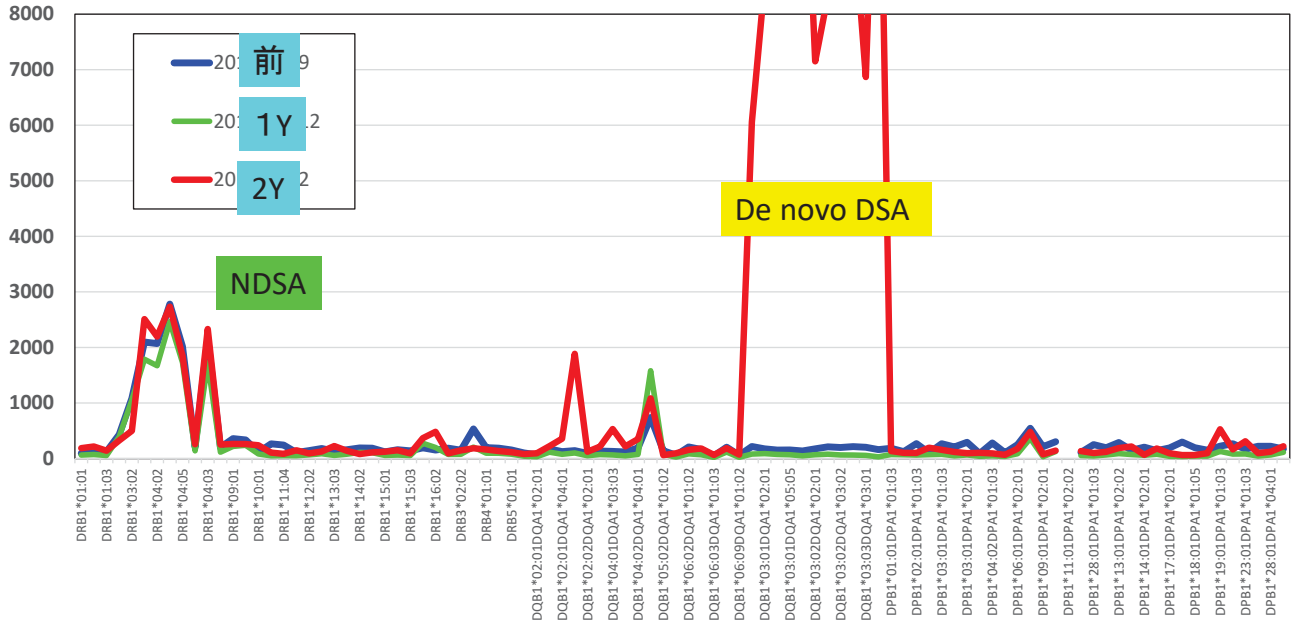
DQ

DSA

SAB
ビーズ番号
HLA（4桁）
(100 + supplement)

移植前後のHLA抗体の推移(LABScreen SAB)

(LD-101，2016/4/12 移植)



Department of Renal Transplant Surgery　Aichi Medical University School of Medicine

2019年

報告書作成日：　2019年8月19日　　検査担当者：
報告書確認者：

抗HLA抗体シングル同定検査(ClassⅡ)結果報告書 1/3

報告書ＩＤ	AI19-086	患者氏名	
採血日	2019/7/5	ドナー氏名	

●スクリーニング検査結果 (LABScreen PRAを使用)

ClassⅡ判定	陽性

●同定検査結果 (LABScreen Single Beadsを使用)

ClassⅡ陽性抗体													
	DR14					DR53							
	DQ2	DQ4		DQ6	DQ7	DQ8	DQ9						
	DP1												

●HLAタイピングデータ

遺伝子名	患者		ドナー	
	Allele1	Allele2	Allele1	Allele2
HLA-DRB1	DRB1*01:01	DRB1*15:01	DRB1*01:01	DRB1*12:01
	DR1	DR15	DR1	DR12
HLA-DRB345		DRB5*01:01		DRB3*01:01
		DR51		DR52
HLA-DQB1	DQB1*05:01	DQB1*06:02	DQB1*03:01	DQB1*05:01
	DQ5	DQ6	DQ7	DQ5
HLA-DPB1				

●クロス反応が予想される抗HLA抗体

DSA	DQ7

DSA DQB1*03:01 MFI (24209)
2018年8月　MFI (1801)

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コメント	DSA DQB1*03:01 MFI(24209) 2018年8月 測定時DQB1*03:01 MFI(1801)

2019年

報告書作成日： 2019年8月16日　　検査担当者：
報告書確認者：

抗HLA抗体シングル同定検査(ClassⅡ)結果報告書 1/3

報告書ＩＤ	AI19-034	患者氏名	
採血日	2019/6/20	ドナー氏名	

●スクリーニング検査結果 (LABScreen PRAを使用)

ClassⅡ判定	陽性

●同定検査結果 (LABScreen Single Beadsを使用)

ClassⅡ 陽性抗体												
					DQ7	DQ8	DQ9					

●HLAタイピングデータ

遺伝子名	患者		ドナー	
	Allele1	Allele2	Allele1	Allele2
HLA-DRB1	DRB1*14:54	DRB1*15:02	DRB1*12:01	DRB1*14:54
	DR14	DR15	DR12	DR14
HLA-DRB345	DRB3*02:02	DRB5*01:02	DRB3*01:01	DRB3*02:02
	DR52	DR51	DR52	DR52
HLA-DQB1	DQB1*05:02	DQB1*06:01	DQB1*03:03	DQB1*05:02
	DQ5	DQ6	DQ9	DQ5
HLA-DPB1				

●クロス反応が予想される抗HLA抗体

DSA	DQ9

DSA DQB1*03:03 MFI (2331)
2018年　MFI (10055)

コメント	DSA DQB1*03:03 MFI(2331) 2018年測定時 DQB1*03:03 MFI(10055)

Department o　　hool of Medicine

2019年

報告書作成日： 2019年8月16日　　検査担当者：
報告書確認者：

抗HLA抗体シングル同定検査(ClassⅡ)結果報告書 1/3

報告書ＩＤ	AI19-069	患者氏名	
採血日	2019/6/28	ドナー氏名	

●スクリーニング検査結果 (LABScreen PRAを使用)

ClassⅡ判定	陽性

●同定検査結果 (LABScreen Single Beadsを使用)

ClassⅡ 陽性抗体	DR1	DR103	DR17		DR4		DR8	DR9		DR11		DR13
	DR14			DR51	DR52	DR53						
	DQ2	DQ4	DQ5	DQ6		DQ8						
			DP3			DP6		DP10	DP11	DP13		
	DP17	DP18										

●HLAタイピングデータ

遺伝子名	患者		ドナー	
	Allele1	Allele2	Allele1	Allele2
HLA-DRB1	DRB1*04:10	DRB1*13:02	DRB1*01:01	DRB1*13:02
	DR4	DR13	DR1	DR13
HLA-DRB345	DRB4*01:03	DRB3*03:01		DRB3*03:01
	DR53	DR52		DR52
HLA-DQB1	DQB1*04:02	DQB1*06:04	DQB1*05:01	DQB1*06:04
	DQ4	DQ6	DQ5	DQ6
HLA-DPB1				

●クロス反応が予想される抗HLA抗体

DSA	DR1,DQ5

DSA DQB1*01:01 MFI (1927)
2018年8月　MFI (149)

コメント	DSA DRB1*01:01 MFI(1927) DSA DQB1*05:01 MFI(50)　NDSA DQB1*05:02 MFI(3222) 2018年8月測定時　DRB1*01:01　MFI（149）でDSA陰性

Department　　hool of Medicine

● MFI（Normal）とHLAタイプ

MFI >1000

DSA DQB1*01:01 MFI (1927)

Cntl	PC	NC	PC/NC
Single	12,629	79	159
Supplement	9,654	57	168

Single Antigen

抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele	抗体名	アレル	MFI(Nomal)	患者 Sero	患者 Allele	ドナー Sero	ドナー Allele
DR1	DRB1*01:01	1927			○	○	DQ6	DQA1*01:02,DQB1*06:02	249	○		○	
DR1	DRB1*01:02	2888			○		DQ6	DQA1*01:01,DQB1*06:02	480	○		○	
DR103	DRB1*01:03	1159					DQ6	DQA1*01:03,DQB1*06:03	65	○		○	
DR17	DRB1*03:01	2134					DQ6	DQA1*01:02,DQB1*06:04	2463	○	○	○	○
DR18	DRB1*03:02	851					DQ6	DQA1*01:02,DQB1*06:09	11	○		○	
DR4	DRB1*04:01	1033	○				DQ7	DQA1*03:01,DQB1*03:01	404				
DR4	DRB1*04:02	5321	○				DQ7						
DR4	DRB1*04:04	179	○				DQ7						
DR4	DRB1*04:05	1872	○				DQ7						
DR7	DRB1*07:01	160					DQ7						
DR4	DRB1*04:03	334	○				DQ8	DQA1*02:01,DQB1*03:02	833				
DR8	DRB1*08:01	566					DQ8	DQA1*03:01,DQB1*03:02	2624				
DR9	DRB1*09:01	2280					DQ8	DQA1*03:02,DQB1*03:02	24				
DR9	DRB1*09:02	2810					DQ9	DQA1*02:01,DQB1*03:03	399				
DR10	DRB1*10:01	978					DQ9	DQA1*03:01,DQB1*03:03	897				
DR11	DRB1*11:01	2448					DQ9	DQA1*03:02,DQB1*03:03	10				
DR11	DRB1*11:04	76					DP1	DPA1*01:03,DPB1*01:01	79				
DR12	DRB1*12:01	544					DP1	DPA1*02:01,DPB1*01:01	17				
DR12	DRB1*12:02	631					DP2	DPA1*01:03,DPB1*02:01	83				
DR13	DRB1*13:01	4527	○		○		DP5	DPA1*02:02,DPB1*05:01	76				
DR13	DRB1*13:03	2267	○		○		DP3	DPA1*01:03,DPB1*03:01	1430				
DR14	DRB1*14:01	144					DP3	DPA1*01:05,DPB1*03:01	887				
DR14	DRB1*14:02	2800					DP3	DPA1*02:01,DPB1*03:01	315				
DR14	DRB1*14:54	74					DP4	DPA1*01:03,DPB1*04:01	81				
DR15	DRB1*15:01	231					DP4	DPA1*01:03,DPB1*04:02	136				
DR15	DRB1*15:02	179					DP5	DPA1*02:01,DPB1*05:01	45				
DR15	DRB1*15:03	350					DP6	DPA1*02:01,DPB1*06:01	2710				
DR16	DRB1*16:01	170					DP6	DPA1*01:03,DPB1*06:01	2484				
DR16	DRB1*16:02	768					DP9	DPA1*02:01,DPB1*09:01	249				
DR52	DRB3*01:01	72	○		○		DP10	DPA1*02:02,DPB1*10:01	1179				
DR52	DRB3*02:02	3511	○		○		DP11	DPA1*01:03,DPB1*11:01	3629				
DR52	DRB3*03:01	48	○	○	○	○	DP28	DPA1*01:03,DPB1*28:01	382				
DR53	DRB4*01:01	1349	○				DP13	DPA1*02:01,DPB1*13:01	139				
DR53	DRB4*01:03	742	○	○			DP13	DPA1*02:02,DPB1*13:01	160				
DR51	DRB5*01:01	4104					DP13	DPA1*03:01,DPB1*13:01	5252				
DR51	DRB5*02:02	3355					DP14	DPA1*02:01,DPB1*14:01	67				
DQ2	DQA1*02:01,DQB1*02:01	1149					DP15	DPA1*02:01,DPB1*15:01	107				
DQ2	DQA1*03:01,DQB1*02:01	405					DP17	DPA1*02:01,DPB1*17:01	1298				
DQ2	DQA1*04:01,DQB1*02:01	494					DP18	DPA1*02:01,DPB1*18:01	104				
DQ2	DQA1*05:01,DQB1*02:01	277					DP18	DPA1*01:05,DPB1*18:01	1427				
DQ2	DQA1*02:01,DQB1*02:02	397					DP18	DPA1*01:04,DPB1*18:01	1866				
DQ4	DQA1*02:01,DQB1*04:01	3402	○				DP19	DPA1*01:03,DPB1*19:01	528				
DQ4	DQA1*03:03,DQB1*04:01	0	○				DP20	DPA1*03:01,DPB1*20:01	532				
DQ4	DQA1*02:01,DQB1*04:02	850	○	○			DP23	DPA1*01:03,DPB1*23:01	72				
DQ4	DQA1*04:01,DQB1*04:02	312	○	○			DP28	DPA1*01:05,DPB1*28:01	473				
DQ5	DQA1*01:01,DQB1*05:01	50			○	○	DP28	DPA1*04:01,DPB1*28:01	559				
DQ5	DQA1*01:02,DQB1*05:02	3222			○		DP11	DPA1*02:02,DPB1*11:01	1442				
DQ6	DQA1*01:03,DQB1*06:01	9	○		○								

AUTO（自己）にも MFI (2463)

Dep Medicine

電子カルテ 掲示板に記入

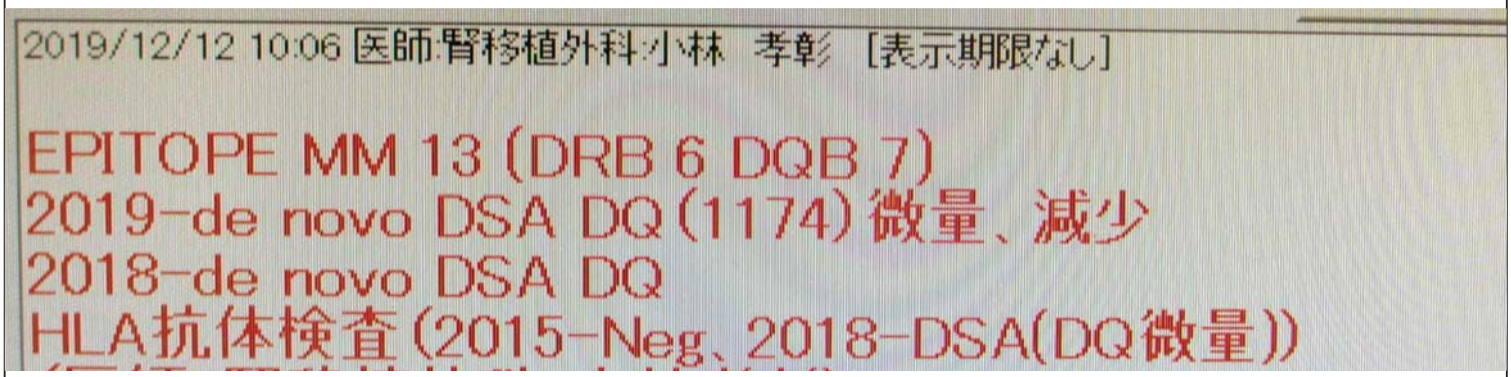
2019/11/26 11:28 医師:腎移植外科:小林 孝彰 [表示期限なし]

EPITOPE MM 17 (DRB 13 DQB 4)
HLA抗体検査(2015Neg-2019Neg)

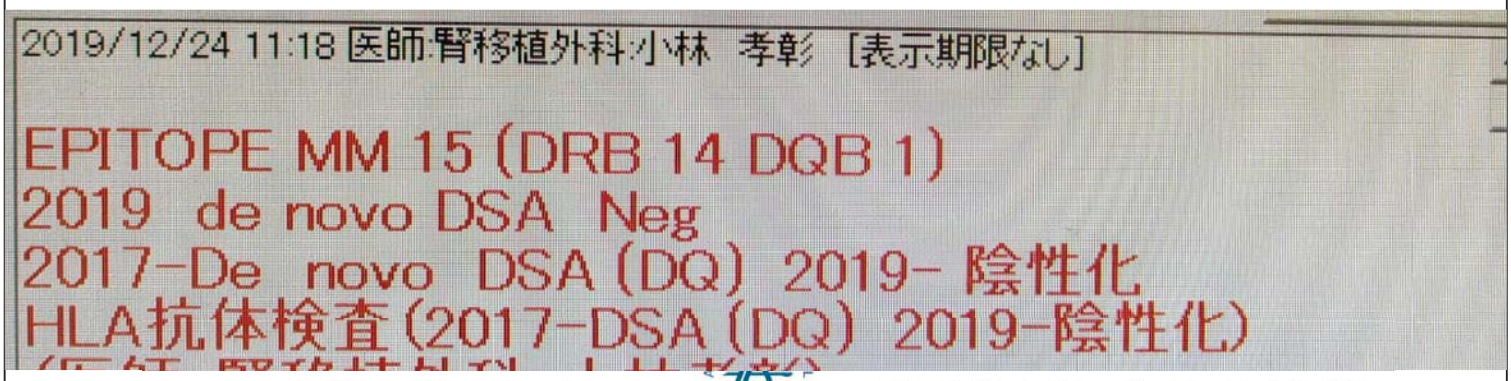
2019/11/26 11:36 医師:腎移植外科:小林 孝彰 [表示期限なし]

EPITOPE MM 23 (DRB 13 DQB 10)
HLA抗体検査(2015Neg-2019Neg)

電子カルテ　掲示板に記入



電子カルテ　掲示板に記入



電子カルテ　掲示板に記入

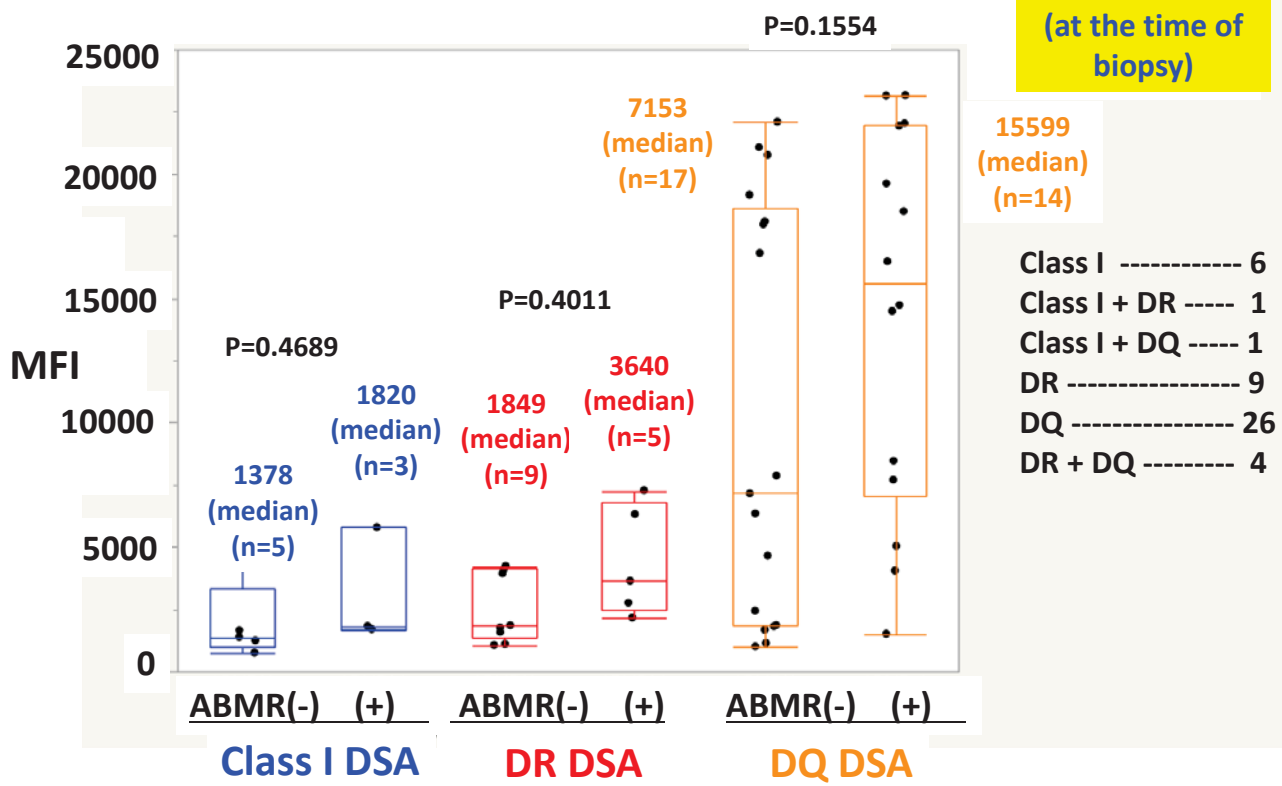
DSA　全てが
ABMRを引き起こすのではない

Biopsy has been conducted in patients with Class I, DR or DQ DSA (n=47)

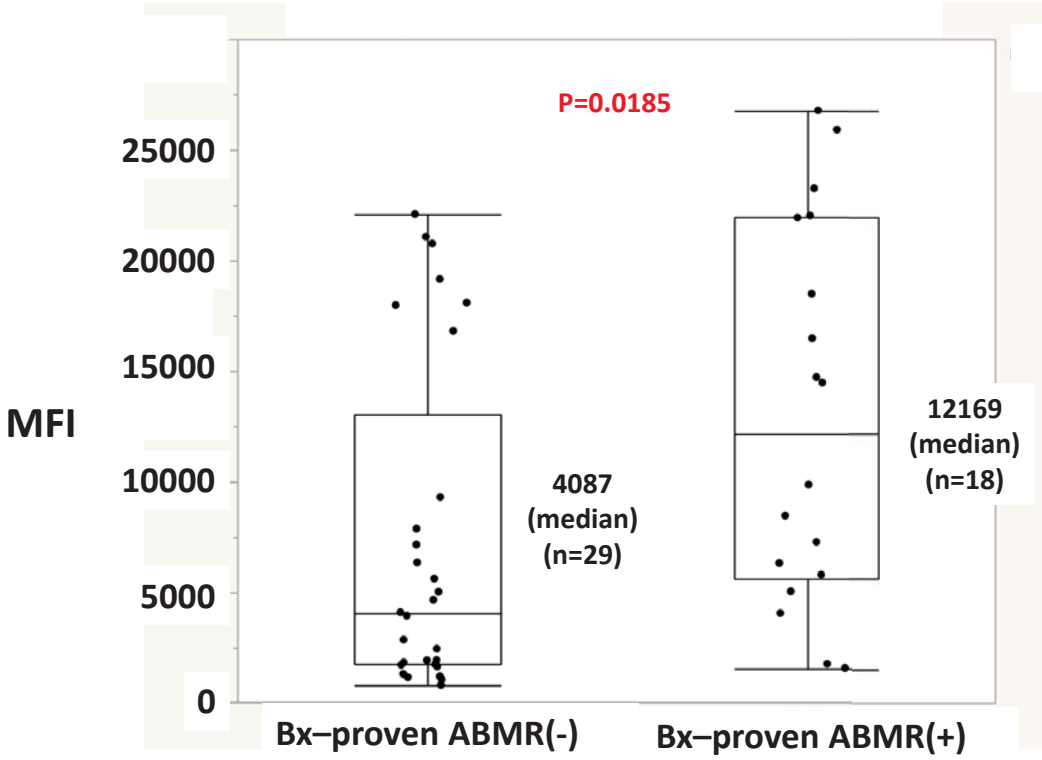
Bx proven ABMR (n=18)
Bx proven non ABMR (n=29)

MFI levels of DSA

DSA MFI levels in Bx-proven ABMR and non-ABMR (n=47)



Sum of DSA MFI levels in Bx-proven ABMR and non-ABMR (n=47)



(at the time of biopsy)

Class I ---------- 6
Class I + DR ----- 1
Class I + DQ ----- 1
DR ---------- 9
DQ ---------- 26
DR + DQ --------- 4

Biopsy proven ABMR and de novo DSA (sum of MFI levels)

(n=47)

	Bx-proven ABMR	
	(+)	(-)
Total MFI < 5000	3 (15.8%)	16
Total MFI ≥ 5000	15 (53.6%)	13

(Sum of DSA MFI levels at the time of biopsy)

P=0.008925

HLA抗体モニタリング
De novo DSA 出現

Blood concentration
Non-adherence check

Renal dysfunction (-)

- ✓ Sum of MFI < 5000 -- > 3-6ヶ月後再検査
- ✓ Sum of MFI ≥ 5000 -- > Graft Biopsy
  - ➢ ABMR (+) -- > Treatment
  - ➢ ABMR (-) -- > F/U

運用の課題（自施設 vs 外注）

自施設での検査、管理
　患者ごとにファイル（記録）（Excel　など）
　担当医に連絡、電子カルテ反映

外注での検査（依頼、管理は大変！）
　データ管理も含めて外部に委託　？
　（個人情報の問題）
　毎年、血液を送れば、結果を送ってくれる
　（HLA情報2回目から不要）
　臨床医の負担軽減

本日の内容

- モニタリングの意義？
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- 課題（問題点）
- 今後について（将来展望）

課題（問題点）

- ✓ HLA タイピング　どこまで？　DQA1？　C，DPB1/DPA1？
- ✓ Complement，　IgG subclass
- ✓ Non HLA Ab
- ✓ Pathogenicity of DSA　（ABMRとの関連）

→　結果の解釈

Transplant International

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

REVIEW

Caveats of HLA antibody detection by solid-phase assays

Caroline Wehmeier[1], Gideon Hönger[1,2,3] & Stefan Schaub[1,2,3]

1 Clinic for Transplantation Immunology and Nephrology, University Hospital Basel, Basel, Switzerland
2 Transplantation Immunology, Department of Biomedicine, University of Basel, Basel, Switzerland
3 HLA-Diagnostics and Immunogenetics, Department of Laboratory Medicine, University Hospital Basel, Basel, Switzerland

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Tel.: 0041 61 265 45 33;
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e-mail: stefan.schaub@usb.ch

SUMMARY

Solid-phase assays for human leukocyte antigens (HLA) antibody detection have clearly revolutionized the field of HLA diagnostics and transplantation. The key advantages are a high sensitivity and specificity for detection of HLA antibodies compared with cell-based assays, as well as the potential for standardization. Solid-phase assays enabled the broad introduction of tools such as "virtual crossmatching" and "calculated panel reactive antibodies," which are essential components in many organ allocation systems, kidney-paired donation programs, and center-specific immunological risk stratification procedures. The most advanced solid-phase assays are the so-called single antigen beads (SAB). They are available now for more than 15 years, and the transplant community embraced their significant advantages. However, SAB analysis and interpretation is complex and many pitfalls have to be considered. In this review, we will discuss problems, limitations, and challenges using SAB. Furthermore, we express our wishes for improvements of SAB as well as their future use for immunological assessment and research purposes.

Transplant International 2020; 33: 18–29

Solid phase assays

Virtual crossmatching, Calculated PRA (cPRA)

- ✓ **Organ allocation system**
- ✓ **Kidney –paired donation**
- ✓ **Center-specific immunological risk stratification**

Limitations

- **Quantification**
  - ＊MFI 基準　＊メーカーによる違い
  - ＊ビーズの質　＊DSAの反応性 (public epitopes, IgM, IgA) ＊過飽和
- **Interference of serum components**
  - ＊高濃度のアルブミン、免疫グロブリン、ハプトグロビン、トランスフェリン、アンチトリプシン、フィブリノーゲン　--- > HLA抗体の接着↓　negative control接着↑
  - ＊補体結合　--- > HLA抗体の接着↓
- **Interference of medicinal drugs**
  - ＊サイモグロブリン (HLA抗体含んでいる可能性）　＊IVIG
- **Denatured HLA molecules**
  - ＊Problematic beads

Table 1. Main reasons for false-negative and false-positive reactions in the IgG SAB assay.

False-negative	False-positive
• "Dilution" of the MFI signal across multiple beads sharing the same epitope • Serum matrix masks binding of HLA antibodies • High amount of bound HLA antibodies leading to accumulation of complement components, which interfere with binding of the detection antibody (i.e., complement interference) • Massive excess of HLA antibodies leading to steric hindrance of binding (i.e., hook or prozone effect) • IgA and/or IgM antibodies competing for binding sites of IgG antibodies • Rabbit anti-HLA antibodies present in medical products (e.g., polyclonal anti-thymocyte globulin) competing for binding sites of human HLA antibodies • IgG antibodies with low concentration or affinity unable to sufficiently bind during the 30 min incubation time	• Exposure of neo-epitopes • Unspecific binding of serum matrix components • HLA antibodies present in medical products (e.g., polyclonal anti-thymocyte globulin)

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

Critical issues for data interpretation

- R/D HLA typing
- Sensitization history
- CREG (cross reactive groups)
  --- > EPITOPE (HLA Matchmaker)
  * Public epitope, private epitope
  * Current SAB panels cover 98.5% of eplets
- Ethnicity-adapted SAB panels
- Pathogenicity factors
  * epitope specificity of DSA, magnitude and durability of memory response, density of antigen expression in graft, regulation of effector functions ---

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

Complement binding, IgG subclass

- C1q, C3d --- > pros and cons
- IgG1, IgG3 vs IgG2, IgG4 --- > pros and cons

Overall, it is still a matter of debate whether the modified SAB assays (C1q/C3d-binding; IgG subclasses; IgA/IgM; titration studies) enhance risk prediction beyond the generic SAB assay in a clinically significant way. It is conceivable that they provide very important information in specific cases, but their general application might not be necessary and they are currently expensive as well as labor-intensive.

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

Screening for HLA antibodies

- **Pre-Tx（waiting list 含む）** — Memory immune response (preformed DSA)

  ＊意義あり --- Immunological risk

- **Post-Tx** — Primary immune response (de novo DSA)

Banff 2017 meeting report [83]. Routine screening in the absence of allograft dysfunction is, however, more debatable and cannot, in terms of cost-benefit considerations, be commonly recommended within the first year post-transplant. Wiebe *et al.* [84] have nicely shown that the frequency of *de novo* DSA in the first year is only 2%, but steadily increases over the subsequent years at a rate of about 2%/year.

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

But how to deal with the detection of *de novo* DSA in a transplant recipient with stable allograft function beyond the first year post-transplant? Unfortunately, in case of evidence of allograft injury in a biopsy following *de novo* DSA detection, therapeutic options are currently very limited as neither the proteasome inhibitor bortezomib nor a combination of intravenous immunoglobulin and rituximab was effective in two randomized-controlled trials [85–87]. Detection of *de novo* DSA might, nevertheless, still be helpful to identify patient having insufficient immunosuppression (e.g., nonadherence or physician-induced minimization) and to tailor immunosuppression on an individual basis [88].

Wehmeier C, et al. Transpl Int 2020; 33: 18–29

Department of Renal Transplant Surgery Aichi Medical University School of Medicine

本日の内容

- モニタリングの意義？
- どのように実施、臨床に役立てる？
- 課題（問題点）
- 今後について（将来展望）

将来展望

- EPITOPE (B cell, T cell)
  - ＊DSA産生　risk stratification --- > 予防 (individualized IS)
- Non HLA antibody
- Early diagnosis
  - ＊DSA産生よりも早期に
  - ＊Tfh, Bmem, High Throughput Sequence
- Graft Accommodation

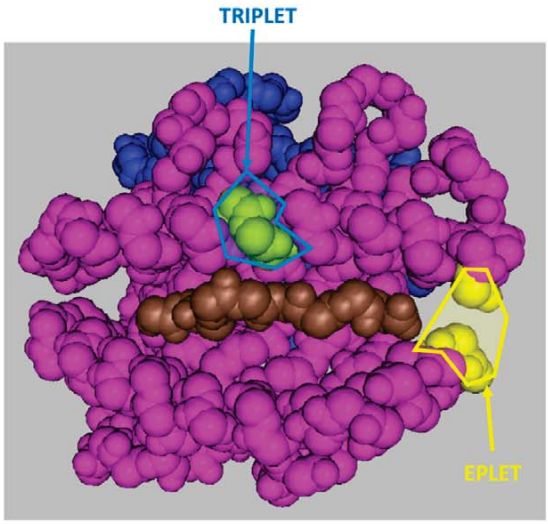
EPITOPE

B cell epitope --- BCR (HLA antibody)

Eplet mismatch number Difference between donor and recipient HLA

determined by HLA matchmaker (website)

TRIPLET: 3 linearly consecutive amino acid residues



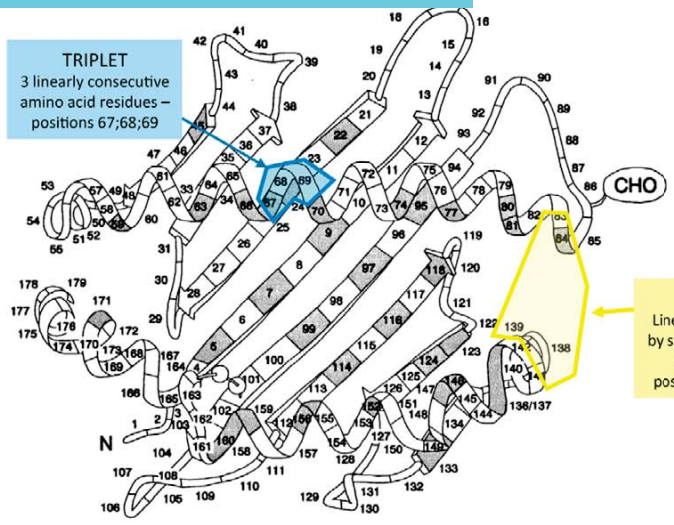


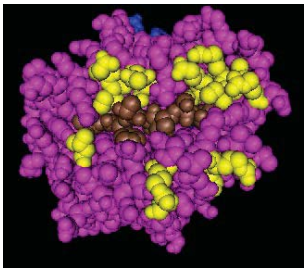
Figure 2: Depiction of the principle difference between a triplet and an eplet.

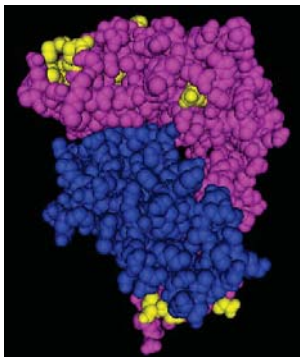
EPLET: Linearly discontinuous spatially close amino acid residues

Tambur AR, Claas FHJ. AJT 2015, 15: 1148-54.

HLAMatchmaker algorithm predicts the immunogenicity of an HLA alloantigen.

Principles of HLAMatchmaker:





1. **Antigen has many potentially immunogenic epitopes (triplets/eplets) but some of these are shared with the patients' own HLA molecules**

2. **Patient will not make antibodies to epitopes present on the own HLA antigens and therefore:**

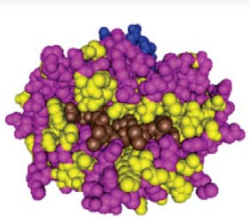
3. **Polymorphism of an HLA mismatch should be considered in the context of the HLA type of the potential antibody producer.**

Duquesnoy, Human Immunol. 2002

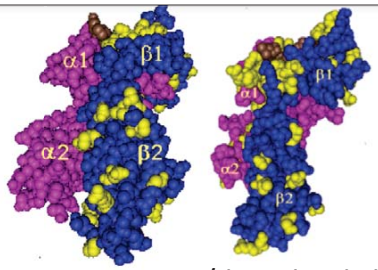
Dr Frans Claasより提供

Department of Renal Transplant Surgery

Aichi Medical University School of Medicine



HLAMatchmaker
An Algorithm for Epitopes



http://www.epitopes.net/downloads.html

Welcome to the Program Download Section

All HLAMatchmaker programs are in Microsoft Excel format and they consider two groups of epitopes (1) those verified experimentally with informative HLA antibodies and (2) eplets that are theoretically predicted as potential epitopes but we do not know yet which ones become antibody-verified. All epitopes are annotated according to the system in the International HLA Epitope Registry

The HLAMatchmaker programs consider two groups of epitopes (1) those verified experimentally with informative HLA antibodies and (2) eplets that are theoretically predicted as potential epitopes but we do not know yet which ones can be considered as antibody-verified.

There are two types of HLAMatchmaker programs: epitope specificity analysis of HLA antibodies and HLA matching at the epitope level. The antibody analysis programs are based on the reactivity with single allele panels. All programs on this website are listed as version 01 but HLA-ABC and HLA-DRDQDP antibody analysis programs have been updated to version 02 which include recently antibody-verified epitopes and incorporate additional features aimed to analyze complex reactivity patterns. There is also a new manual for version 02 that can be downloaded as a PDF.

Two programs are for HLA matching at the epitope level. They use reduced eplet repertoires that avoid similar overlapping sequences (see Instructions) and they can be used for a single donor-recipient combination or a meta-analysis of up to 1000 combinations. The data provide quantitative (eplet loads) and qualitative information about antibody-verified and theoretically predicted epitopes. These programs will be updated soon.

All programs are based of high-resolution allele types. The four-digit converter program can be used to make predictions from two-digit antigen types but actual four-digit typing is preferred.

To download a program, you must be signed in to google.

Downloads have been optimized for Google Chrome

CLINICAL RESEARCH www.jasn.org

Wiebe C, et al. J Am Soc Nephrol. 2017; 28: 3353-3362

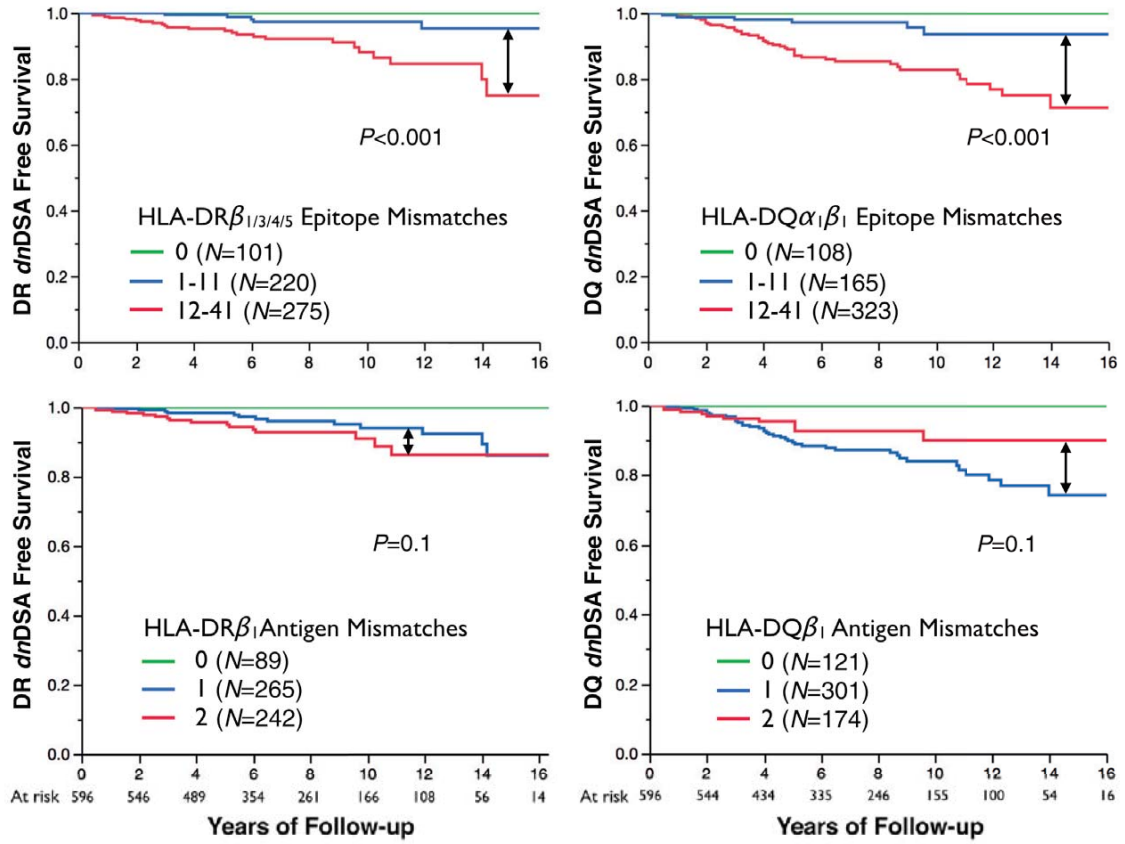
Class II Eplet Mismatch Modulates Tacrolimus Trough Levels Required to Prevent Donor-Specific Antibody Development

Chris Wiebe,*† David N. Rush,* Thomas E. Nevins,‡ Patricia E. Birk,§ Tom Blydt-Hansen,‖ Ian W. Gibson,†¶ Aviva Goldberg,§ Julie Ho,*** Martin Karpinski,* Denise Pochinco,† Atul Sharma,§ Leroy Storsley,* Arthur J. Matas,†† and Peter W. Nickerson*†**

Departments of *Medicine, §Pediatrics and Child Health, ¶Pathology, and **Immunology, University of Manitoba, Winnipeg, Manitoba, Canada; †Diagnostic Services of Manitoba, Winnipeg, Manitoba, Canada; Departments of ‡Pediatrics and ††Surgery, University of Minnesota, Minneapolis, Minnesota; and ‖Department of Pediatrics, University of British Columbia, Vancouver, British Columbia, Canada

Department of Renal Transplant Surgery — Aichi Medical University School of Medicine

Wiebe C, et al. J Am Soc Nephrol. 2017; 28: 3353-3362



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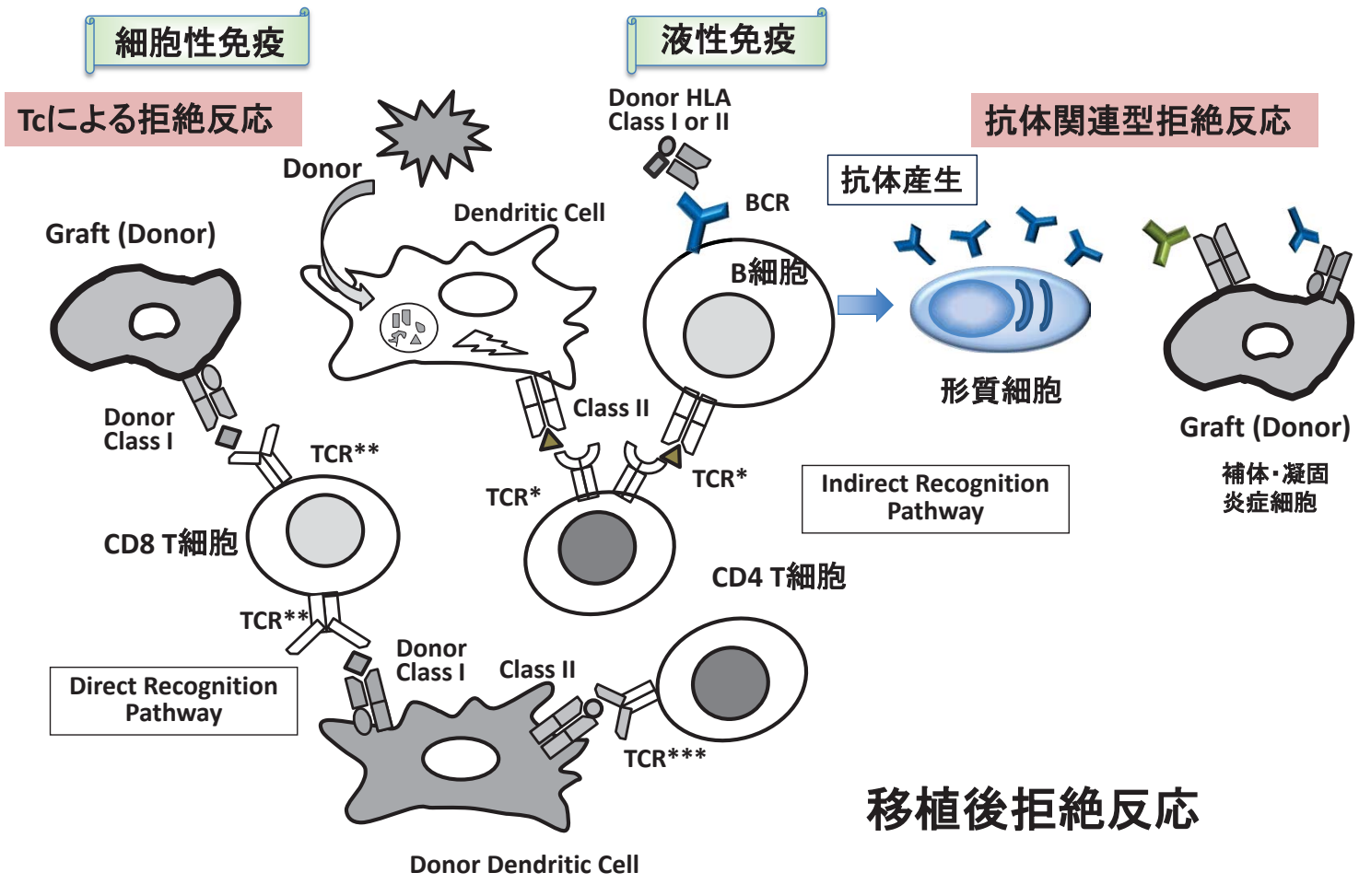
EPITOPE

B cell epitope --- BCR (HLA antibody)

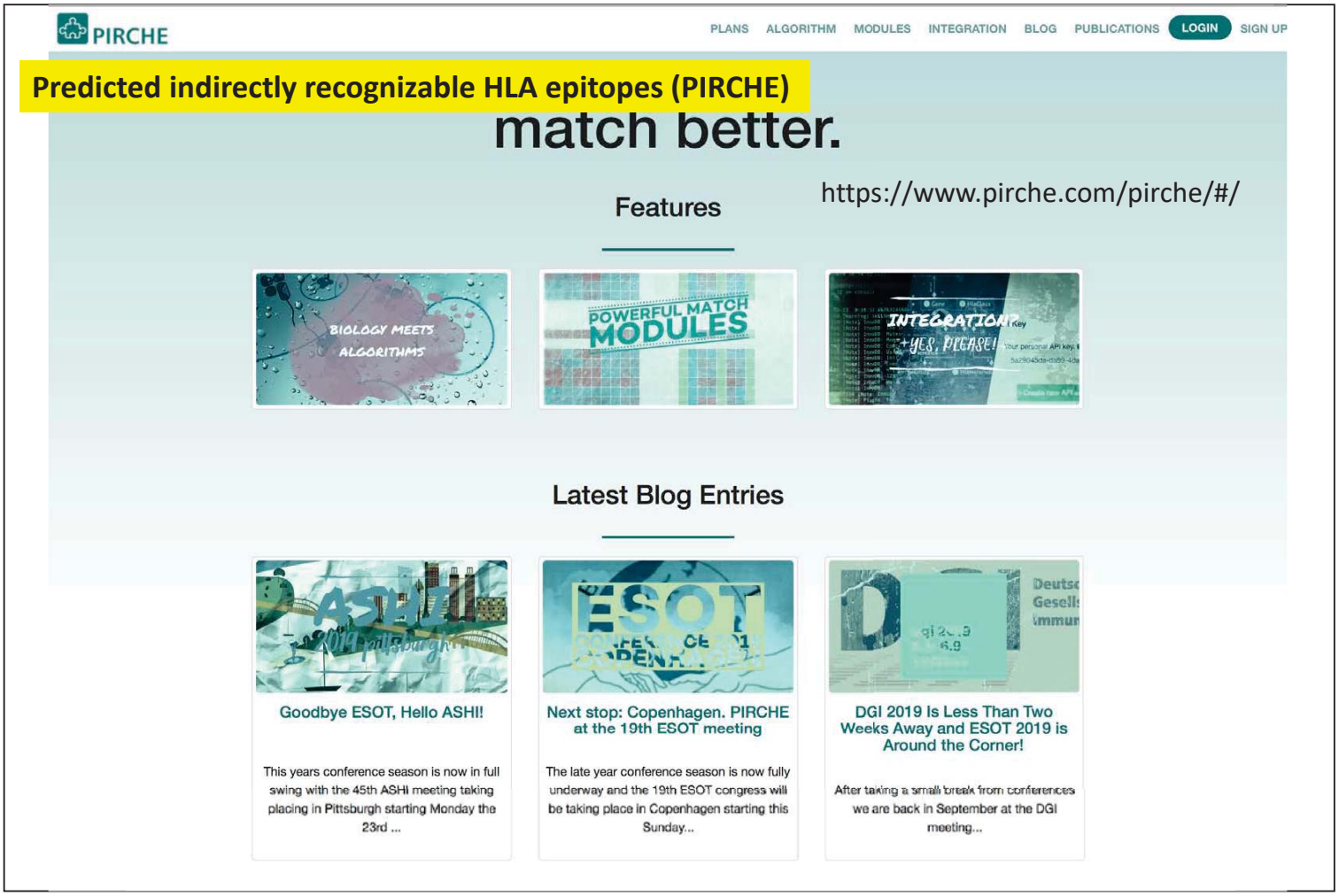
Eplet mismatch Number Difference between donor and recipient HLA

determined by HLA matchmaker (website)

T cell epitope --- TCR



Predicted indirectly recognizable HLA epitopes (PIRCHE)



American Journal of Transplantation 2017; 17: 3076–3086
Wiley Periodicals Inc.

doi: 10.1111/ajt.14393

Predicted indirectly recognizable HLA epitopes (PIRCHE)

Donor–Recipient Matching Based on Predicted Indirectly Recognizable HLA Epitopes Independently Predicts the Incidence of *De Novo* Donor-Specific HLA Antibodies Following Renal Transplantation

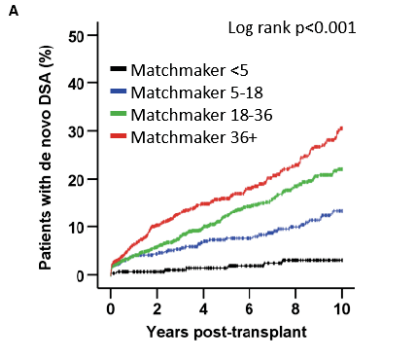
Lachmann N, et al. AJT 2017; 17: 3076-3086

N. Lachmann[1,*], M. Niemann[2], P. Reinke[3,†], K. Budde[3], D. Schmidt[3], F. Halleck[3], A. Pruß[4], C. Schönemann[1], E. Spierings[5] and O. Staeck[3]

[1]Center for Tumor Medicine, H&I Laboratory, Charité University Medicine Berlin, Berlin, Germany
[2]PIRCHE AG, Berlin, Germany
[3]Department of Nephrology, Charité University Medicine Berlin, Berlin, Germany
[4]Universitary Tissue Bank, Charité University Medicine Berlin, Berlin, Germany
[5]UMC Utrecht, Laboratory of Translational Immunology

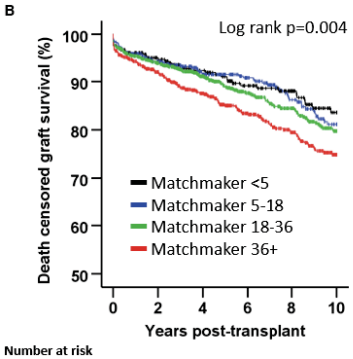
Abbreviations: AA, amino acid; AMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity test; CI, confidence interval; dnDSA, *de novo* donor-specific HLA antibodies; DSA, donor-specific HLA antibody(ies); HLAab, HLA antibody(ies); IQR, interquartile range; SAB, single antigen bead(s); PIRCHE, predicted indirectly recognizable HLA epitopes

Received 15 December 2016, revised 26 May 2017 and accepted for publication 04 June 2017



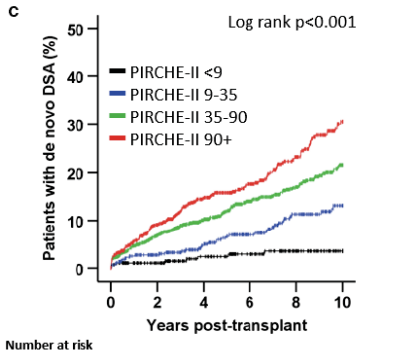
Number at risk

Years post-transplant	0	2	4	6	8	10
Matchmaker <5	323	280	236	193	145	101
Matchmaker 5-18	476	397	326	263	201	150
Matchmaker 18-36	1136	917	683	510	388	249
Matchmaker 36+	852	644	495	371	265	162



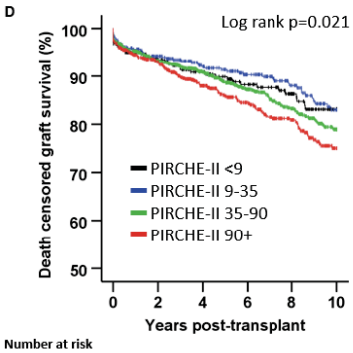
Number at risk

Years post-transplant	0	2	4	6	8	10
Matchmaker <5	323	268	222	179	136	90
Matchmaker 5-18	476	391	320	255	190	138
Matchmaker 18-36	1136	926	703	524	399	250
Matchmaker 36+	852	672	523	388	278	168



Number at risk

Years post-transplant	0	2	4	6	8	10
PIRCHE <9	285	246	203	168	126	87
PIRCHE 9-35	446	378	311	245	182	131
PIRCHE 35-90	1222	984	763	578	451	301
PIRCHE 90+	834	630	463	346	240	143



Number at risk

Years post-transplant	0	2	4	6	8	10
PIRCHE <9	285	234	190	155	116	77
PIRCHE 9-35	446	370	301	238	179	123
PIRCHE 35-90	1222	1001	787	590	450	299
PIRCHE 90+	834	652	490	363	258	147

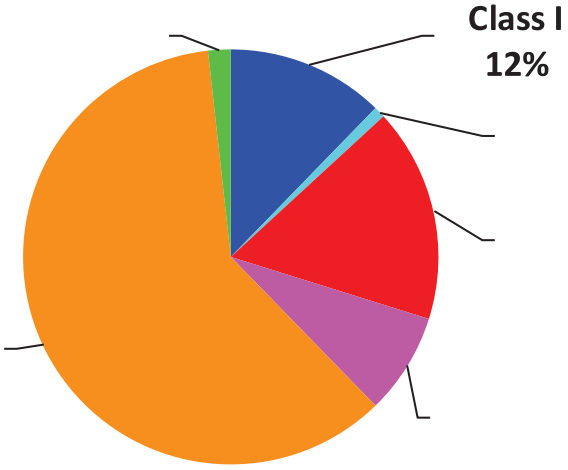
Lachmann N, et al. AJT 2017; 17: 3076-3086

de novo DSA

114/691 (16.5%)

Class I ----------14
Class I + DR ----- 1
Class I + DQ ----- 2
DR ----------19
DQ ---------- 69
DR + DQ --------- 9

Class I-associated -----17
DR-associated----------29
DQ-associated ---------80



De novo DSA (DR, DQ) and Eplet MM

(n=691)

		de novo DSA (DR,DQ)	
		(+)	(-)
Eplet MM (DRB, DQB)	0--13	8 (3.4%)	227
	14--67	92 (20.2%)	364

P<0.0001

De novo DSA (DR, DQ) and PIRCHE score

(n=691)

		de novo DSA (DR,DQ)	
		(+)	(-)
PICHE score	0--175	26 (8.2%)	292
	176--763	74 (19.8%)	299

P<0.0001

Non HLA

Transplant International

REVIEW ARTICLE

Novel insights into non-HLA alloimmunity in kidney transplantation

Roman Reindl-Schwaighofer[1], Andreas Heinzel[1], Guido A. Gualdoni[1], Laurent Mesnard[2], Frans H.J. Claas[3] & Rainer Oberbauer[1]

Transpl Int 2020, 33: 5-17.

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SUMMARY

Recognition of non-self structures on donor cells represents the main immunological barrier in solid organ transplantation. The human leukocyte antigens (HLA) are considered the most important non-self (allo)antigens in transplantation. Long-term graft attrition is mainly caused by the formation of alloreactive antibodies that are directed against non-self structures (i.e., epitopes) on cell surface proteins. Recently published data provided evidence for a similar importance of non-HLA mismatches between donors and recipients in acute rejection as well as long-term kidney allograft survival. These data suggest a broader concept of immunological non-self that goes beyond HLA incompatibility and expands the current concept of polymorphic non-self epitopes on cell surface molecules from HLA to non-HLA targets. Amino acid substitutions caused by single nucleotide variants in protein-coding genes or complete loss of gene expression represent the basis for polymorphic residues in both HLA and non-HLA molecules. To better understand these novel insights in non-HLA alloimmunity, we will first review basic principles of the alloimmune response with a focus on the HLA epitope concept in donor-specific antibody formation before discussing key publications on non-HLA antibodies.

Early Diagnosis

Tfh

ORIGINAL ARTICLE

AJT

Circulating T follicular helper cells are a biomarker of humoral alloreactivity and predict donor-specific antibody formation after transplantation

Glenn Michael La Muraglia II | Maylene E. Wagener | Mandy L. Ford | Idelberto Raul Badell

La Muraglia GM II, et al. Am J Transplant 2020; 20: 75-87

Department of Renal Transplant Surgery Aichi Medical University School of Medicine

KI REPORTS
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TRANSLATIONAL RESEARCH

Impact of Induction Therapy on Circulating T Follicular Helper Cells and Subsequent Donor-Specific Antibody Formation After Kidney Transplant

Camila Macedo[1,2], Kevin Hadi[1,2], John Walters[1,2], Beth Elinoff[1,2], Marilyn Marrari[1,3], Adriana Zeevi[1,3,4], Bala Ramaswami[1,2], Geetha Chalasani[1,4,5], Douglas Landsittel[1,6], Adele Shields[7], Rita Alloway[8], Fadi G. Lakkis[1,4,5], E. Steve Woodle[7] and Diana Metes[1,2,4]

[1]Thomas E. Starzl Transplantation Institute, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA; [2]Department of Surgery, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [3]Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [4]Department of Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [5]Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [6]Department of Biomedical Informatics, University of Pittsburgh, Pittsburgh, Pennsylvania, USA; [7]Division of Transplantation, University of Cincinnati, Cincinnati, Ohio, USA; and [8]Division of Nephrology, University of Cincinnati, Cincinnati, Ohio, USA

Macedo C, et al. Kidney Int Rep 2019; 4: 455-469

Department of Renal Transplant Surgery — Aichi Medical University School of Medicine

REVIEW ARTICLE

HLA Immune Response Genetics WILEY

van Besouw NM, et al. HLA 2019; 94: 407-414.

The role of follicular T helper cells in the humoral alloimmune response after clinical organ transplantation

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Over the past decade, antibody-mediated or humoral rejection in combination with development of de novo donor-specific antibodies (DSA) has been recognized as a distinct and common cause of transplant dysfunction and is responsible for one-third of the failed allografts. Detailed knowledge of the mechanisms that initiate and maintain B-cell driven antidonor reactivity is required to prevent and better treat this antidonor response in organ transplant patients. Over the past few years, it became evident that this response largely depends on the actions of both T follicular helper (Tfh) cells and the controlling counterparts, the T follicular regulatory (Tfr) cells. In this overview paper, we review the latest insights on the functions of circulating (c)Tfh cells, their subsets Tfh1, Tfh2 and Tfh17 cells, IL-21 and Tfr cells in antibody mediated rejection (ABMR). This may offer new insights in the process to reduce de novo DSA secretion resulting in a decline in the incidence of ABMR. In addition, monitoring these cell populations could be helpful for the development of biomarkers identifying patients at risk for ABMR and provide novel therapeutic drug targets to treat ABMR.

Bmem

Transplant International

REVIEW

B cells in transplant tolerance and rejection: friends or foes?

Robin Schmitz, Zachary W. Fitch, Paul M. Schroder, Ashley Y. Choi, Annette M. Jackson, Stuart J. Knechtle & Jean Kwun

Transpl Int 2020, 33: 30-40

Department of Surgery, Duke Transplant Center, Duke University Medical Center, Durham, NC, USA

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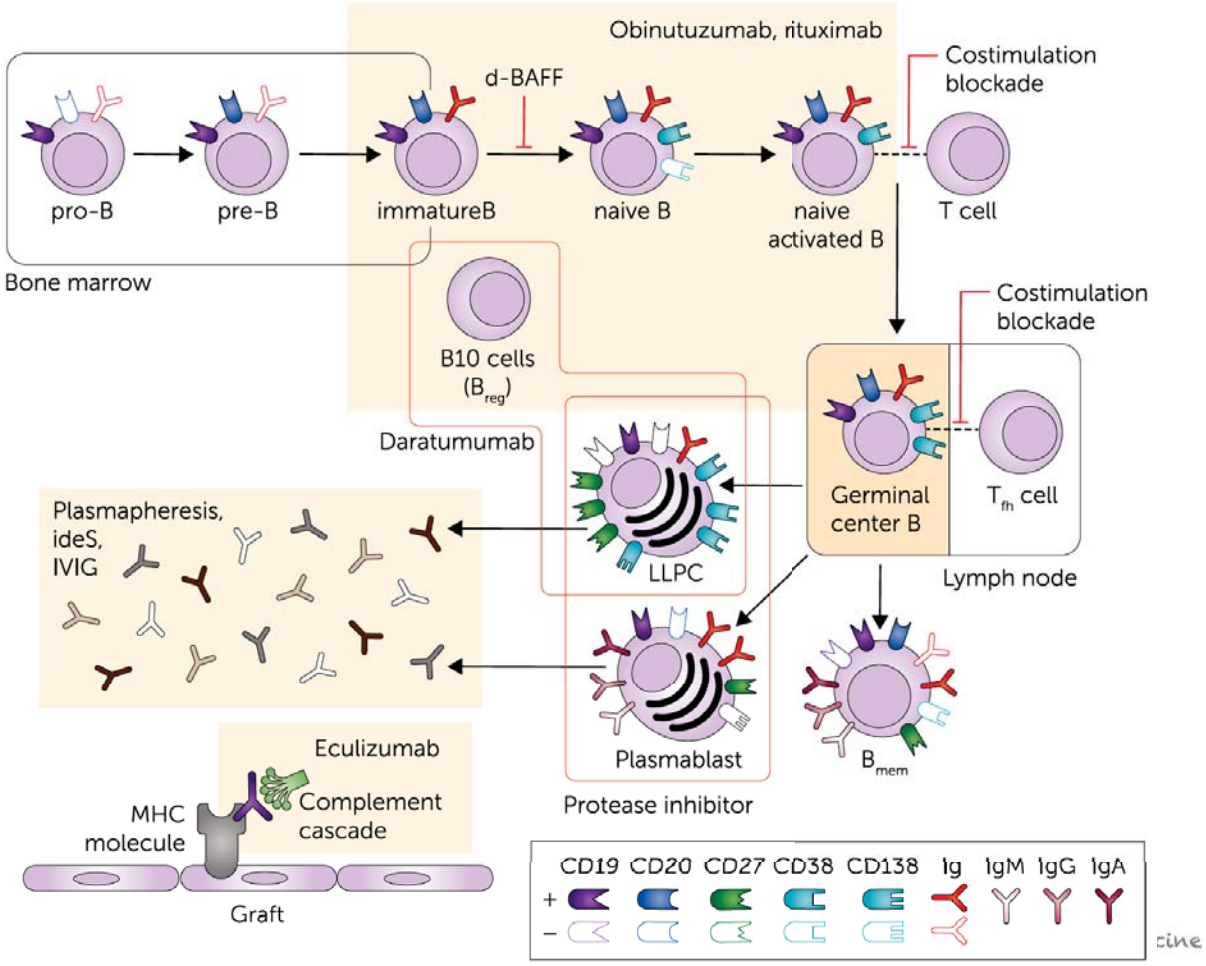
SUMMARY

Our understanding of the role of B cells in organ transplantation remains incomplete and continues to grow. The majority of research has focused on the detrimental role of antibodies that drive the development of pathogenesis of the transplanted organ. However, it has been shown that not all donor-specific antibodies are harmful and in some circumstances can even promote tolerance through the mechanism of accommodation. Furthermore, B cells can have effects on transplanted organs through their interaction with T cells, namely antigen presentation, cytokine production, and costimulation. More recently, the role and importance of Bregs was introduced to the field of transplantation. Due to this functional and ontogenetic heterogeneity, targeting B cells in transplantation may bring undesired immunologic side effects including increased rejection. Therefore, the selective control of B cells that contribute to the humoral response against donor antigens will continue to be an important and challenging area of research and potentially lead to improved long-term transplant outcomes.



Am J Transplant 2019; 19: 368-380.

ORIGINAL ARTICLE

AJT

Value of monitoring circulating donor-reactive memory B cells to characterize antibody-mediated rejection after kidney transplantation

Sergi Luque[1] | Marc Lúcia[1] | Edoardo Melilli[2] | Carmen Lefaucheur[3] | Marta Crespo[4] | Alex Loupy[3] | David Bernal-Casas[1,5] | Montse Gomà[6] | Marta Jarque[1] | Elena Crespo[1] | Núria Montero[2] | Anna Manonelles[2] | Josep M. Cruzado[1,2] | Salvador Gil-Vernet[2] | Josep M. Grinyó[1,2] | Oriol Bestard[1,2]

Department of Renal Transplant Surgery

Aichi Medical University School of Medicine

Liquid biopsy ----

Biomarker for ABMR (2018-2019)

mRNA, miRNA (PBMC, plasma)

- Development and validation of a peripheral blood mRNA assay for the assessment of antibody-mediated kidney allograft rejection: A multicentre, prospective study. Van Loon E, Gazut S, Yazdani S, et al. EBioMedicine. 2019 Aug;46:463-472.
  (BIOMARGIN) study (www.biomargin.eu) 8-gene assay (CXCL10, FCGR1A, FCGR1B, GBP1, GBP4, IL15, KLRC1, TIMP1) in blood samples (PBMC mRNA)
- Development of a multivariable gene-expression signature targeting T-cell-mediated rejection in peripheral blood of kidney transplant recipients validated in cross-sectional and longitudinal samples. Christakoudi S, Runglall M, Mobillo P, et al. EBioMedicine. 2019 Mar;41:571-583
  (KALIBRE) study 22-genes in PBMC mRNA for TCMR (IFNG, IP-10, ITGA4, MARCH8, RORc, SEMA7A, WDR40A)
- Peripheral blood transcriptome analysis and development of classification model for diagnosing antibody-mediated rejection vs accommodation in ABO-incompatible kidney transplant. Jeon HJ, Lee JG, Kim K, Jang JY, Han SW, Choi J, Ryu JH, Koo TY, Jeong JC, Lee JW, Ishida H, Park JB, Lee SH, Ahn C, Yang J. Am J Transplant. 2019 Aug 1.
  PBMC mRNA COX7A2L, CD69, CD14, CFD, and FOXJ3 ABO-i
- The regulation of interferon type I pathway-related genes RSAD2 and ETV7 specifically indicates antibody-mediated rejection after kidney transplantation. Matz M, Heinrich F, Zhang Q, Lorkowski C, Seelow E, Wu K, Lachmann N, Addo RK, Durek P, Mashreghi MF, Budde K. Clin Transplant. 2018 Dec;32(12):e13429.
  PBMC mRNA, IFN type I and II signature gene, .ETV7, RSAD2
- MicroRNA regulation in blood cells of renal transplanted patients with interstitial fibrosis/tubular atrophy and antibody-mediated rejection. Matz M, Heinrich F, Lorkowski C, Wu K, Klotsche J, Zhang Q, Lachmann N, Durek P, Budde K, Mashreghi MF. PLoS One. 2018 Aug 13;13(8):e0201925.
  PBMC miRNA miR-145-5p as IFTA specific marker miR-223-3p, miR-424-3p and miR-145-5p in TCMR and ABMR
- Cell-free microRNA-148a is associated with renal allograft dysfunction: Implication for biomarker discovery. Nariman-Saleh-Fam Z, Bastami M, Ardalan M, et al. J Cell Biochem 2019; 120: 5737-5746.
  Plasma cell-free miRNA-148a correlated with renal function and histological grades

Biomarker for ABMR (2018-2019)

OMICS (urine)

- Urinary proteomics to diagnose chronic active antibody-mediated rejection in pediatric kidney transplantation - a pilot study. Kanzelmeyer NK, Zürbig P, Mischak H, Metzger J, Fichtner A, Ruszai KH, Seemann T, Hansen M, Wygoda S, Krupka K, Tönshoff B, Melk A, Pape L. Transpl Int. 2019 Jan;32(1):28-37
  Urine proteomics identify 79 significant biomarkers CKD273
- Non-invasive staging of chronic kidney allograft damage using urine metabolomic profiling. Landsberg A, Sharma A, Gibson IW, Rush D, Wishart DS, Blydt-Hansen TD. Pediatr Transplant. 2018 Aug;22(5):e13226.
  Urine metabolomics profiling IFTA GS (not ABMR)

Biomarker for ABMR (2018-2019)

Cell free DNA (Blood)

- Diagnostic application of kidney allograft-derived absolute cell-free DNA levels during transplant dysfunction. Whitlam JB, Ling L, Skene A, Kanellis J, Ierino FL, Slater HR, Bruno DL, Power DA. Am J Transplant. 2019 Apr;19(4):1037-1049
  graft-derived cell-free DNA for ABMR.
- Donor-derived Cell-free DNA Identifies Antibody-mediated Rejection in Donor Specific Antibody Positive Kidney Transplant Recipients. Jordan SC, Bunnapradist S, Bromberg JS, Langone AJ, Hiller D, Yee JP, Sninsky JJ, Woodward RN, Matas AJ. Transplant Direct. 2018 Aug 20;4(9):e379.
  Combined use of dd-cfDNA and DSA for ABMR
- Donor-specific Cell-free DNA as a Biomarker in Solid Organ Transplantation. A Systematic Review. Knight SR, Thorne A, Lo Faro ML. Transplantation. 2019 Feb;103(2):273-283
  Discriminatory power of dd-cfDNA was greatest for higher grades of T cell-mediated and antibody-mediated acute rejection, with high negative predictive values.

まとめ

HLA抗体モニタリング（年1回）のポイント

- ✓ ABMR早期診断（腎機能障害の出現しないうちに）
- ✓ 変化があれば再検査
- ✓ 必要時には腎生検で確認、治療を開始
- ✓ 血中濃度、non-adherence チェック、適正な免疫抑制

→ 予後、治療効果の解析