Lecture 2

Antigenicity of HLA-ABC epitopes

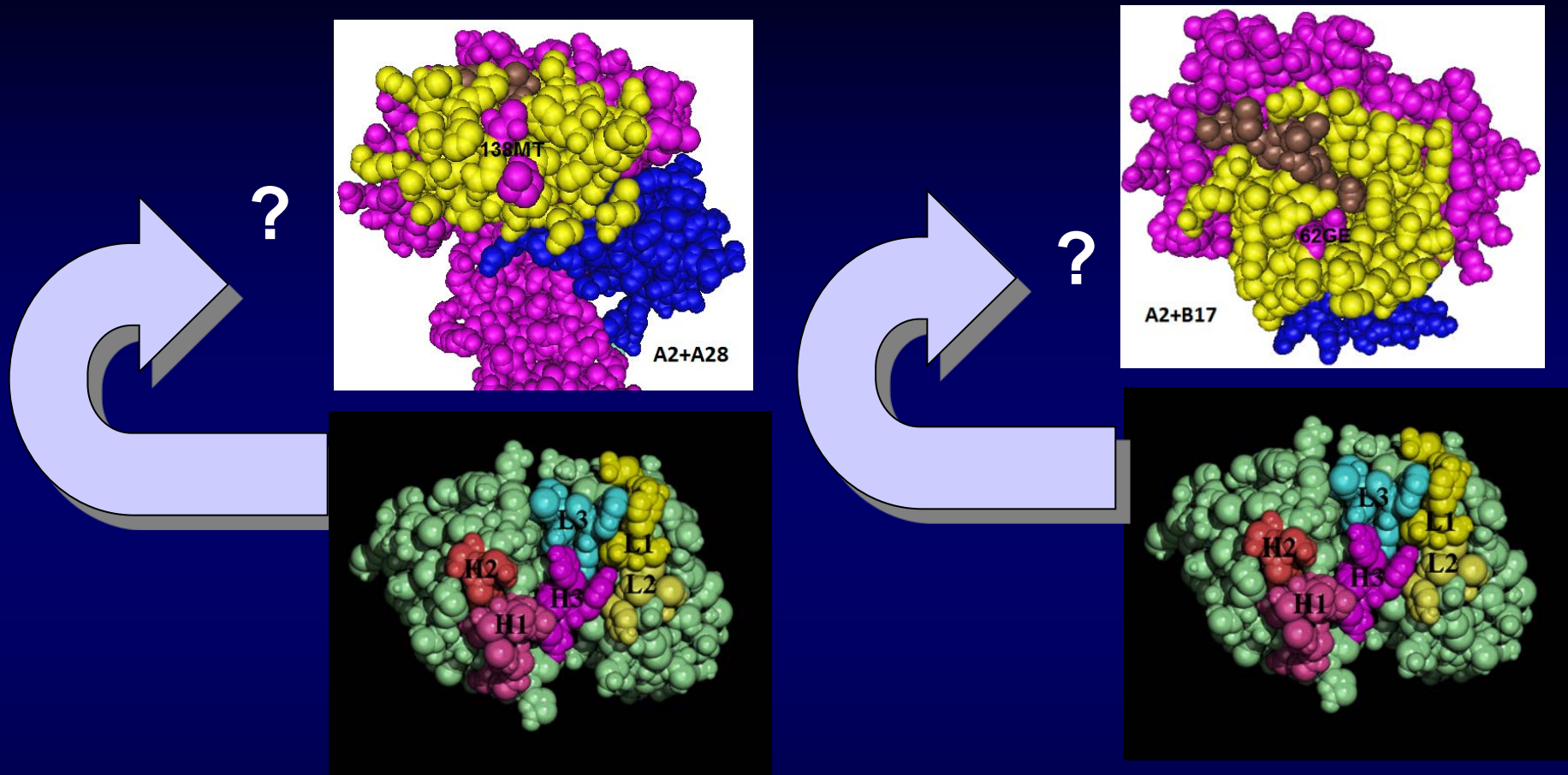Antibody reactivity analysis in different assays

HLA Epitopes

Distinguish between epitope

Antigenicity (reactivity with antibody)

and

Immunogenicity (ability to induce an antibody response)
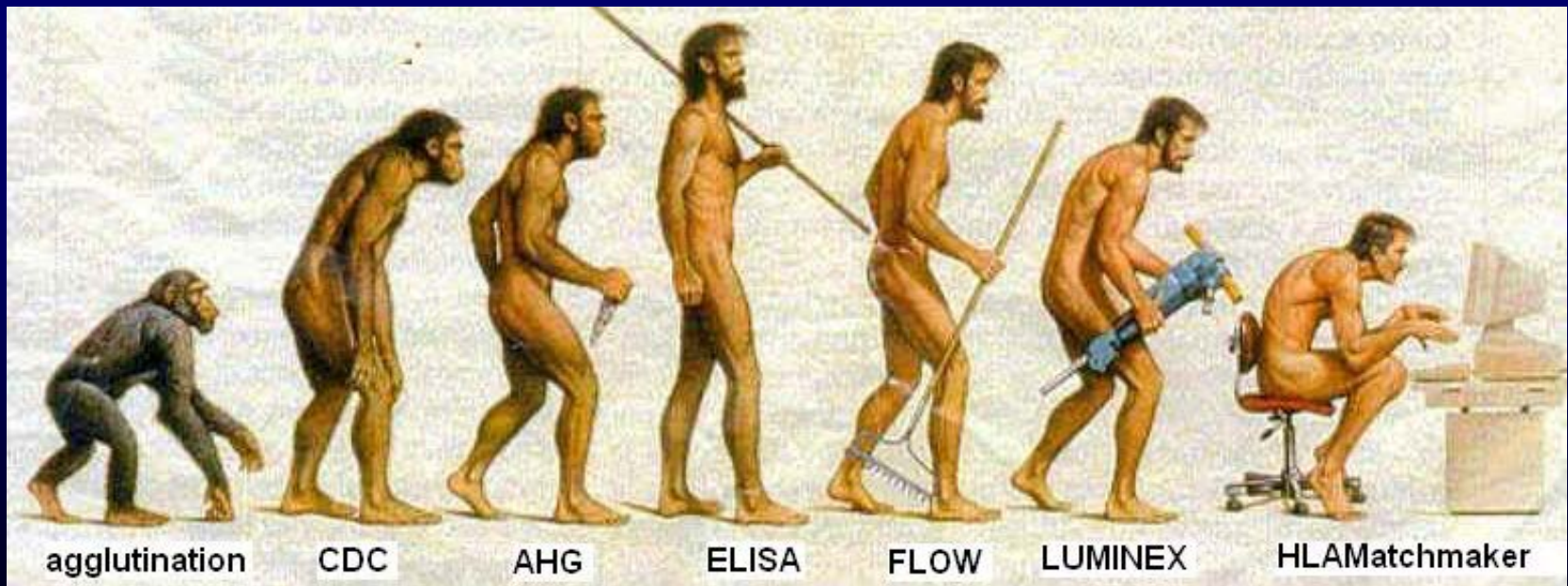
How do antibodies react with HLA epitopes?

Influence of residues in the structural epitope on
1. Binding with antibody
2. Activation of biological activity of antibody

HLA Epitope Antigenicity

Evolution of HLA antibody testing methods

agglutination CDC AHG ELISA FLOW LUMINEX HLAMatchmaker

Serum Testing for HLA Antibodies

Methods in the clinical laboratory

- Ig-binding with Luminex beads coated with single alleles or HLA phenotypes

- C1q- (and C3) -binding (Luminex) with single alleles

- Direct (and AHG-augmented) complement-dependent lymphocytotoxicity with HLA panels and in cross-matching

- Flow cytometry (mostly for cross-matching)

Primary Purpose of Serum Analysis of HLA Antibodies

- Select donors with acceptable HLA mismatches

- Avoid donors with unacceptable HLA mismatches.

QUESTIONS:

- Do we consider mismatch acceptability for: HLA-A,B,C; DRB1/3/4/5; DQA,B; DPA,B; MICA? Which epitopes are important?

But, we may also want to know what epitopes these HLA antibodies recognize

What Do We Need to Know for Epitope-Based Analysis of Serum HLA Antibody Reactivity?

As an example, consider three alleles in a Luminex panel; each one has lots of eplets!

Eplet repertoires of selected alleles

A*24:02 44RME,62EE,63EK,65GK,66K,66KA,66KAH,70H,69AT,70HT,69ATN,71STN,73TD,
73TDE,76EN,76ENR,76ENI,80I,81ALR ,82LR,102DV,105S,109F,127K,138MI,
144K,144TKR,144KA,149AAH,149AH,151H,151HV,156QA,163T,163TD,166DG,
170RY,182TDP,193PI,9S,11SV,94TL,97M,99F,113YH,116Y,152V

A*32:01 44RME,62QE,63ER,63EN,65RN,65RA,66N,66NV,66NAH,70H,69AT,70HT,69ATS,73TD,
73TDE,76ES,76ESI,80I,81ALR ,82LR,102DV,138MI,142ITQ,151RV,156LA,163T,163TE,170RY,
182TDA,193AV,194V,207S,245AS,253Q,9F,11SV,94TI,97M,99Y,113YQ,116D,152V,156L

B*37:01 44RT,63EI,66I,66IS,69TNT,71TTD,73TY,76ED,76ET,80T,80TL,
80TLR,81LLR,82LR,102DV,131S,142ITQ,151RV,156DA,163T,163TE,
170RY,193PI,9H,11SV,24S,94TI,97R,99S,113YN,116F,152V

What Do We Need to Know for Epitope-Based Analysis of Serum HLA Antibody Reactivity?

- HLA type of antibody producer will distinguish between non-self from self eplets

Eplet repertoires of selected alleles

A*24:02	44RME,62EE,63EK,65GK,66K,66KA,66KAH,70H,69AT,70HT,69ATN,71STN,73TD, 73TDE,76EN,76ENR,76ENI,80I,81ALR ,82LR,102DV,105S,109F,127K,138MI, 144K,144TKR,144KA,149AAH,149AH,151H,151HV,156QA,163T,163TD,166DG, 170RY,182TDP,193PI,9S,11SV,94TL,97M,99F,113YH,116Y,152V
A*32:01	44RME,62QE,63ER,63EN,65RN,65RA,66N,66NV,66NAH,70H,69AT,70HT,69ATS,73TD, 73TDE,76ES,76ESI,80I,81ALR ,82LR,102DV,138MI,142ITQ,151RV,156LA,163T,163TE,170RY, 182TDA,193AV,194V,207S,245AS,253Q,9F,11SV,94TI,97M,99Y,113YQ,116D,152V,156L
B*37:01	44RT,63EI,66I,66IS,69TNT,71TTD,73TY,76ED,76ET,80T,80TL, 80TLR,81LLR,82LR,102DV,131S,142ITQ,151RV,156DA,163T,163TE, 170RY,193PI,9H,11SV,24S,94TI,97R,99S,113YN,116F,152V

Patient Type: HLA-A*02:01, A*31:01; B*40:01, B*44:02; C*03:04, C*05:01

Non-self epitopes for this patient

A*24:02	62EE,65GK,69ATN,71STN,73TDE,76ENI,80I,144TKR,156QA,163TD,166DG,9S,99F
A*32:01	69ATS,73TDE,76ESI,80I
B*37:01	44RT,71TTD,76ED,81LLR,24S,99S

What Do We Need to Know for Epitope-Based Analysis of Serum HLA Antibody Reactivity?

- HLA type of antibody producer will distinguish between non-self from self eplets

- HLA type of immunizer will distinguish "donor-specific" eplets

  Patient type: A*02:01,A*31:01; B*40:01, B*44:02; C*03:04, C*05:01

  and has been sensitized by B*51:01

Eplet repertoire of Immunizing allele	
B*51:01	44RT,62RN,62RNQ,66I,66IF,69TNT,71TTN,73TY,76EN,76ENR,76ENI,80I,81ALR, 82LR,102DV,131S,142ITQ,151RE,156LA,163L,163LE,163LW,170RH,193PV,194V, 9Y,11AM,94TW,97T,99Y,113HN,116Y,152E,156L
For patient:	44RT,62RN,62RNQ,66IF,76ENI,80I,170RH,94TW,97T

What Do We Need to Know for Epitope-Based Analysis of Serum HLA Antibody Reactivity?

- HLA type of antibody producer will distinguish between non-self from self epitopes

- HLA type of immunizer will distinguish "donor-specific" eplets

Eplet repertoire of Immunizing allele	
B*51:01	44RT,62RN,62RNQ,66I,66IF,69TNT,71TTN,73TY,76EN,76ENR,76ENI,80I,81ALR,
	82LR,102DV,131S,142ITQ,151RE,156LA,163L,163LE,163LW,170RH,193PV,194V,
	9Y,11AM,94TW,97T,99Y,113HN,116Y,152E,156L
For patient:	44RT,62RN,62RNQ,66IF,76ENI,80I,170RH,94TW,97T

Patient Type:HLA-A*02:01, A*31:01; B*40:01,B*44:02; C*03:04, C*05:01	Donor-Specific
A*24:02 ,62EE,65GK,69ATN,71STN,73TDE,76ENI,80I,144TKR,156QA,163TD,166DG,9S,99F	76ENI,80I
A*32:01 ,69ATS,73TDE,76ESI,80I,	76ESI,80I
B*37:01 ,44RT,71TTD,76ED,81LLR,24S,99S,	44RT

What Do We Need to Know for Epitope-Based Analysis of Serum HLA Antibody Reactivity?

- HLA type of antibody producer will distinguish between non-self from self epitopes

- The immunizing event: HLA type of immunizer will distinguish "donor-specific" from "third-party" (irrelevant?) epitopes

- IMPORTANT CONCEPT: Antibody specificity patterns are restricted to small numbers of epitopes

- Luminex assays with single allele panels

  – Is the panel informative enough? Need for absorption/elution studies?

  – How do we interpret MFI values?

MFI Values in Luminex Assays

- High MFI values comparable to positive controls indicate positive reactions

- Extremely low MFI values comparable to those with self alleles of the antibody producer and negative controls indicate negative reactions.

How do we determine cut-off values?

MFI>100? MFI>500?   MFI>1000? MFI>3000? Etc.

Classify MFI Values in Single Allele Bead Assays of Monospecific Antibodies

MFI	Description	Reactivity
High	Comparable to Positive Control	Positive
Intermediate	Significantly lower than the Positive Control	Positive
Low	Low but significantly higher than the MFI of self alleles of the antibody producer	Weakly Positive
Very Low	Comparable to the MFI of self alleles and the Negative Control	Borderline or Negative?

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Low	Low but significantly higher than the MFI of self alleles of the antibody producer	Weakly Positive
Very Low	Comparable to the MFI of self alleles and the Negative Control	Borderline or Negative?

Important considerations:
Some alleles in a SAB kit may have non-HLA specific reactivity
Some alleles might generally be weakly reactive

MFI Values in Luminex Assays

- High MFI values comparable to positive controls indicate positive reactions

- Extremely low MFI values comparable to those with self alleles of the antibody producer and negative controls indicate negative reactions

- Suggestion: *Use MFI values with self-alleles to determine cut-off values for positive and negative reactions*

- Hypothesis: **Certain eplet-carrying alleles have low MFI values because they have significant amino acid differences with the structural epitope of the immunizing allele**

82LR-Defined Epitope (Bw4)

- 82LR-carrying antigens:

A23,A24,A25,A32,B13,B27(not B*27:08),
B37,B38,B44,B47,B49,B51,B52,
B53,B57,B58,B59,B63,B77

Antibody-Verified 82LR Epitope

PANEL		OL MFI	GP MFI
	Positive control	14108	10434
	Negative control	56	633
	82LR+ A*23:01	24030	nt
	82LR+ A*23:02	nt	9730
Immunizing allele:	82LR+ A*24:02	23808	17620
	82LR+ A*24:03	24023	18614
	82LR+ A*25:01	24414	11838
	82LR+ A*32:01	24388	13852
	82LR+ B*13:01	23778	nt
	82LR+ B*13:02	23502	14792
	82LR+ B*15:13	23694	8964
	82LR+ B*15:16	24007	17645
	82LR+ B*27:03	nt	13390
	82LR+ B*27:05	24075	18546
	82LR+ B*37:01	24100	18629
	82LR+ B*38:01	23888	13537
	82LR+ B*44:02	22433	6969
	82LR+ B*44:03	23884	12520
	82LR+ B*47:01	20840	15903
	82LR+ B*49:01	23951	13574
	82LR+ B*51:01	23535	13662
	82LR+ B*51:02	24112	nt
	82LR+ B*52:01	23723	10645
	82LR+ B*53:01	24202	15858
	82LR+ B*57:01	24109	15853
	82LR+ B*57:03	24064	nt
	82LR+ B*58:01	23576	18862
	82LR+ B*59:01	22231	nt
	Self Alleles	30 ± 3	470 ± 30
	Other 82LR-negative Alleles	41 ± 19	516 ± 154

Antibody-Verified 82LR+self145RA-Defined Epitope

	PANEL	OL MFI	GP MFI
	Positive control	nt	nt
	Negative control	1	7
82LR+/145RA+	A*23:01	11511	5602
82LR+/145RA+	A*24:02	11391	5936
82LR+/145RA+	A*24:03	9895	5636
82LR+/145RA+	A*32:01	11340	7712
82LR+/145RA+	B*15:13	1118	26
82LR+/145RA+	B*15:16	2894	6108
82LR+/145RA+	B*27:03	nt	4167
82LR+ /145RA+	B*27:05	11414	9349
Immunizing allele: 82LR+/145RA+	B*37:01	9240	11610
82LR+/145RA+	B*38:01	10268	1738
82LR+/145RA+	B*44:02	9742	7141
82LR+/145RA+	B*44:03	9111	6991
82LR+/145RA+	B*47:01	6775	5191
82LR+/145RA+	B*49:01	2794	3528
82LR+/145RA+	B*51:01	5765	160
82LR+/145RA+	B*51:02	7305	nt
82LR+/145RA+	B*52:01	3093	240
82LR+/145RA+	B*53:01	13127	4126
82LR+/145RA+	B*57:01	6650	6882
82LR+/145RA+	B*57:03	10143	nt
82LR+/145RA+	B*58:01	5537	9431
82LR+/145RA+	B*59:01	10178	nt
82LR+	A*25:01	2	13
82LR+	B*13:01	7	nt
82LR+	B*13:02	4	7
	Self Alleles	8 ± 6	9 ± 4
	Other 82LR-negative Alleles	160 ± 650	37 ± 149

Antibody-Verified 82LR-Defined Epitopes recorded in the International Registry

Epitope	Reactive 82LR-carrying antigens	Nonreactive 82LR- carrying epitopes
82LR	All	None
82LR+90A	All, except A25	82LR+90D (A25)
82LR+144QR	All, except A24 and B13	82LR+144KR (A24) 82LR+144QL (B13)
82LR+145R	All, except B13	82LR+145L (B13)
82LR+145RA	All, except A25 and B13	82LR+145RT (A25) 82LR+145LA (B13)
82LR+138T	Only HLA-B antigens	82LR+138M
82LR+138M	Only HLA-A antigens	82LR+138T
80I+65QI	B38,B49,B51,B52,B53,B59,B77	80I+65GK (A23,A24) 80I+65RN (A25,A32,B57,B58,B63)
80I+90A	A23,A24,A32,B38,B49,B51,B52,B53, B57,B58,B59,B63,B77	80I+90D
80I+151RE	B49,B51,B52,B59,B77	80I+151HV (A24,A25) 80I+151RV (A23,A32,B38,B53,B57,B58)

Antibody-Verified 82LR-Defined Epitopes (contd.)

Epitope	Reactive 82LR-carrying antigens	Nonreactive 82LR- carrying epitopes
82LR	All	None
82LR+90A	All, except A25	82LR+90D (A25)
82LR+144QR	All, except A24 and B13	82LR+144KR (A24) 82LR+144QL (B13)
82LR+145R	All, except B13	82LR+145L (B13)
82LR+145RA	All, except A25 and B13	82LR+145RT (A25) 82LR+145LA (B13)
82LR+138T	Only HLA-B antigens	82LR+138M
82LR+138M	Only HLA-A antigens	82LR+138T
80I+65QI	B38,B49,B51,B52,B53,B59,B77	80I+65GK (A23,A24) 80I+65RN (A25,A32,B57,B58,B63)
80I+90A	A23,A24,A32,B38,B49,B51,B52,B53, B57,B58,B59,B63,B77	80I+90D
80I+151RE	B49,B51,B52,B59,B77	80I+151HV (A24,A25) 80I+151RV (A23,A32,B38,B53,B57,B58)
80T (80TLR)	B13,B27,B37,B44,B47	
80I (80ILR)	A23,A24,A25,A32,B38,B49,B51,B52, B53,B57,B58,B59,B63,B77	

Effect of Position 80 Residue on 82LR Epitope

Allele	Eplet	Residue	Case 13	Case 14	Case 15	Case 16
A*23:01	82LR	80I	10483	11634	2609	12601
A*24:02	82LR	80I	8916[IM]	11190[IM]	3760	14744
A*24:03	82LR	80I	8966	11210	3633	14899
A*25:01	82LR	80I	11850	13917	8024	8041
A*32:01	82LR	80I	11570	12411	5050	13091
B*15:13	82LR	80I	8736	12519	4490	6132
B*15:16	82LR	80I	10384	14057	4836	6967
B*38:01	82LR	80I	12522	14977	7165	11071
B*49:01	82LR	80I	11240	11228	6682	13172[IM]
B*51:01	82LR	80I	8448	13744	5500[IM]	6403
B*51:02	82LR	80I	11258	14186	7297	9178
B*52:01	82LR	80I	10274	14728	6674	7570
B*53:01	82LR	80I	10994	14370	5699	9049
B*57:01	82LR	80I	10461	13501	3217	9968
B*57:03	82LR	80I	10132	13559	2969	9236
B*58:01	82LR	80I	8695	13245	2380	8115
B*59:01	82LR	80I	8664	14135	5093	7745
		Mean+SD	10211+1275	13212+1272	5004+1751*	9881+2866'
B*13:01	82LR	80T	11531	7171	670	700
B*13:02	82LR	80T	12031	12628	1081	2651
B*27:05	82LR	80T	7684	12074	779	3690
B*37:01	82LR	80T	6007	12041	1062	2251
B*44:02	82LR	80T	12112	11722	623	1753
B*44:03	82LR	80T	12149	11861	685	1860
B*47:01	82LR	80T	9968	7935	326	605
		Mean+SD	10212+2468	10776+2231	747+263*	1930+1082'
Self Alleles			67	35	13	36

[IM] MFI with immunizing allele   * p< 0.0001   ' P < 0.00001

Antibody-Verified 80I-Defined Epitope

	PANEL	OL MFI	GP MFI
	Positive control	10446	10086
	Negative control	4	312
	80I+ A*23:01	9066	nt
	80I+ A*23:02	nt	1431
	80I+ A*24:02	9779	8607
	80I+ A*24:03	10706	9080
	80I+ A*25:01	13356	3883
	80I+ A*32:01	13720	6727
	80I+ B*15:13	13071	4570
	80I+ B*15:16	18064	14917
	80I+ B*38:01	15974	7947
Immunizing allele:	80I+ B*49:01	13463	11612
	80I+ B*51:01	18003	12009
	80I+ B*51:02	20161	nt
	80I+ B*52:01	16044	8572
	80I+ B*53:01	20423	11916
	80I+ B*57:01	7354	9042
	80I+ B*57:03	13060	nt
	80I+ B*58:01	7538	13222
	80I+ B*59:01	16391	nt
	Self Alleles	8 ± 7	332 ± 34
	Other 80I-negative Alleles	14 ± 20	352 ± 220

Antibody-Verified 82LR-Defined Epitopes (contd.)

Epitope	Reactive 82LR-carrying antigens	Nonreactive 82LR- carrying epitopes
82LR	All	None
82LR+90A	All, except A25	82LR+90D (A25)
82LR+144QR	All, except A24 and B13	82LR+144KR (A24) 82LR+144QL (B13)
82LR+145R	All, except B13	82LR+145L (B13)
82LR+145RA	All, except A25 and B13	82LR+145RT (A25) 82LR+145LA (B13)
82LR+138T	Only HLA-B antigens	82LR+138M
82LR+138M	Only HLA-A antigens	82LR+138T
80I+65QI	B38,B49,B51,B52,B53,B59,B77	80I+65GK (A23,A24) 80I+65RN (A25,A32,B57,B58,B63)
80I+90A	A23,A24,A32,B38,B49,B51,B52,B53, B57,B58,B59,B63,B77	80I+90D
80I+151RE	B49,B51,B52,B59,B77	80I+151HV (A24,A25) 80I+151RV (A23,A32,B38,B53,B57,B58)
80T (80TLR)	B13,B27,B37,B44,B47	
80I (80ILR)	A23,A24,A25,A32,B38,B49,B51,B52, B53,B57,B58,B59,B63,B77	

Molecular model of the 82LR-defined structural epitope and locations of residues that can play a critical role in determining reactivity with antibodies specific for eplet pairs involving 82LR

CDRs of Antibody
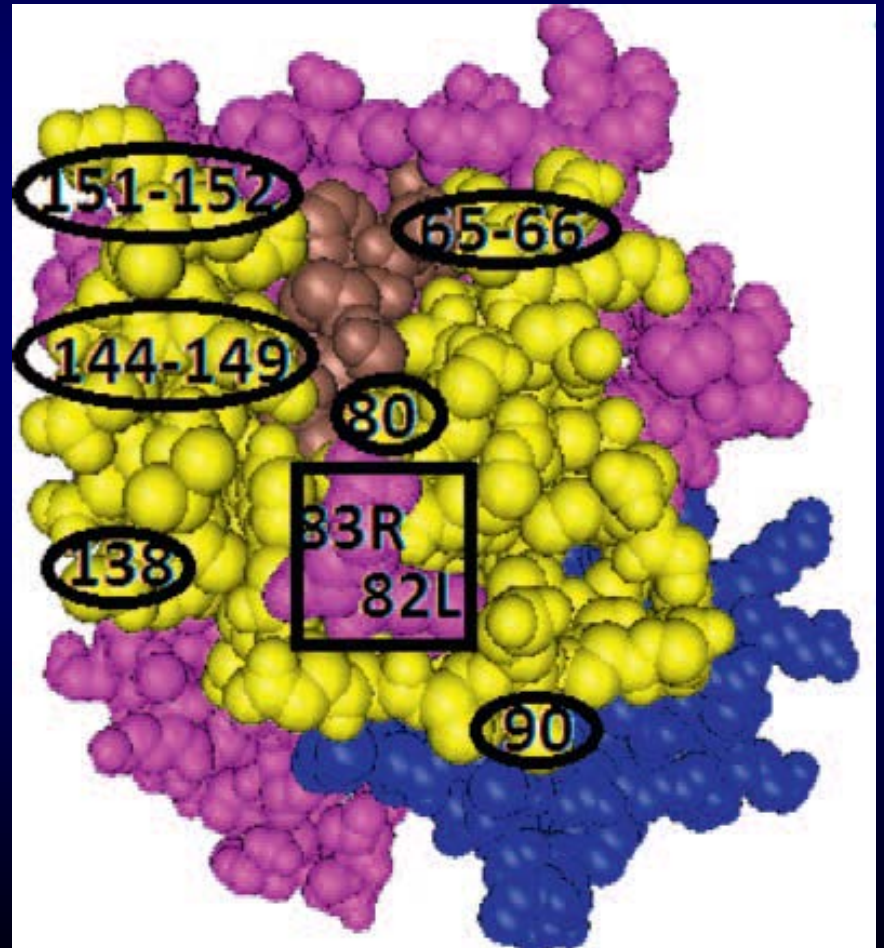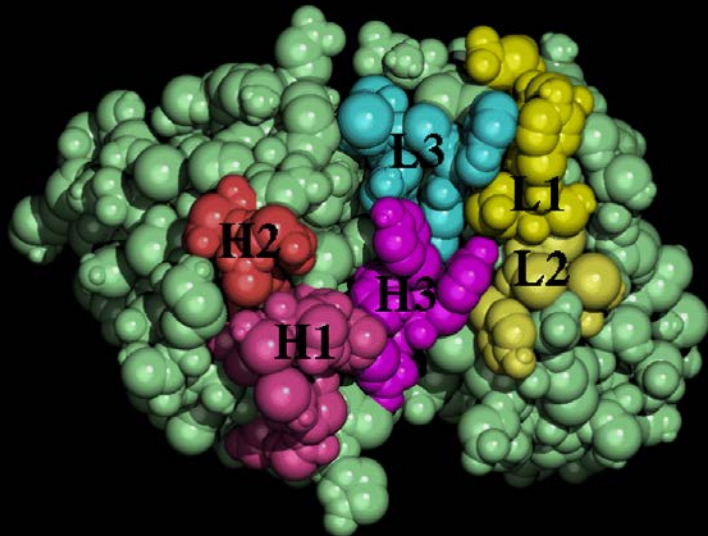
Antibody-Verified Epitope Defined by nonself 65RN + self 80I

	PANEL	OL MFI	GP MFI
	Positive control	10144	10718
	Negative control	10	334
65RN+/80I+	A*25:01	20769	8715
Immunizing allele: 65RN+/80I+	A*32:01	20157	12337
65RN+/80I+	B*15:16	18565	14272
65RN+/80I+	B*57:01	10931	8210
65RN+/80I+	B*57:03	15821	nt
65RN+/80I+	B*58:01	10006	12586
65RN+	A*01:01	4	355
65RN+	A*03:01	18	287
65RN+	A*11:01	5	302
65RN+	A*11:02	6	321
65RN+	A*26:01	7	334
65RN+	A*29:01	7	nt
65RN+	A*29:02	7	353
65RN+	A*30:01	5	295
65RN+	A*30:02	2045	nt
65RN+	A*31:01	5	308
65RN+	A*33:01	8	335
65RN+	A*33:03	7	337
65RN+	A*34:01	7	nt
65RN+	A*34:02	4	323
65RN+	A*36:01	7	353
65RN+	A*43:01	8	342
65RN+	A*66:01	8	316
65RN+	A*66:02	6	310
65RN+	A*68:01	9	296
65RN+	A*68:02	11	368
65RN+	A*69:01	7	324
65RN+	A*74:01	8	327
65RN+	A*80:01	7	383
	Self Alleles	12 ± 5	335 ± 53
	Other 65RN-negative Alleles	14 ± 7	351 ± 53

# Antibody-Verified 80N-Defined Epitope (Bw6-Associated)

PANEL	OL MFI	GP MFI
Positive control	nt	nt
Negative control	3	9
Immunizing allele: 80N+ B*07:02	11198	12614
80N+ B*27:08	13714	12826
80N+ B*42:01	13143	12983
80N+ B*54:01	10529	12460
80N+ B*55:01	9731	13270
80N+ B*56:01	10605	12283
80N+ B*67:01	13359	nt
80N+ B*81:01	13838	11977
80N+ B*82:01	12309	nt
80N+ B*82:02	nt	12730
80N+ B*07:03	nt	4897
80N+ B*08:01	12887	11516
80N+ B*14:01	10326	nt
80N+ B*14:02	8687	nt
80N+ B*14:05	nt	5722
80N+ B*14:06	nt	6095
80N+ B*15:01	12789	12499
80N+ B*15:02	13353	12381
80N+ B*15:03	12632	12080
80N+ B*15:10	10698	nt
80N+ B*15:11	11858	nt
80N+ B*15:12	13071	12524
80N+ B*15:18	nt	10147
80N+ B*18:01	13420	12593
80N+ B*35:01	13664	11553

| PANEL	OL MFI	GP MFI
80N+ B*35:08	nt	12407
80N+ B*39:01	11589	12059
80N+ B*40:01	7473	11123
80N+ B*40:02	10877	12418
80N+ B*40:06	9864	nt
80N+ B*41:01	12380	9641
80N+ B*45:01	12475	11142
80N+ B*48:01	11598	7559
80N+ B*50:01	11299	7840
80N+ B*78:01	12261	11442
80N+ B*73:01	10682	3975
80N+ B*46:01	10585	4951
80N+ C*01:02	9042	5616
80N+ C*03:02	9146	nt
80N+ C*03:03	9713	7689
80N+ C*03:04	12024	8531
80N+ C*07:01	nt	5272
Immunizing allele: 80N+ C*07:02	8880	5021
80N+ C*08:01	6918	9345
80N+ C*08:02	nt	8966
80N+ C*12:02	nt	10781
80N+ C*12:03	6939	nt
80N+ C*14:02	8652	8680
80N+ C*16:01	7068	1046
Self alleles	347 ± 283	10 ± 4
Other 80N-negative alleles	188 ± 262	12 ± 5

162LW Eplet

On HLA-B*13:04, *15:01, *15:02, *15:03, *15:04, *15:05, *15:07, *15:08, *15:09, *15:10, *15:11, *15:13, *15:16, *15:17, *15:18, 15:20, *15:21, *15:23, *15:24, *15:25, *15:27, *15:29, *15:30, *15:31, *15:32, *15:35, *15:39, *15:65, *15:70, *35:01, *35:02, *35:03, *35:05, *35:08, *35:10, 35:12, *35:17, *35:19, *35:20, *35:23, *46:01, *49:01, *49:03, *50:01, *51:01, *51:02, *51:04, *51:06, *51:07, *51:08, *51:24, *51:28, *52:01, *53:01, *53:06, *55:08, *56:01, *56:02, *56:03, *56:04, *56:05, *56:07, *57:01, *57:02, *57:03, *57:04, *58:01, *58:02, HLA-C*03:02, C*03:03, C*03:04

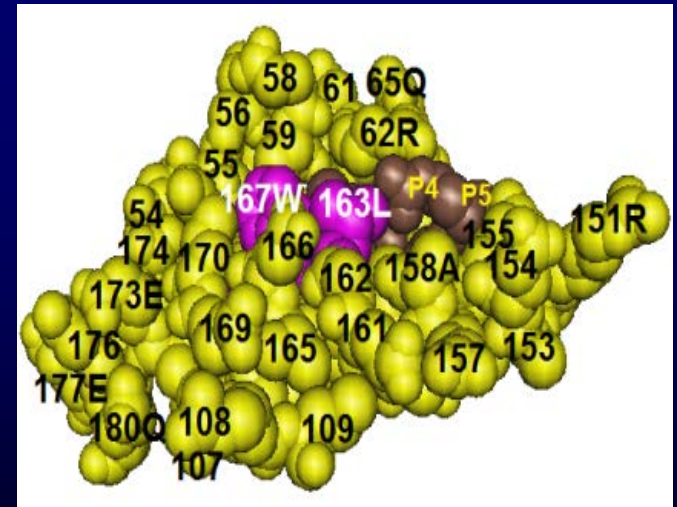# Surface residues within 15 Ångstroms of the 163LW eplet

163LW on B*35:01
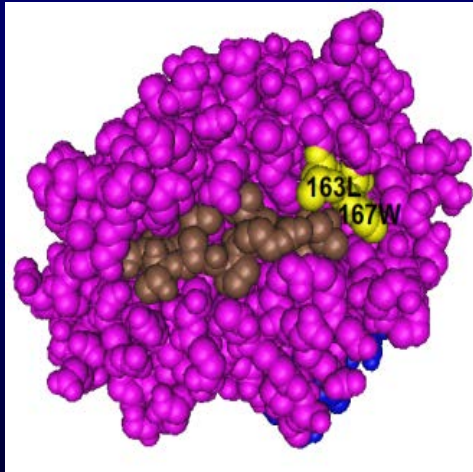
Top view
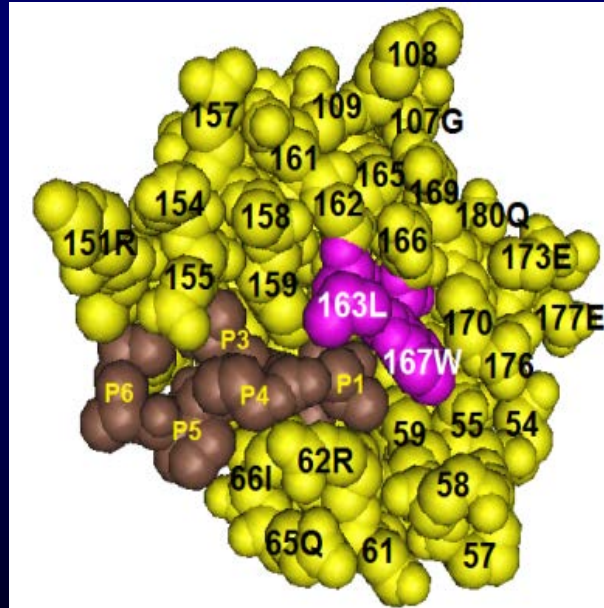
Side view

Polymorphic residue positions are shown with standard single letter amino acid code

Human Monoclonal Antibody* OK6H12 Defined 163LW Epitope

OK6H12	Eplet:	**163LW**		Polymorphic surface residues within 15 Å of 163LW								
Sequence position		163	167	62	65	66	107	109	151	173	177	180
IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q

Human Monoclonal Antibody* OK6H12 Defined 163LW Epitope

OK6H12	Eplet:	163LW		Polymorphic surface residues within 15 Å of 163LW								
Sequence position		163	167	62	65	66	107	109	151	173	177	180
IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q
OL MFI	PANEL											
8125	B*15:01	L	W	-	-	-	-	-	-	-	-	-
8972	B*15:02	L	W	-	-	-	-	-	-	-	-	-
7638	B*15:03	L	W	-	-	-	-	-	-	-	-	-
8241	B*15:10	L	W	-	-	-	-	-	-	-	-	-
7300	B*15:13	L	W	-	-	-	-	-	-	-	-	-
8625	B*35:01	L	W	-	-	-	-	-	-	-	-	-
6438	B*49:01	L	W	-	-	-	-	-	-	-	-	-
9862	B*50:01	L	W	-	-	-	-	-	-	-	-	-
7292	B*53:01	L	W	-	-	-	-	-	-	-	-	-
6252	B*56:01	L	W	-	-	-	-	-	-	-	-	-
1903	B*51:01	L	W	-	-	-	-	-	-	-	-	-
8339	B*51:02	L	W	-	-	-	-	-	-	-	-	-
3899	B*52:01	L	W	-	-	-	-	-	-	-	-	-
3620	B*78:01	L	W	-	-	-	-	-	-	-	-	-
3702	B*57:01	L	W	G	R	N	-	-	-	-	-	-
3087	B*57:03	L	W	G	R	N	-	-	-	-	-	-
3554	B*58:01	L	W	G	R	N	-	-	-	-	-	-
2055	B*15:16	L	W	-	-	N	-	-	-	-	-	-
111	B*46:01	L	W	-	-	K	-	-	-	-	-	-
796	C*03:02	L	W	-	-	K	-	-	-	K	-	-
438	C*03:03	L	W	-	-	K	-	-	-	K	-	-
554	C*03:04	L	W	-	-	K	-	-	-	K	-	-
10 ± 4	Self alleles											
10 ± 5	163LW-negative alleles											

OK6H12 and VD1F11 Defined 163LW Epitopes

VD1F11	OK6H12	Eplet:	**163LW**		Polymorphic surface residues within 15 Å of 163LW								
	Sequence position		163	167	62	65	66	107	109	151	173	177	180
Unknown	IMM	B*15:03	L	W	R	Q	I	G	L	R	E	E	Q
OL MFI	OL MFI	PANEL											
8362	8125	B*15:01	L	W	-	-	-	-	-	-	-	-	-
7226	8972	B*15:02	L	W	-	-	-	-	-	-	-	-	-
6474	7638	B*15:03	L	W	-	-	-	-	-	-	-	-	-
8361	8241	B*15:10	L	W	-	-	-	-	-	-	-	-	-
5416	7300	B*15:13	L	W	-	-	-	-	-	-	-	-	-
7244	8625	B*35:01	L	W	-	-	-	-	-	-	-	-	-
4577	6438	B*49:01	L	W	-	-	-	-	-	-	-	-	-
7812	9862	B*50:01	L	W	-	-	-	-	-	-	-	-	-
6010	7292	B*53:01	L	W	-	-	-	-	-	-	-	-	-
5275	6252	B*56:01	L	W	-	-	-	-	-	-	-	-	-
5586	1903	B*51:01	L	W	-	-	-	-	-	-	-	-	-
7879	8339	B*51:02	L	W	-	-	-	-	-	-	-	-	-
2049	3899	B*52:01	L	W	-	-	-	-	-	-	-	-	-
5547	3620	B*78:01	L	W	-	-	-	-	-	-	-	-	-
3749	3702	B*57:01	L	W	G	R	N	-	-	-	-	-	-
3749	3087	B*57:03	L	W	G	R	N	-	-	-	-	-	-
2783	3554	B*58:01	L	W	G	R	N	-	-	-	-	-	-
5480	2055	B*15:16	L	W	-	-	N	-	-	-	-	-	-
5172	111	B*46:01	L	W	-	-	K	-	-	-	-	-	-
8056	796	C*03:02	L	W	-	-	K	-	-	-	K	-	-
7453	438	C*03:03	L	W	-	-	K	-	-	-	K	-	-
7532	554	C*03:04	L	W	-	-	K	-	-	-	K	-	-
19 ± 10	10 ± 4	Self alleles											
101 ± 262*	10 ± 5	163LW-negative alleles											

* Includes A*11:02=416, A*66:01=1299, C*06:02=566, C*08:01=1127, C*12:03=1195 and C*14:02=455

* All human mAbs were generated by Arend Mulder, Leiden, The Netherlands)

Structural Epitope-Based Interpretations of MFI Values of Single Allele Beads Reacting with Monospecific Antibodies

MFI	Description	Comparison with immunizing allele
High	Comparable to Positive Control	The eplet-carrying allele has a similar structural epitope or there are residue differences that are not important
Intermediate	Significantly lower than the Positive Control	The structural epitope of the eplet-carrying allele may have some residue differences but they have a minor effect on antibody binding
Low	Low but significantly higher than the MFI of self alleles of the antibody producer	The structural epitope of the eplet-carrying allele has certain residues that interfere with efficient binding but the reaction must still be considered positive
Very Low	Comparable to the MFI of self alleles and the Negative Control	The structural epitope of the eplet-carrying allele lacks critical residues necessary for binding OR the allele lacks the eplet recognized by antibody

Peptide-Dependent HLA Epitopes
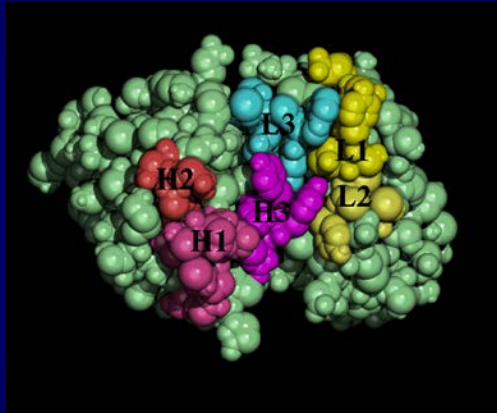
CDRs of Antibody
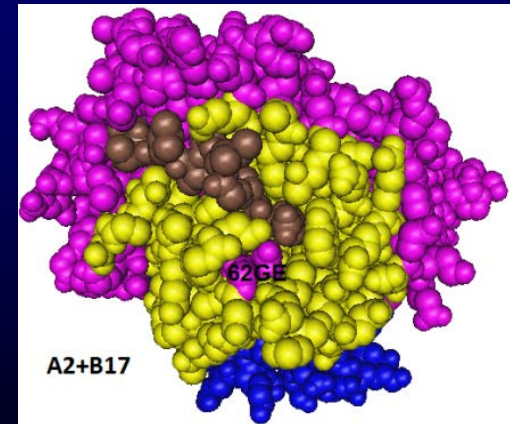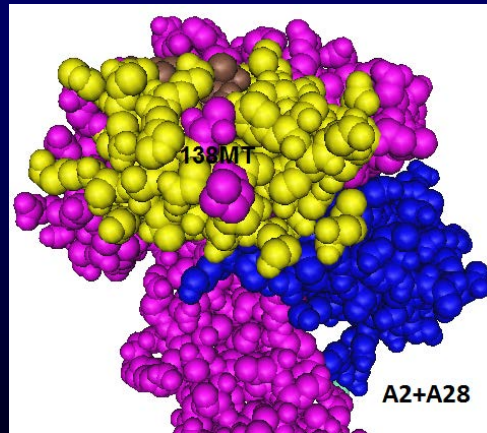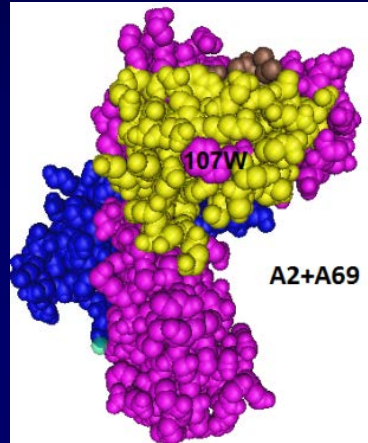
Peptide-Dependent HLA-Specific Antibodies

- Barouch D, Davenport M, McMichael A and Reay P. International Immunology. 7:1599-605, 1995
  - Mouse mAb MA2.1 specific for HLA-A2+B17
  - Very strong binding with HLA-A2 complexed with an HIV peptide SLYNTVATL
  - Moderate binding with HLA-A2 complexed with SLYNTVA**A**L
  - Very low reactivity with HLA-A2 complexed with TLWVDPYEV.

- Wang, J., D. T. Yu, T. Fukazawa, H. Kellner, J. Wen, X. K. Cheng, G. Roth, K. M. Williams, and R. B. Raybourne. 1994. A monoclonal antibody that recognizes HLA-B27 in the context of peptides. *J. Immunol.* 152: 1197–1205

Impact of Peptides on the Recognition of HLA Class I Molecules by Human HLA Antibodies

Arend Mulder, Chantal Eijsink, Michel G. D. Kester, Marry E. I. Franke,
Marrie J. Kardol, Mirjam H. M. Heemskerk, Cees van Kooten, Frank A. Verreck,

Jan Wouter Drijfhout, Frits Koning, Ilias I. N. Doxiadis, and Frans H. J. Claas

Department of Immunohematology and Bloodtransfusion, Department of Experimental
Hematology, and Department of Nephrology, Leiden University Medical Center, Leiden,
The Netherlands

Antibodies Specific for the 62GE Epitope

HLA-A2, B57 and B58 share a well-defined epitope
which corresponds to the 62GE eplet on the $\alpha 1$ helix
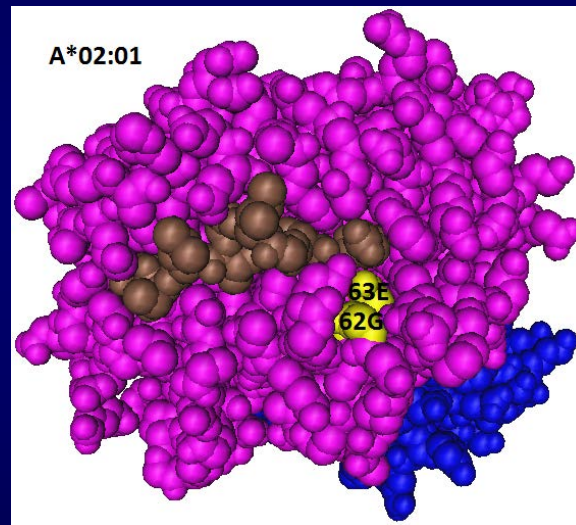
Structural 62GE Epitope
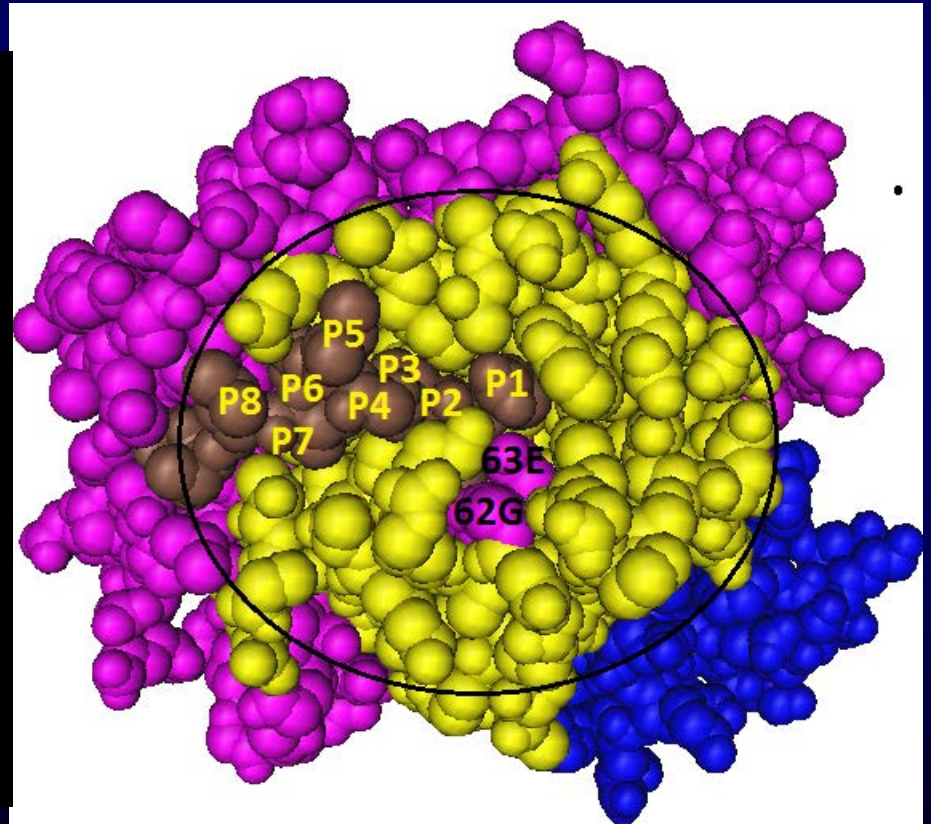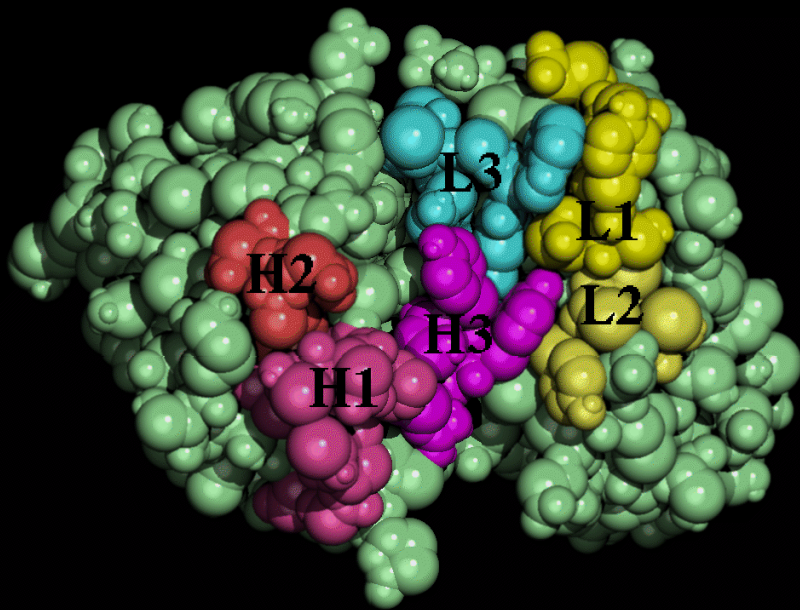
HLA-A2 Loaded with Different Peptides

Peptide	Gene	Amino Acid Sequence
HY	SMCY	FIDSYICQV
PRA	ME	SLYSFPEPEA
HA-1h	KIAA0223	VLHDDLLEA
HA-2	MYO1G	YIGEVLVSV
IB.54	Insulin	HLVEALYLV
HPV	E7 12–20	MLDLQPETT
EII49	FASN	FLFDGSPTYV
EBV	BMLF-1	GLCTLVAML
Flue IMP	Matrix 58–66	GILGFVFTL
hCMV	Pp65	NLVPMVATV
HIV	Pol and RT2	ILKEPVHGV
PR-1	Proteinase-3	VLQELNVTV

Studies by **Arend Mulder** et al. J Immunol. 2005; 175: 5950

62GE-Specific Human Monoclonals

Studies by **Arend Mulder** et al. J Immunol. 2005; 175: 5950
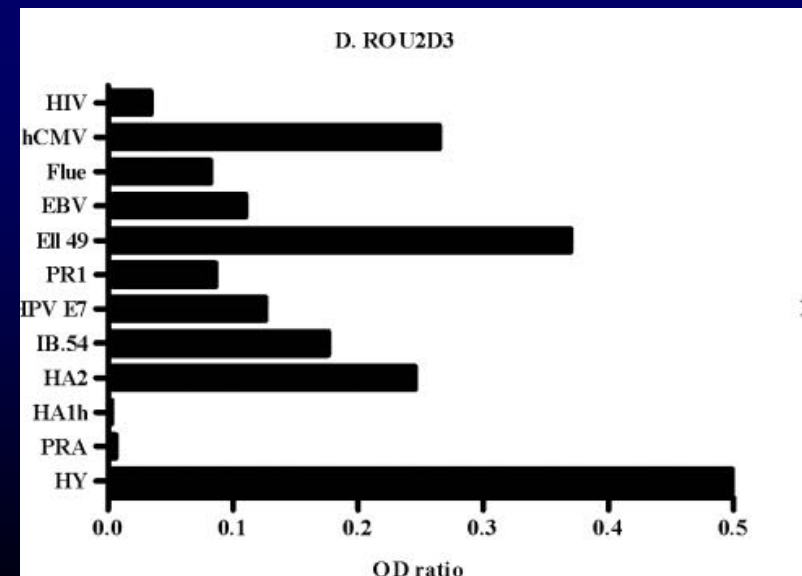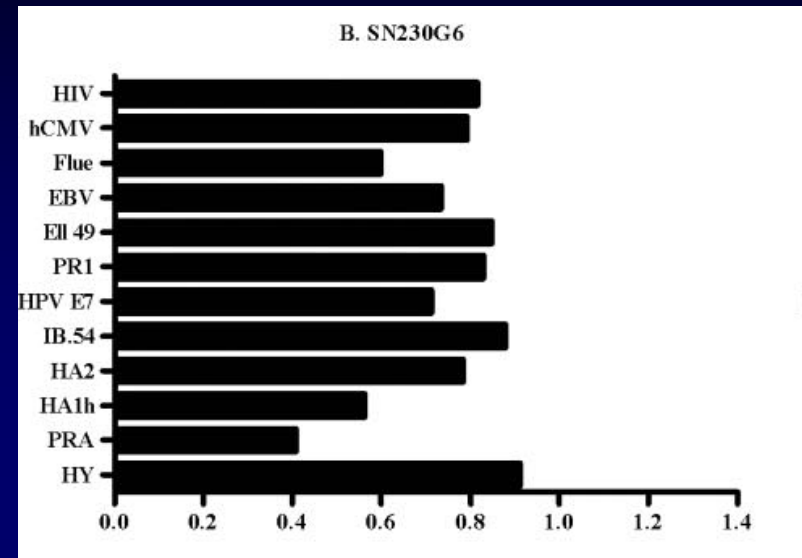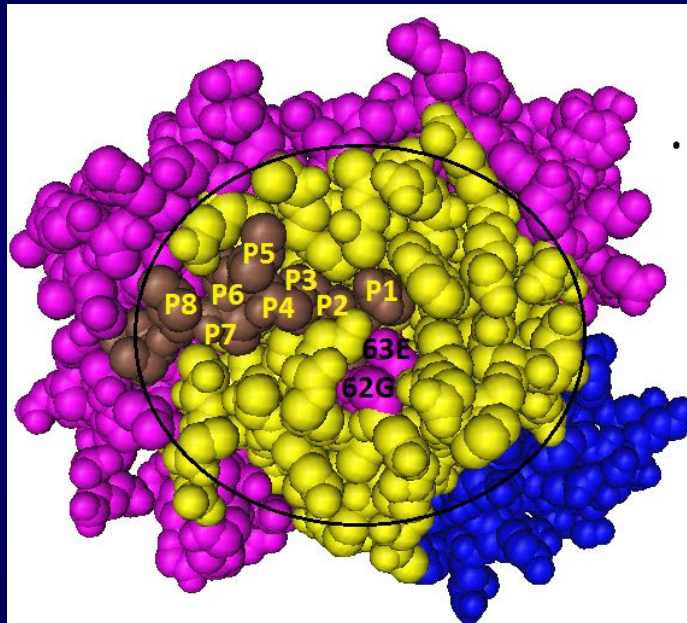
Reactivity of a 163EW-Specific Human Monoclonal Antibody
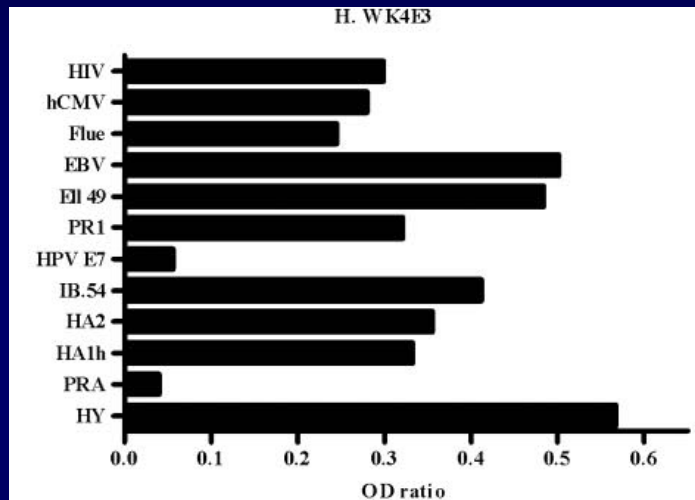
Peptide	Reactivity	Sequence
HY	0.567	FIDSYICQV
HA-1h	0,333	VLHDDLLEA
HA-2	0,355	YIGEVLVSV
IB.54	0.412	HLVEALYLV
EII49	0,484	FLFDGSPTYV
EBV	0.501	GLCTLVAML
Flue IMP	0.245	GILGFVFTL
hCMV	0.280	NLVPMVATV
HIV	0.299	ILKEPVHGV
PR-1	0.321	VLQELNVTV
HPV	**0,056**	MLDLQ**PE**TT
PRA	**0.040**	SLYSF**PE**PEA

Six human monoclonal 62GE-specific antibodies were tested in Luminex assays using One Lambda (OL) and Gen-Probe (GP) single allele kits according to manufacturer's instructions

Consistent Reactivity Patterns of Three 62GE-Specific Human Monoclonals with Two Luminex Kits

| Hu mAb	SN7B12 (IgG)		SN510G11 (IgG)		ROU2D3 (IgM)	
Ab producer	A24,29; B7,44		A24,29; B7,44		A1,25; B8,18	
Immunizer	A2		A2		A2	
	OL	GP	OL	GP	OL	GP
A*02:01	13545	9003	15679	13400	13596	9071
A*02:02	nt	11569	nt	14029	x	9084
A*02:03	17884	11810	21265	15787	14529	10191
A*02:05	nt	16600	nt	16994	x	12339
A*02:06	19101	nt	21981	nt	13571	
B*57:01	13961	8945	14267	10580	13670	6319
B*57:03	17540	nt	18374	nt	13741	
B*58:01	11600	12936	11813	14699	12256	9813
62GE-neg alleles	14 (7-89)	98 (11-211)	25 (13-50)	108 (58-194)	9 (4-14)	13 (1-56)

Inconsistencies Between Reactivity Patterns of Three Other 62GE-Specific Monoclonals Tested with Two Luminex Kits

Hu mAb	WK1D3 (IgM)		VN2F1 (IgM)		WIM1B3 (IgM)	
Ab producer	A1,-; B8,-		?		A3,-; B47,51	
Immunizer	A2		B17?		nd	
	OL	GP	OL	GP	OL	GP
A*02:01	2493	13	3614	165	9663	6457
A*02:02	nt	207	nt	1350	nt	6190
A*02:03	5194	12	6610	223	13865	9888
A*02:05	nt	7898	nt	7979	nt	10173
A*02:06	6374	nt	7254	nt	14387	nt
B*57:01	8234	1998	7850	3398	4184	159
B*57:03	11495	nt	11309	nt	8187	nt
B*58:01	6717	8239	6682	8652	2324	3107
62GE-neg alleles	14 (2-64)	10 (3-71)	19 (2-81)	10 (4-20)	9 (1-258)	19 (3-34)

How can we understand these differences?

Hu mAb	WK1D3 (IgM)		VN2F1 (IgM)		WIM1B3 (IgM)	
Ab producer	A1,-; B8,-		?		A3,-; B47,51	
Immunizer	A2		B17?		nd	
	OL	GP	OL	GP	OL	GP
A*02:01	2493	13	3614	165	9663	6457
A*02:02	nt	207	nt	1350	nt	6190
A*02:03	5194	12	6610	223	13865	9888
A*02:05	nt	7898	nt	7979	nt	10173
A*02:06	6374	nt	7254	nt	14387	nt
B*57:01	8234	1998	7850	3398	4184	159
B*57:03	11495	nt	11309	nt	8187	nt
B*58:01	6717	8239	6682	8652	2324	3107
62GE-neg alleles	14 (2-64)	10 (3-71)	19 (2-81)	10 (4-20)	9 (1-258)	19 (3-34)

|	Eplet		Residue Differences within 15 A Peptide contacting residues in yellow													
	62	63	9	11	43	45	46	66	67	70	71	74	76	77	152	156	163
A*02:01	G	E	F	S	Q	M	E	K	V	H	S	H	V	D	V	L	T
A*02:02	G	E	F	S	R	M	E	K	V	H	S	H	V	D	V	W	T
A*02:03	G	E	F	S	Q	M	E	K	V	H	S	H	V	D	E	W	T
A*02:05	G	E	Y	S	R	M	E	K	V	H	S	H	V	D	V	W	T
A*02:06	G	E	Y	S	Q	M	E	K	V	H	S	H	V	D	V	L	T
B*57:01	G	E	Y	A	P	M	A	N	M	S	A	Y	E	N	V	L	L
B*57:03	G	E	Y	A	P	M	A	N	M	S	A	Y	E	N	V	L	L
B*58:01	G	E	Y	A	P	T	E	N	M	S	A	Y	E	N	V	L	L
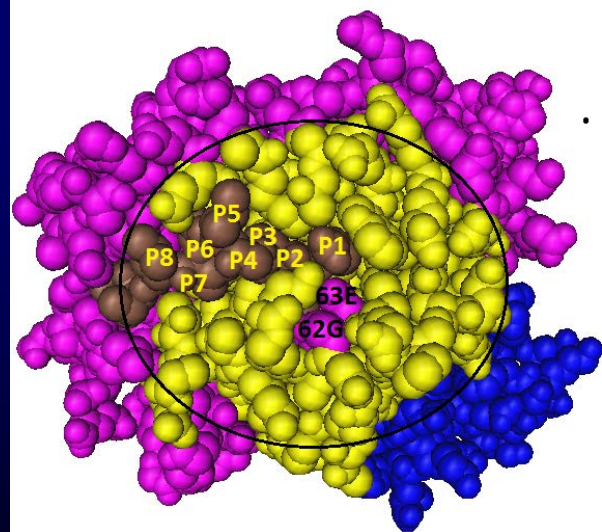
Conclusions

- Some human monoclonal antibodies react differently with alleles in the One Lambda and the Gen-Probe Luminex kits

- Can this be explained with peptide repertoire differences between alleles used in these kits?

- No matter what, we should be aware that some HLA epitope-specific alloantibodies are "peptide-dependent" and will have lower MFI values with certain alleles

How do antibodies react with HLA epitopes?
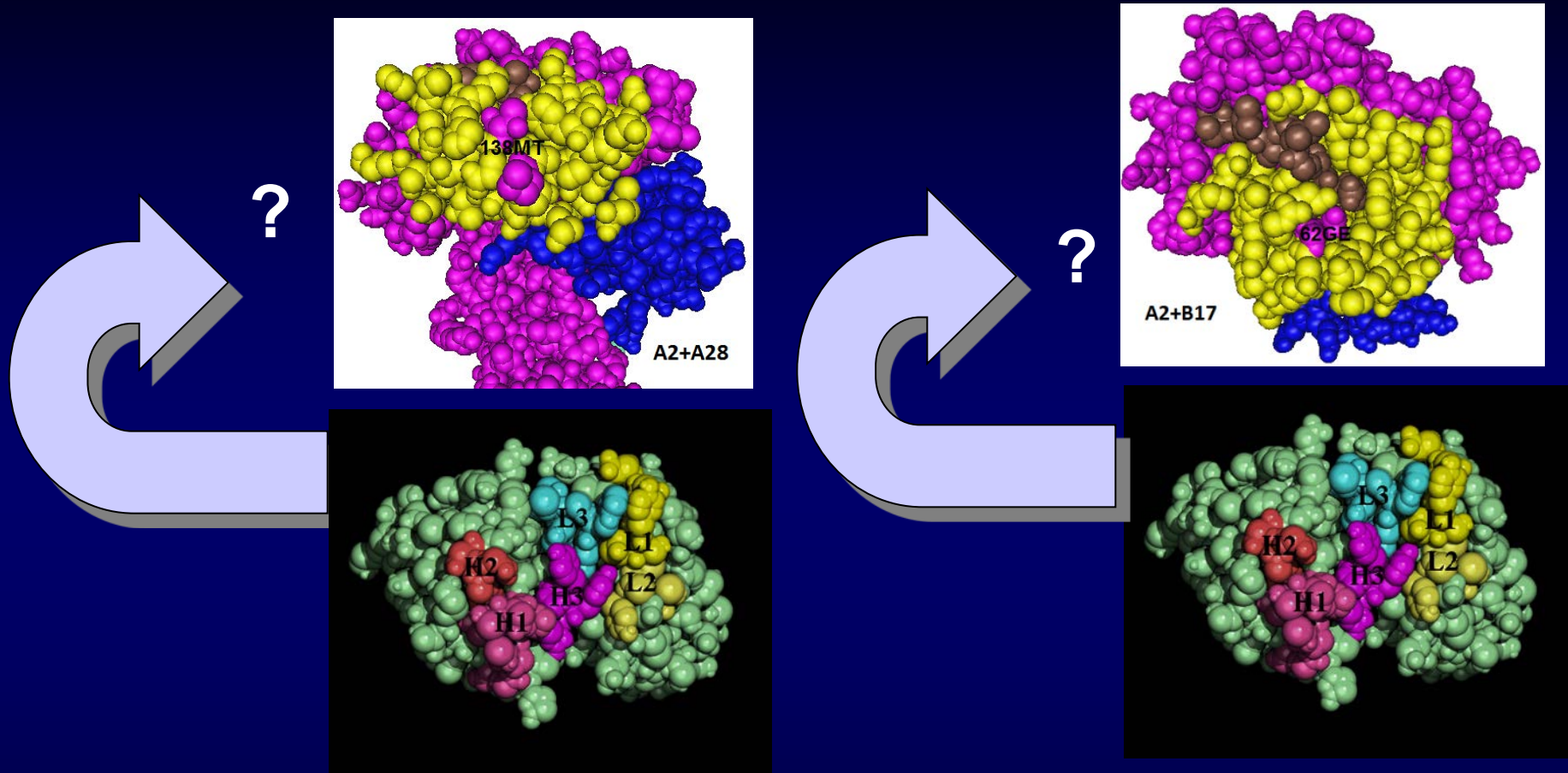
1. Epitopes are defined by eplets and other residue configurations
2. Binding with antibody involves CDRs on heavy and light chains

How do antibodies react with HLA epitopes?
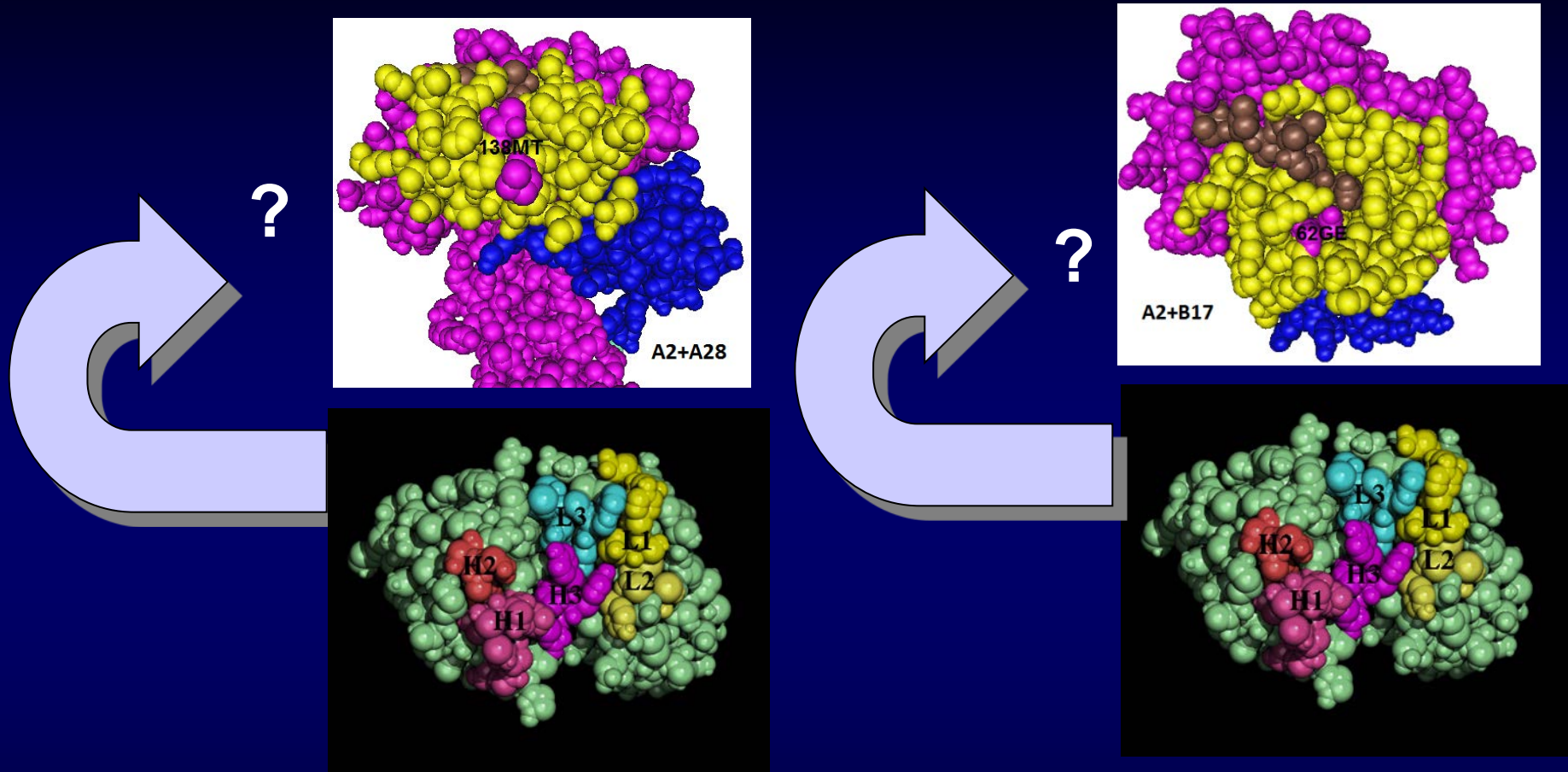
1. Epitopes are defined by eplets and other residue configurations
2. Binding with antibody involves CDRs on heavy and light chains
3. **Binding energy (affinity) and biological activity of antibody**

HLA Antibody Epitope Specificity in Different Assays
(Duquesnoy et al. Human Immunology. 74:1271-1279, 2013)

Human monoclonal antibodies tested with

- Ig-binding (Luminex) with single alleles
- C1q-binding (Luminex) with single alleles
- CDC testing with a large HLA-typed cell panel (N>800; 13th International Workshop)

Human monoclonal antibodies generated by
Arend Mulder
Leiden University Medical Center
The Netherlands

Monoclonal Antibody Reactivity Patterns of Eplet-Carrying Alleles

Pattern	Lum-Ig	Lum-C1q	CDC
I	Positive	Positive	Positive
II	Positive	Negative	Negative
III	Positive	Positive	Negative

Note: All monoclonals in this study had been selected for their lymphocytotoxic reactivity with the immunizing antigen

Antibody Binding Energy (Affinity) and Reactivity in Different Assays

Reactivity	Binding	Interpretation
Negative in all 3 assays	0	No specific epitope recognition and binding
Only Lum-Ig positive	+	Epitope is recognized but insufficient conformational change in antibody
Lum-Ig positive Lum-C1q positive CDC negative	++	Epitope is recognized + conformational change exposes C1q-binding site on antibody but no Complement activation
Positive in all 3 assays	+++	Epitope is recognized + exposed C1q-binding site + conformational change in C1q to activate C1qrs complex, then Complement activation

Pattern I

62GE-Specific mAb ROU2D3

ROU2D3 (IgM) — Sequence positions with residue differences within 15 Angstroms

Allele	Eplet	Lum-Ig	Lum-C1q	CDC score	9	11	43	45	46	66	67	70	71	74	76	77	152	156	163
A*02:01	62GE	13596	22954	7.7 (N=211)	F	S	Q	M	E	K	V	H	S	H	V	D	V	L	T
A*02:03	62GE	14529	23869	7.3 (N=6)	F	S	Q	M	E	K	V	H	S	H	V	D	E	W	T
A*02:06	62GE	13571	20588	8.0 (N=27)	Y	S	Q	M	E	K	V	H	S	H	V	D	V	L	T
B*57:01	62GE	13670	23767	8.0 (N=17)	Y	A	P	M	A	N	M	S	A	Y	E	N	V	L	L
B*57:03	62GE	13741	22101	8.0 (N=6)	Y	A	P	M	A	N	M	S	A	Y	E	N	V	L	L
B*58:01	62GE	12256	4103	8..0 (N=7)	Y	A	P	T	E	N	M	S	A	Y	E	N	V	L	L
Other alleles		9	32	1.3 (N=402)															

Conclusion: all 62GE-carrying alleles are unacceptable mismatches

Pattern II
166DG-Specific mAb BVK5C4

BVK5C4 (IgM)

Allele	Eplet	Lum-Ig	Lum-C1q	CDC score
A*01:01	166DG	10728	967	1.3 (N=82)
A*23:01	166DG	14354	10530	7.9 (N=34)
A*24:02	166DG	13489	3321	7.5 (N=135)
A*80:01	166DG	12911	13013	8.0 (N=6)
B*15:12	166DG	14272	4114	8.0 (N=2)
other alleles		19	26	1.5 (N=434)

Sequence positions with residue differences within 15 Angstroms

	9	56	62	65	66	67	69	99	105	109	113	131	151	152	156	158	163
A*01:01	F	G	Q	R	N	M	A	Y	P	F	Y	R	H	A	R	V	R
A*23:01	S	G	E	G	K	V	A	F	S	F	Y	R	R	V	L	A	T
A*24:02	S	G	E	G	K	V	A	F	S	F	Y	R	H	V	Q	A	T
A*80:01	F	E	E	R	N	V	A	Y	S	F	Y	R	R	R	L	A	E
B*15:12	Y	G	R	Q	I	S	T	Y	P	L	H	S	R	E	W	A	L

A*24:02

156
158
163
166D
167G
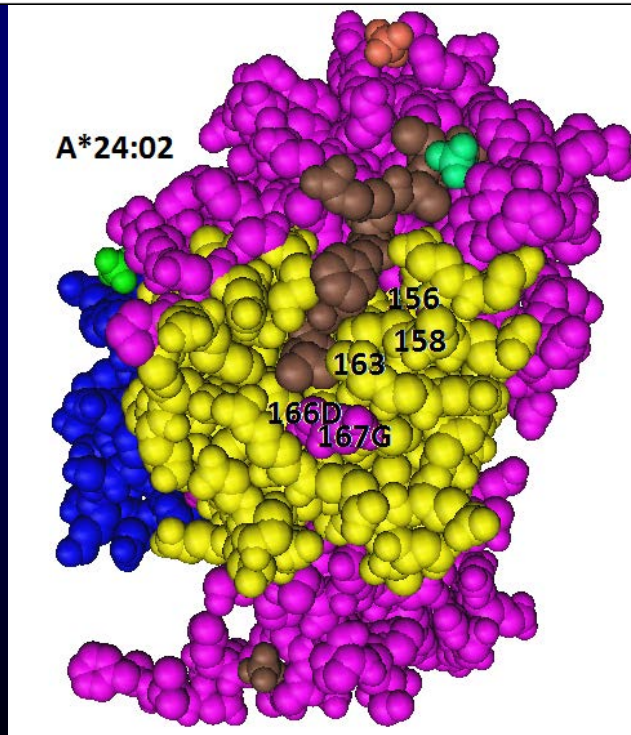
Is A*01:01 an unacceptable mismatch?

Two lymphocytotoxic human monoclonal antibodies specific for 144TKR
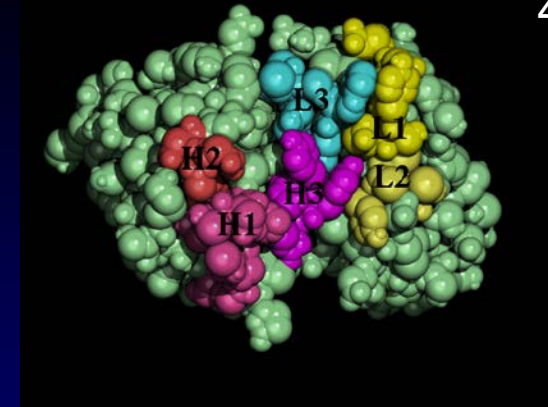
Pattern III

144TKR-Specific mAb OK5A3

OK5A3 (IgM) produced by A2,A68;B7,B27;Cw2,Cw7 — Sequence positions with residue differences within 15 Angstroms

Allele	Eplet	Lum-Ig	Lum-C1q	CDC score	(N)	76	77	79	80	82	83	116	127	150	151	152	156	158
A*0101	144TKR	7462	23994	6.8	(N=89)	A	N	G	T	R	G	D	N	V	H	A	R	V
A*0301 IMM	144TKR	10073	23870	7.8	(N=87)	V	D	G	T	R	G	D	N	A	H	E	L	A
A*1101	144TKR	10261	23522	7.7	(N=77)	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*1102	144TKR	11520	24443	8.0	(N=3)	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*2402	144TKR	9049	21579	7.4	(N=112)	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*2403	144TKR	9541	24294	8.0	(N=4)	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*3601	144TKR	7679	24073	7.7	(N=7)	A	N	G	T	R	G	D	N	V	H	A	R	V
A*8001	144TKR	9803	23992	1.0	(N=5)	A	N	G	T	R	G	D	N	A	R	R	L	A
Other alleles	Others	12	289	1.7	(N=282)													

Lum-Ig- and Lum-C1q reactivity is specific for 144TKR
CDC reactivity is specific for 144TKR+151H

Is A*80:01 an unacceptable mismatch?

Pattern III

Another 144TKR-Specific mAb: BRO11F6

BRO11F6 (IgG) produced by A26,A68;B38, B44; Cw7,-				Sequence positions with residue differences within 15 Angstroms													
Allele	Eplet	Lum-Ig	Lum-C1q	76	77	79	80	82	83	116	127	150	151	152	156	158
A*11:01	144TKR	16907	8929	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*11:02	144TKR	20290	15159	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*01:01	144TKR	20375	14587	A	N	G	T	R	G	D	N	V	H	A	R	V
A*03:01	144TKR	22162	16717	V	D	G	T	R	G	D	N	A	H	E	L	A
A*24:02	144TKR	21608	16188	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*24:03	144TKR	20406	19156	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*36:01	144TKR	14045	9113	A	N	G	T	R	G	D	N	V	H	A	R	V
A*80:01	144TKR	13	22	A	N	G	T	R	G	D	N	A	R	R	L	A
Other alleles	Others	31	44													

Lum-Ig and Lum-C1q reactivity is specific for 144TKR+151H

Pattern III
144TKR-Specific mAb BRO11F6

BRO11F6 (IgG) produced by A26,A68;B38, B44; Cw7,-						Sequence positions with residue differences within 15 Angstroms											
Allele	Eplet	Lum-Ig	Lum-C1q	CDC score		76	77	79	80	82	83	116	127	150	151	152	156	158
A*11:01	144TKR	16907	8929	7.0	(N=66)	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*11:02	144TKR	20290	15159	7.5	(N=4)	V	D	G	T	R	G	D	N	A	H	A	Q	A
A*01:01	144TKR	20375	14587	1.2	(N=78)	A	N	G	T	R	G	D	N	V	H	A	R	V
A*03:01	144TKR	22162	16717	1.7	(N=86)	V	D	G	T	R	G	D	N	A	H	E	L	A
A*24:02	144TKR	21608	16188	1.8	(N=110)	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*24:03	144TKR	20406	19156	1.0	(N=5)	E	N	R	I	L	R	Y	K	A	H	V	Q	A
A*36:01	144TKR	14045	9113	1.0	(N=7)	A	N	G	T	R	G	D	N	V	H	A	R	V
A*80:01	144TKR	13	22	1.0	(N=6)	A	N	G	T	R	G	D	N	A	R	R	L	A
Other alleles	Others	31	44	1.1	(N=288)													

Lum-Ig- and Lum-C1q reactivity is specific for 144TKR+151H
CDC reactivity is specific for 144TKR+150AHA

Are A*11:01 and A*11:02 the only unacceptable mismatches?

Structural Model of HLA Epitope Reactivity of Complement-Fixing Antibodies Tested in Ig-Binding, C1q-Binding and CDC Assays

Binding energy with antibody
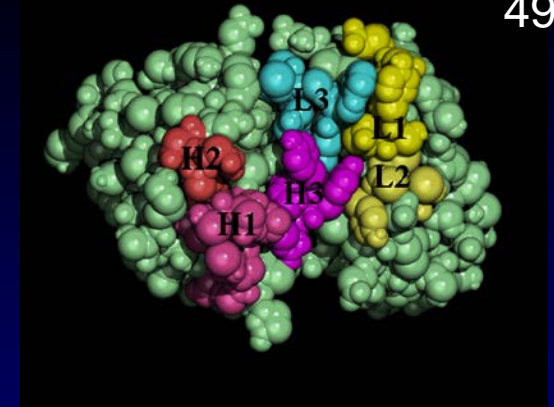+++

Critical Contact Site
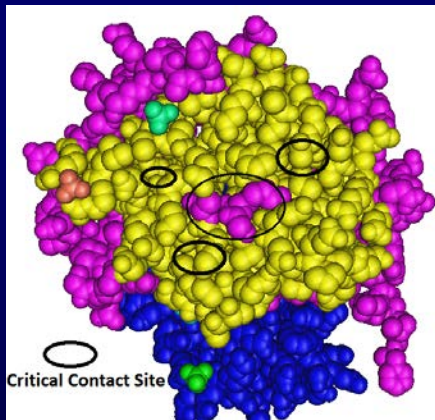
Immunizing allele
Lum-Ig **positive**
Lum-C1q **positive**
CDC **positive**

Structural Model of HLA Epitope Reactivity of Complement-Fixing Antibodies Tested in Ig-Binding, C1q-Binding and CDC Assays
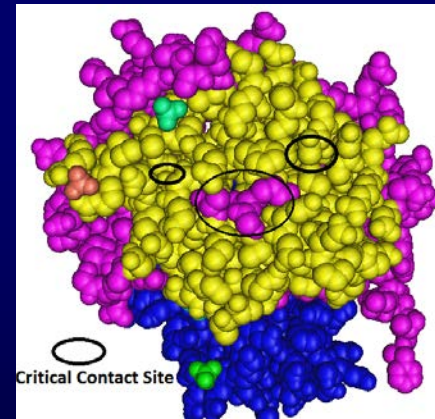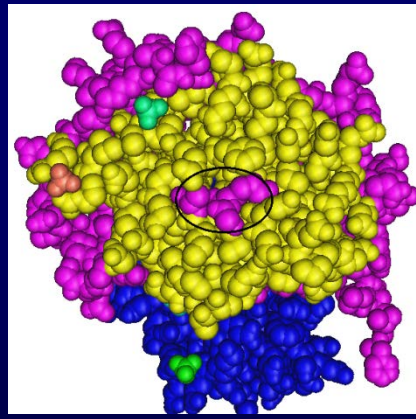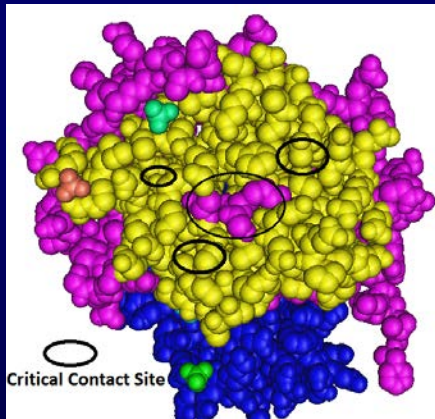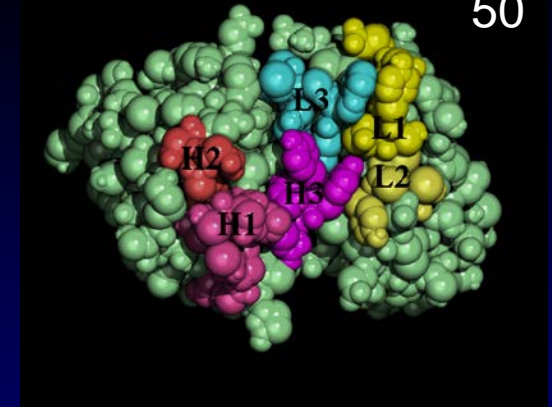

Binding energy with antibody
+++   +

Critical Contact Site
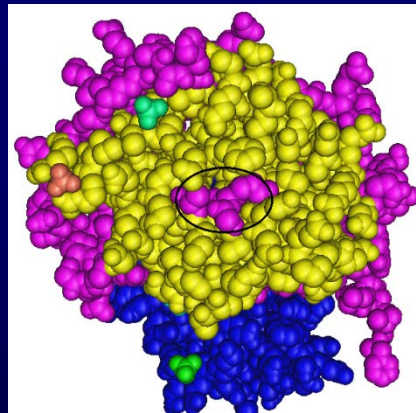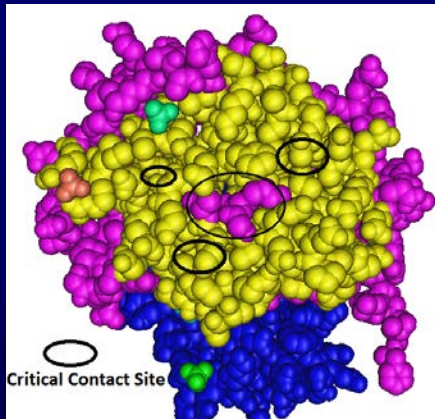
Immunizing allele
Lum-Ig **positive**
Lum-C1q **positive**
CDC **positive**

Allele 1
Lum-Ig **positive**
Lum-C1q negative
CDC negative

Structural Model of HLA Epitope Reactivity of Complement-Fixing Antibodies Tested in Ig-Binding, C1q-Binding and CDC Assays
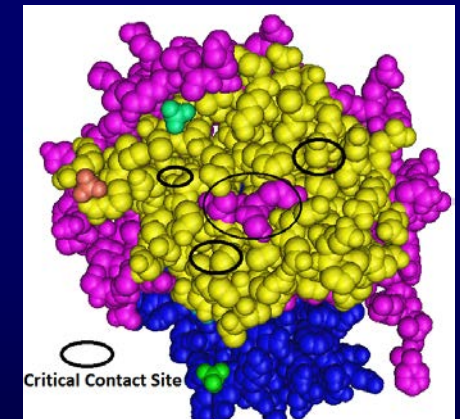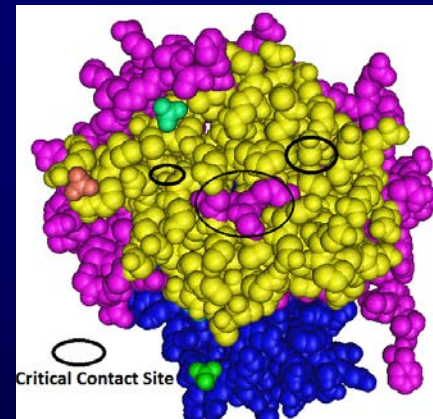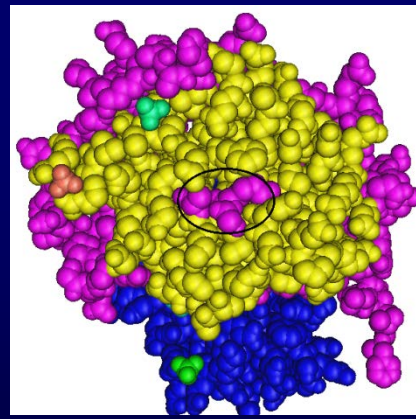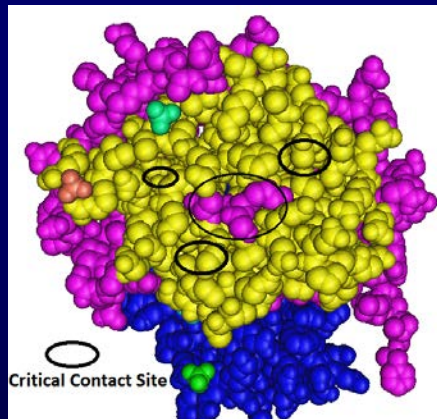

Binding energy with antibody

+++   +   ++

Immunizing allele
Lum-Ig **positive**
Lum-C1q **positive**
CDC **positive**

Allele 1
Lum-Ig **positive**
Lum-C1q negative
CDC negative

Allele 2
Lum-Ig **positive**
Lum-C1q **positive**
CDC negative

Molecular Model of HLA Epitope Reactivity of Complement-Fixing Antibodies Tested in Ig-Binding, C1q-Binding and CDC Assays

Binding energy with antibody

+++	+	++	+++

Critical Contact Site

Immunizing allele
Lum-Ig **positive**
Lum-C1q **positive**
CDC **positive**

Allele 1
Lum-Ig **positive**
Lum-C1q negative
CDC negative

Allele 2
Lum-Ig **positive**
Lum-C1q **positive**
CDC negative

Allele 3
Lum-Ig **positive**
Lum-C1q **positive**
CDC **positive**

Why Do Certain Antibodies React in Luminex but not in CDC?

These antibodies are not Complement-fixing

or (and more likely)

They can fix Complement but will react in CDC only with eplet-carrying alleles with proper structural epitope configurations

The determination of epitope-based mismatch acceptability might be technique dependent

Which method is clinically relevant for determining mismatch acceptability?