

Profiling the Specificity of Neutralizing Antibodies in a Large Panel of Plasmas from Patients Chronically Infected with Human Immunodeficiency Virus Type 1 Subtypes B and C[†]

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Why are we interested in mapping?

to understand the basis of broad neutralization in:

*HIV+ patient sera

-identify valuable targets for vaccine discovery

*vaccine sera

-to determine why present vaccines sometimes elicit type-specific neutralization

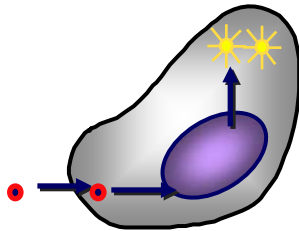
-so we can try to engineer rational vaccine improvements

-to determine how specificity is affected by variables:
e.g. form of Env, choice of adjuvant, animal model

Challenges:

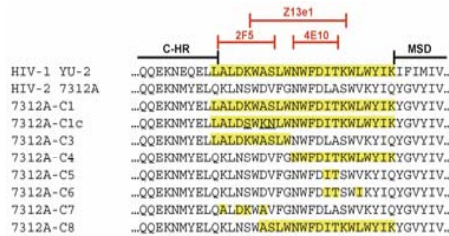
- *distinguishing neutralizing from non-neutralizing fractions
- *deconvoluting mixtures of different neutralization specificities

Epitope mapping methodologies

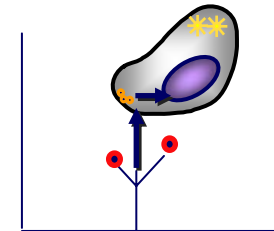


Modified Neutralization Assays

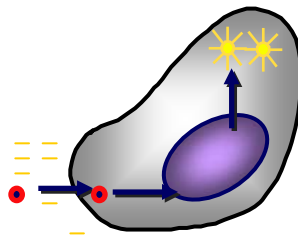
- i) Post-CD4
 - ii) Post CD4/CCR5
- V3, CD4i, MPER epitopes



Epitope-engrafted HIV-2 and SIV V3 and MPER

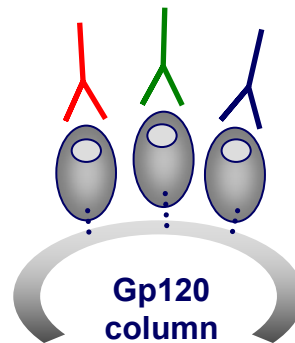


Virus Capture Competition total binding epitopes

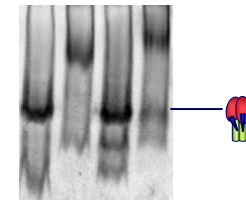


Neutralization Interference Assays using peptides, fusion protein competitors

V3, MPER epitopes



Serum fractionation conformational, CD4bs epitopes



Native PAGE CD4bs, 2G12, others

HIV+ plasma panel

Clade B:

n=8

Z2, Z23, Z48, Z52, Z85, Z86, Z87

From Zeptomatrix.

From chronically infected patients in the US.

Prescreened for neutralizing activity.

Plasma LT2 from Doug Richman

Clade C:

n=16

BB8, BB12, BB14, BB21, BB24, BB28, BB34, BB47, BB55, BB68, BB75,
BB80, BB81, BB87, BB105, BB107.

From South African Blood Bank.

From chronically infected patients.

Prescreened for neutralizing activity.

Provided by Dr. Lynn Morris.

HIV- control plasmas:

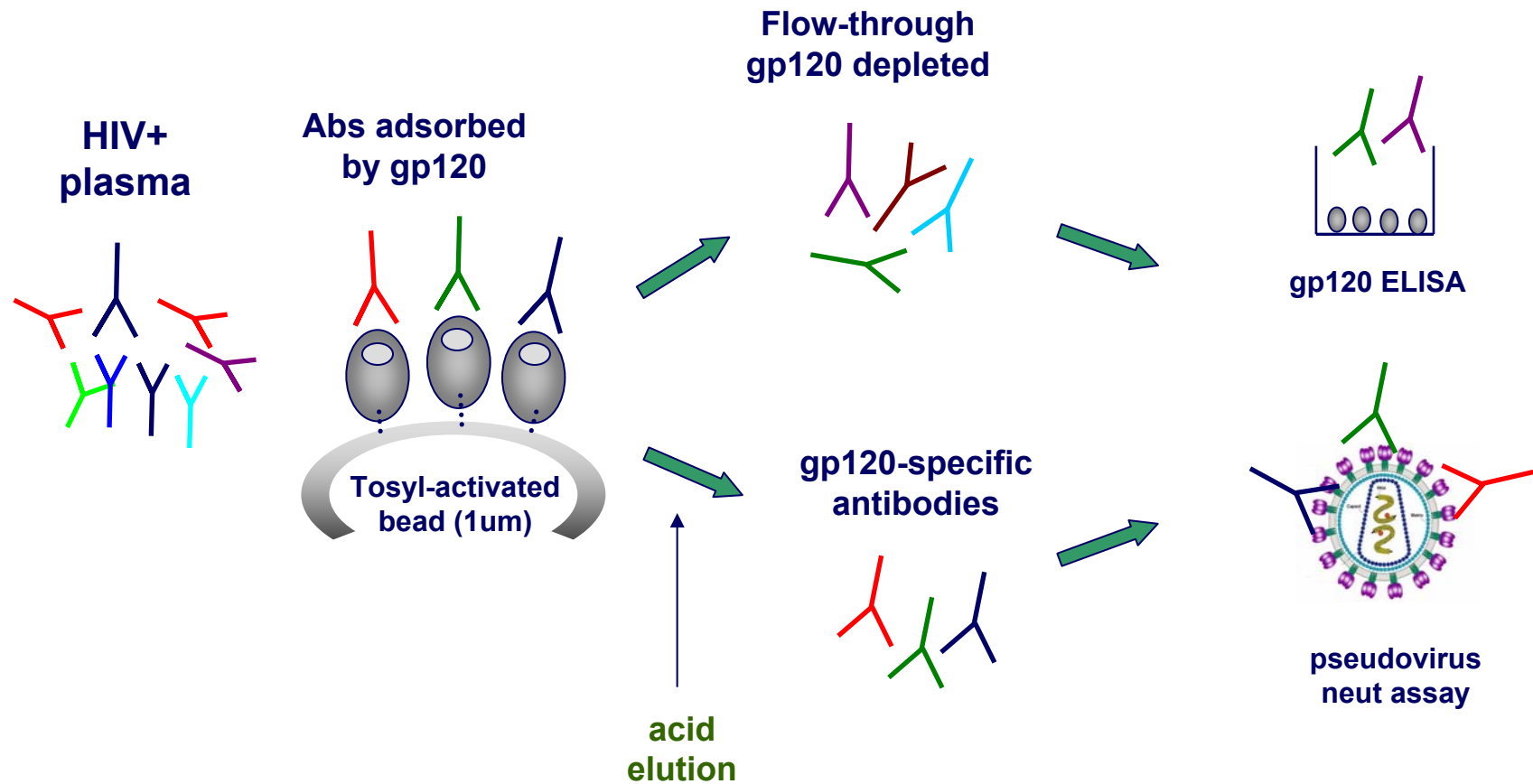
210, 211

From Zeptomatrix

Neutralization breadth analysis

Virus	Clade	Location	clade B																clade C																controls	
			1648	1652	1686	1702	BB8	BB12	BB14	BB21	BB24	BB28	BB34	BB47	BB55	BB68	BB75	BB80	BB81	BB87	BB105	BB107	210	211												
JR-FL_d301	B	USA	188	361	1,914	1,102	8,330	4,675	15,056	11,972	4,750	2,164	27,212	1,686	8,339	1,660	7,111	7,125	712	8,984	10,625	13,714	<20	<20												
SF162.LS	B	USA	3,991	1,307	7,032	4,316	8,330	4,675	15,056	11,972	4,750	2,164	27,212	1,686	8,339	1,660	7,111	7,125	712	8,984	10,625	13,714	<20	<20												
HXB2	B	France	278	1,109	314	424	1,634	6,070	1,744	2,374	2,846	10,101	17,288	5,717	3,743	1,171	5,418	2,651	1,513	9,742	1,429	332	<20	<20												
Bal.26	B	USA	533	375	934	1,493	498	460	903	559	255	130	798	235	499	153	160	346	154	747	405	1,399	<20	<20												
6535.3	B	USA	143	311	120	257	408	858	349	321	626	197	306	214	605	140	107	517	145	1,269	315	2,691	<20	<20												
RHPA4259.7	B	USA	141	112	503	149	258	728	354	426	177	1,029	192	366	175	152	101	182	210	457	219	883	<20	<20												
AC10.0.29	B	USA	425	267	119	162	275	520	403	281	284	142	370	216	460	218	118	253	205	504	331	1,120	<20	<20												
REJO4541.67	B	USA	619	870	217	624	320	126	324	670	115	116	331	199	93	224	43	99	97	268	425	158	<20	<20												
SHIV-89.6P	B	USA	441	335	172	448	313	389	180	312	155	80	131	136	207	144	91	224	93	332	290	900	<20	<20												
WITO4160.33	B	USA	106	780	204	392	293	277	389	237	307	126	243	238	193	133	95	168	141	307	307	146	<20	<20												
SS1196.1	B	USA	146	178	187	367	161	298	509	189	211	53	416	258	363	80	125	176	90	804	136	145	<20	<20												
SC422661.8	B	Trinidad	203	201	121	150	197	289	250	224	246	97	800	240	210	140	130	130	162	408	180	140	<20	<20												
JR-FL	B	USA	40	143	1,329	192	131	413	40	35	20	35	292	100	100	20	150	50	80	600	70	600	<20	<20												
TRO.11	B	Italy	110	130	195	171	564	476	66	251	66	72	250	80	200	73	41	331	61	404	101	519	<20	<20												
CAAN5342.A2	B	USA	263	180	110	125	199	175	157	338	201	81	150	305	229	104	71	108	96	527	303	279	<20	<20												
THRO4156.18	B	USA	262	259	138	173	180	89	213	409	171	105	131	207	239	109	50	131	85	132	140	88	<20	<20												
SHIV-SF162.P3	B	USA	237	315	138	243	155	126	115	226	115	88	66	56	94	75	38	85	64	146	139	453	<20	<20												
TRJ04551.58	B	USA	99	55	83	316	155	172	77	182	50	735	73	73	33	54	58	91	36	109	84	30	<20	<20												
QH0692.42	B	Trinidad	117	216	124	114	65	204	64	48	120	44	110	91	63	65	75	94	75	167	65	57	<20	<20												
PVO.4	B	Italy	67	97	141	118	38	225	31	54	50	40	38	55	76	32	26	31	35	41	44	93	<20	<20												
geometric mean titer clade B			182	219	190	245	206	285	179	218	141	111	203	155	173	96	77	142	95	319	170	293	<20	<20												
MW965.26	C	Malawi	5,619	3,011	2,375	2,186	25,388	12,353	110,682	98,928	80,906	15,271	461,999	21,189	33,569	11,606	12,016	14,707	4,061	42,052	11,813	12,002	<20	<20												
CAP45.2.00.G3	C	S. Africa	922	995	153	394	480	1,514	749	578	494	1,420	768	1,024	478	1,126	109	1,050	446	1,175	1,855	203	<20	<20												
Du151.2	C	S. Africa	64	60	72	108	98	998	423	177	378	160	102	193	616	62	9,320	302	102	336	152	1,076	<20	<20												
Du156.12	C	S. Africa	380	358	149	333	242	666	175	93	299	154	1,263	328	389	254	312	349	258	957	217	761	<20	<20												
ZM135M.PL10a	C	Zambia	422	353	128	171	433	372	479	651	483	142	1,056	360	444	317	200	325	296	487	539	287	<20	<20												
CAP210.2.00.E8	C	S. Africa	546	228	171	313	471	269	562	632	627	267	329	733	408	253	145	259	416	433	470	224	<20	<20												
ZM233M.PB6	C	Zambia	146	190	86	156	434	230	369	327	596	272	269	786	462	231	148	419	725	1,222	341	160	<20	<20												
ZM214M.PL15	C	Zambia	2,028	390	132	470	359	382	142	209	483	233	268	414	204	220	113	154	271	291	261	71	<20	<20												
ZA012	C	Zambia	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	168	382	n.d.	50	1,081	n.d.	n.d.	n.d.	11	n.d.	n.d.	<20	<20											
ZM109F.PB4	C	Zambia	282	185	183	320	467	280	472	685	395	136	384	501	363	253	133	236	213	336	285	249	<20	<20												
ZM249M.PL1	C	Zambia	296	161	227	568	270	172	255	378	398	263	141	391	222	169	120	168	417	490	312	159	<20	<20												
ZM197M.PB7	C	Zambia	414	402	141	276	274	137	375	348	302	126	877	275	271	167	83	160	148	155	218	92	<20	<20												
Du172.17	C	S. Africa	35	99	39	86	298	954	167	105	230	288	94	551	195	95	232	297	231	428	138	248	<20	<20												
TV1.29	C	S. Africa	96	69	22	123	192	185	93	170	247	127	286	462	264	158	137	161	131	274	178	136	<20	<20												
ZM53M.PB12	C	Zambia	232	112	209	247	230	82	138	275	115	286	68	201	131	258	35	114	77	83	151	104	38	<20	<20											
Du422.1	C	S. Africa	<20	31	36	55	92	221	170	147	99	112	89	305	113	251	30	224	167	140	120	487	<20	<20												
Du123.6	C	S. Africa	98	118	56	103	60	210	64	73	186	77	226	158	109	112	133	106	118	258	82	237	<20	<20												
geometric mean titer clade C			250	209	121	236	333	403	363	370	444	256	437	501	369	242	228	310	268	489	262	287	<20	<20												
overall geometric mean titer			212	214	152	240	260	337	252	282	246	168	298	273	250	152	132	207	157	392	211	290	<20	<20												
SIVmac239CS.23			<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20												
HIV-2 KR			<500	<500	<500	<500	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20												
HIV-2 7312A			<20	166	25	100	<20	<20	<20	<20	<20	26	<20	<20	27	<20	<20	<20	<20	52	<20	<20	<20	<20												

Fractionation of HIV+ Plasmas



Forms of gp120 for plasma fractionations

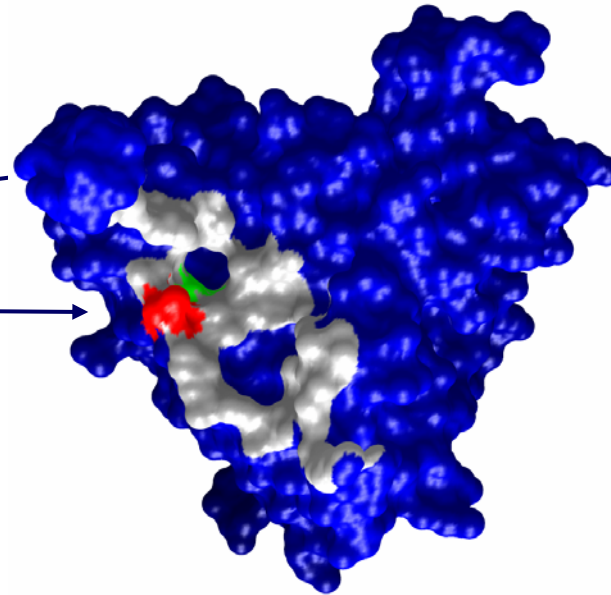
-gp120 wt protein (BaL, YU2)

-gp120 wt protein (denatured)

-gp120 core $\Delta V12V3$

-gp120 point mutant **D368R**

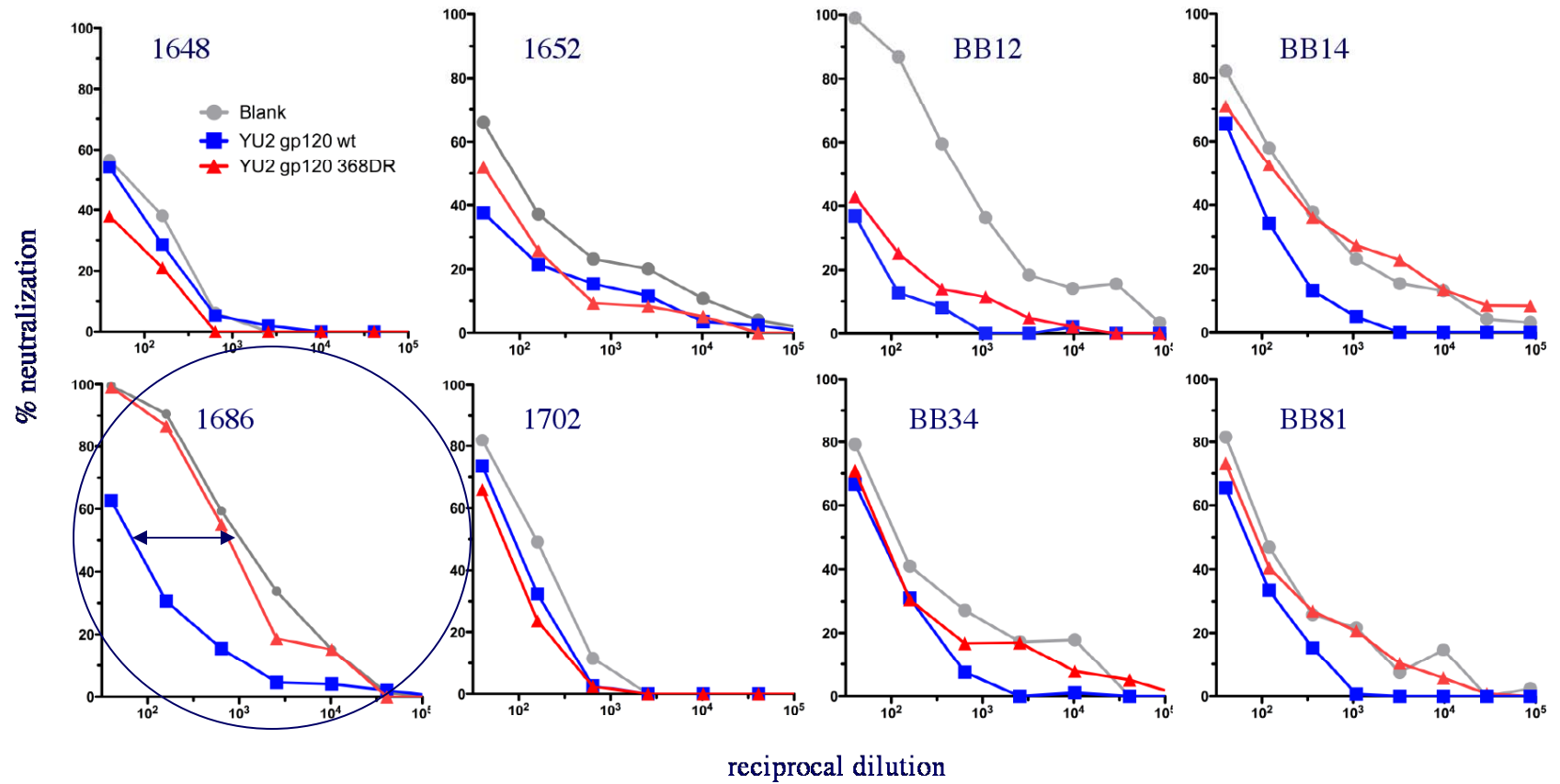
-gp120 point mutant **D368R +
E370A**



gp120 368DR and 368/370 point mutations:

- Eliminate CD4 binding site antibodies (e.g. b12, F105, sCD4)
- Retain binding to other gp120 antibodies (*Olshevsky et al, 1990; Thali et al, 1991*)

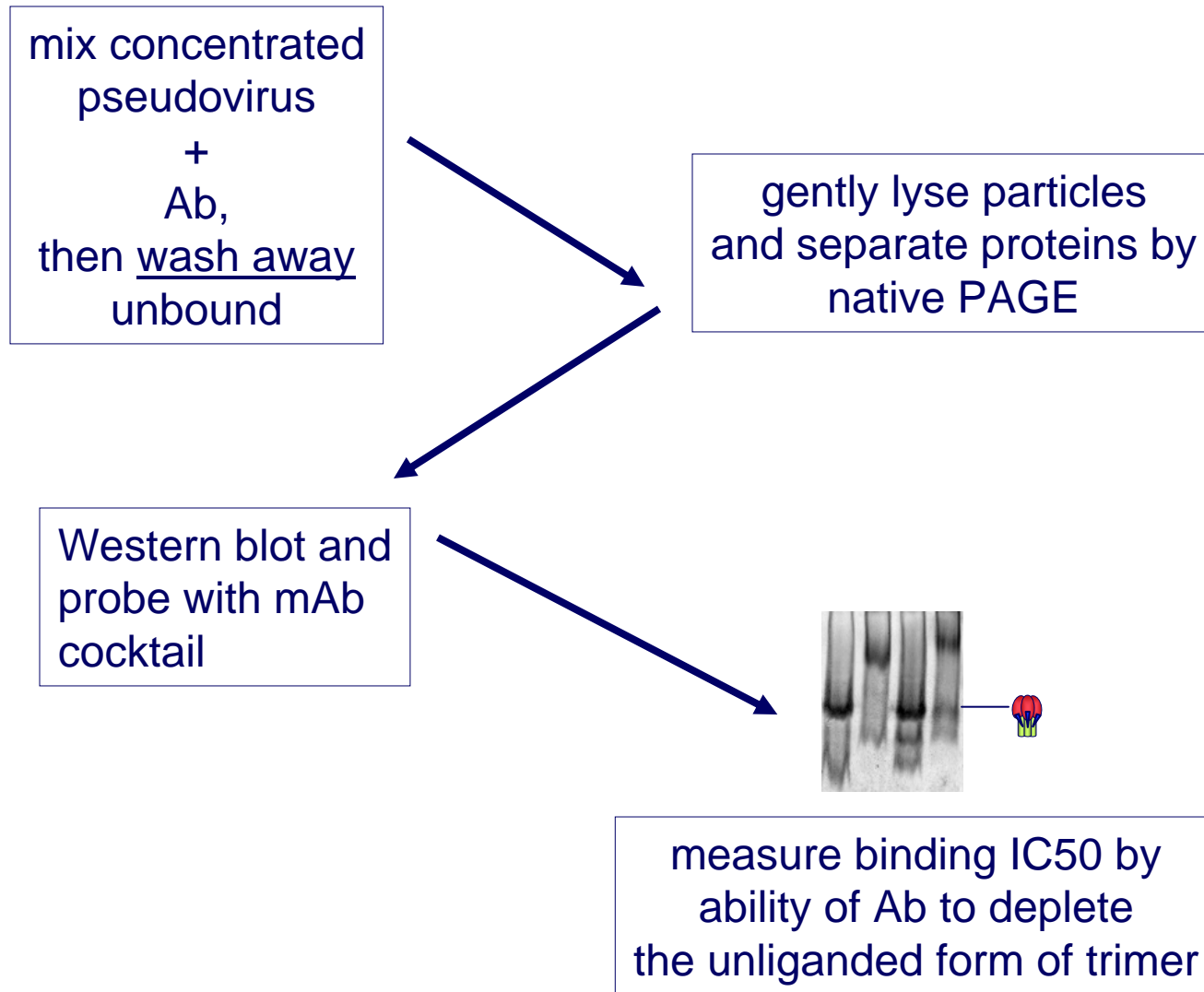
Gp120 bead fractionation



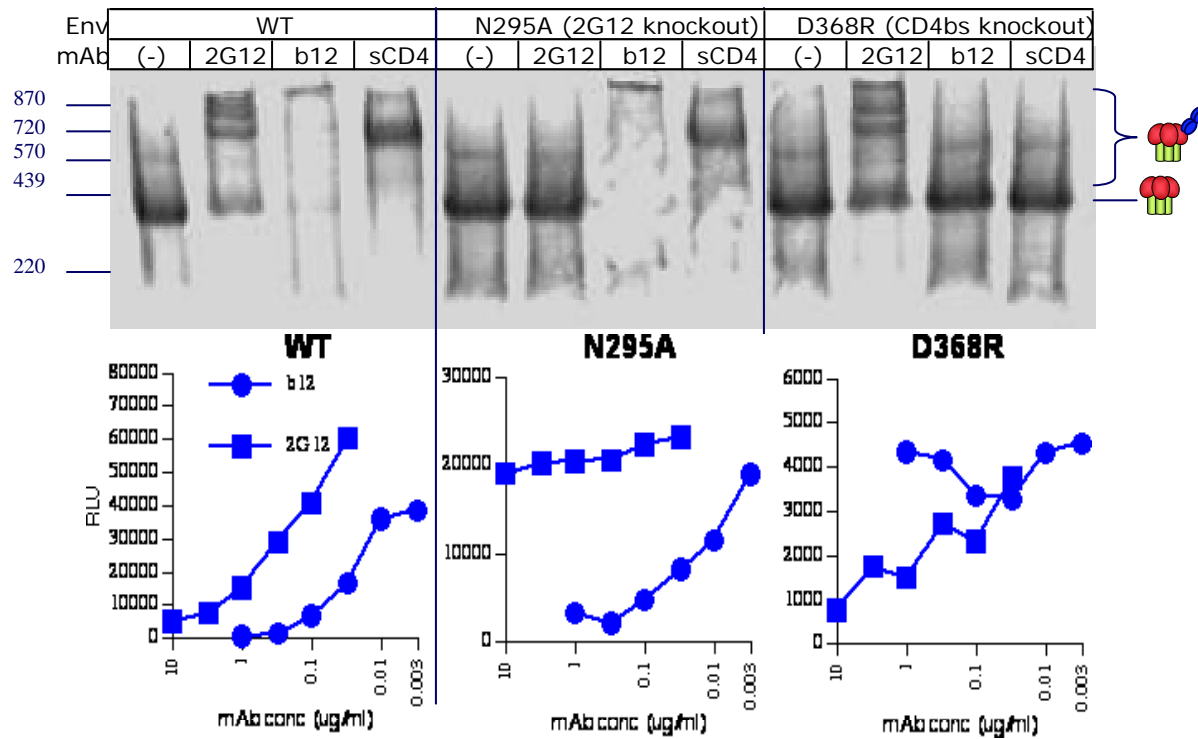
Summary of gp120 fractionations

plasma ID	virus	subtype	blank ^a	ID50		% gp120- directed ^d	% CD4bs- directed ^e
				gp120 wt ^b	gp120 D368R ^c		
1648	HxB2	B	354	20	40	94%	6%
	SF162	B	1330	173	150	87%	0%
	BaL.01	B	63	40	20	37%	
	JRFL	B	64	51	40	20%	
1652	HxB2	B	1080	531	1105	51%	51%
	SF162	B	615	249	280	60%	5%
	BaL.01	B	55	40	20	27%	
	JRFL	B	93	20	43	78%	25%
1686	HxB2	B	408	40	40	90%	0%
	SF162	B	928	158	93	83%	0%
	BaL.01	B	1069	40	778	96%	69%
	JRFL	B	1185	70	797	94%	61%
	RHPA	B	365	40	456	89%	89%
	RW020	A	121	20	104	83%	69%
	ZM249M	C	432	40	174	91%	25%
1702	HxB2	B	456	284	320	38%	
	SF162	B	804	182	141	77%	0%
	BaL.01	B	161	130	95	19%	
	JRFL	B	146	88	66	40%	
BB12	HxB2	B	982	60.5	807	94%	76%
	SF162	B	2144	80.4	219	96%	7%
	JRFL	B	676	20	20	97%	0%
	Du151	C	2285	166	362	93%	7%
	Du156	C	859	185	367	78%	21%
BB14	HxB2	B	2666	55.8	1820	98%	67%
	SF162	B	769	59.9	276	92%	28%
	JRFL	B	218	69.3	166	68%	44%
	Du151	C	739	285	525	61%	32%
	Du156	C	281	140	274	50%	48%
BB34	HxB2	B	9426	2592	6364	73%	40%
	SF162	B	2304	174	362	92%	8%
	JRFL	B	160	76	88	53%	7%
	Du151	C	174	81	133	54%	30%
	Du156	C	605	645	673	0%	
BB81	HxB2	B	671	130	463	81%	50%
	SF162	B	458	123	249	73%	28%
	JRFL	B	139	68.9	106	50%	27%
	Du151	C	145	88	175	39%	
	Du156	C	441	375	302	15%	

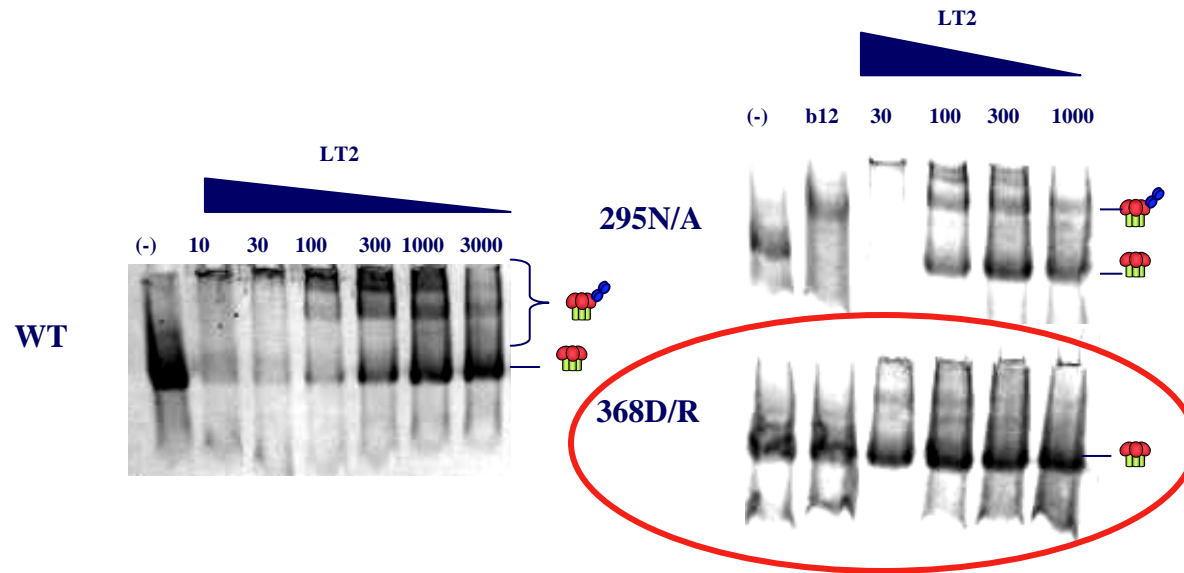
Blue Native PAGE



BN-PAGE: analysis of mutants



BN-PAGE analysis of plasma LT2 binding



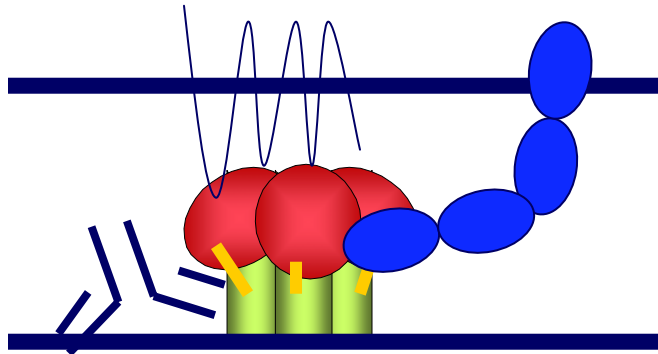
*368 mutant eliminates most trimer binding

Summary of BN-PAGE mapping with HIV+ plasmas

*4 of 8 clade B plasmas (50%)
5 of 16 clade C plasmas (31%)
contain >50% 368 sensitive (CD4bs) activity

*no plasma contained >50% 295 sensitive (2G12-like) activity,
although some competed with mAb 2G12 in virus capture,
suggesting non-neutralizing Abs that overlap the 2G12 epitope

Assays for measuring MPER activity



Modified Neutralization Assay
Post CD4/CCR5
using SOS pseudovirions

	C-HR	2F5	Z13e1	4E10	MSD
HIV-1 YU-2	...QQEKNEQEL	LALDKWASLWNWFDITKWLWYIKIFIMIV...			
HIV-2 7312A	...QQEKNMYELQKLN	SDVFGNWF	DLASWVKYIQYGVYIV...		
7312A-C1	...QQEKNMYEL	LALDKWASLWNWFDITKWLWYIKYGVYIV...			
7312A-C1c	...QQEKNMYEL	LALDSWKNLWNWFDITKWLWYIKYGVYIV...			
7312A-C3	...QQEKNMYEL	LALDKWASLWNWFDLASWVKYIQYGVYIV...			
7312A-C4	...QQEKNMYELQKLN	SDVFGNWF	ITKWLWYIKYGVYIV...		
7312A-C5	...QQEKNMYELQKLN	SDVFGNWF	ITSWVKYIQYGVYIV...		
7312A-C6	...QQEKNMYELQKLN	SDVFGNWF	ITSWIKYIQYGVYIV...		
7312A-C7	...QQEKNMYELQ	ALDKWAVFGNWF	DLASWVKYIQYGVYIV...		
7312A-C8	...QQEKNMYELQKLN	SWASLWNWFDITKWLWYIKYGVYIV...			

Epitope-engrafted HIV-2

Summary of MPER activity

plasma	JR-FL neutralization			HIV-2/YU2 C1 chimera neutralization
	standard	post-CD4/CCR5	% MPER	
1648	40	8	20	1,044
1652	143	19	13	884
1685	368	<2	<1	225
1686	1,329	<2	<1	55
1687	911	<2	<1	73
1688	605	3	1	78
1702	192	31	16	641
LT2	3,609	28	8	<20
BB8	131	<2	<2	31
BB12	413	<2	<1	46
BB14	40	<2	<5	32
BB21	35	<2	<6	35
BB24	20	<2	<10	170
BB28	35	<2	<6	353
BB34	292	200	69	6,734
BB47	100	<2	<2	453
BB55	100	<2	<2	42
BB68	<20	<2	<10	124
BB75	150	<2	<1	83
BB80	50	<2	<4	31
BB81	80	5	6	326
BB87	600	<2	<1	70
BB105	70	3	4	62
BB107	600	<2	<1	20
210	<20	<2	N/A	
211	<20	<2	N/A	
2F5	0.2	0.3		0.138
4E10	2	3		0.0731
Z13e1	26	6		0.282
IgG1b12	0.04	>30		N/A

MPER chimera mapping

clade B plasmas
reactivity scattered
throughout MPER

HIV-2 7312A MPER grafts:

7312A (HIV-2)
YU2
7312A C1 (YU2)
7312A C3 (5' inc. 2F5)
7312A C4 (small 3' inc. 4E10)
7312A C4GW (small 3' inc. Z13, 4E10)
7312A C6 (minimal 4E10)
7312A C7 (minimal 2F5)
7312A C8 (big 3' inc. Z13, 4E10)

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NMYELQKLNSDVFGNWFDLASWVKYIQY  
NEQELLALDKWASLWNWFDITKWLWYIKI  
NMYELLALDKWASLWNWFDITKWLWYIKY  
NMYELLALDKWASLWNWFDLASWVKYIQY  
NMYELQKLNSDVFGNWFDITKWLWYIKY  
NMYELQKLNSDVFGNWFDITKWLWYIKY  
NMYELQKLNSDVFGNWFDITSWIKYIQY  
NMYELQALDKWAVFGNWFDLASWVKYIQY  
NMYELQKLNSWASLWNWFDITKWLWYIKY
```

clade C plasmas
reactivity focused
on 3' part of MPER
Z13e1 and MPER

HIV-2 7312A MPER grafts:

7312A (HIV-2)
YU2
7312A C1 (YU2)
7312A C3 (5' inc. 2F5)
7312A C4 (small 3' inc. 4E10)
7312A C4GW (small 3' inc. Z13, 4E10)
7312A C6 (minimal 4E10)
7312A C7 (minimal 2F5)
7312A C8 (big 3' inc. Z13, 4E10)

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NMYELQKLNSDVFGNWFDLASWVKYIQY  
NEQELLALDKWASLWNWFDITKWLWYIKI  
NMYELLALDKWASLWNWFDITKWLWYIKY  
NMYELLALDKWASLWNWFDLASWVKYIQY  
NMYELQKLNSDVFGNWFDITKWLWYIKY  
NMYELQKLNSDVFGNWFDITKWLWYIKY  
NMYELQKLNSDVFGNWFDITSWIKYIQY  
NMYELQALDKWAVFGNWFDLASWVKYIQY  
NMYELQKLNSWASLWNWFDITKWLWYIKY
```

Sequences of V3 scaffold viruses and V3 peptides

Clade B/C V3.01 and V3.02

JR-FL.V3.01	TRPNNNTRKSIHIGPGRAFYTTG
MW965.V3.01	TRPNNNTRKSVRIGPGQTFYATG
TV1.V3.01	TRPNNNTRKSVRIGPGQAFYATN
JR-FL.V3.02	YTTGEIIGDIRQAHC
MW965.V3.02	YATGAIIGDIRQAHC
TV1.V3.02	YATNDVIGDIRQAHC

Summary of V3 activity

clade B virus (JR-FL d301)

clade C virus (TV1)

clade B
plasma

C H A Q R D I G N N P R T
R T N N P R T
K S I H I G P G R A F Y
T T G E I I G D

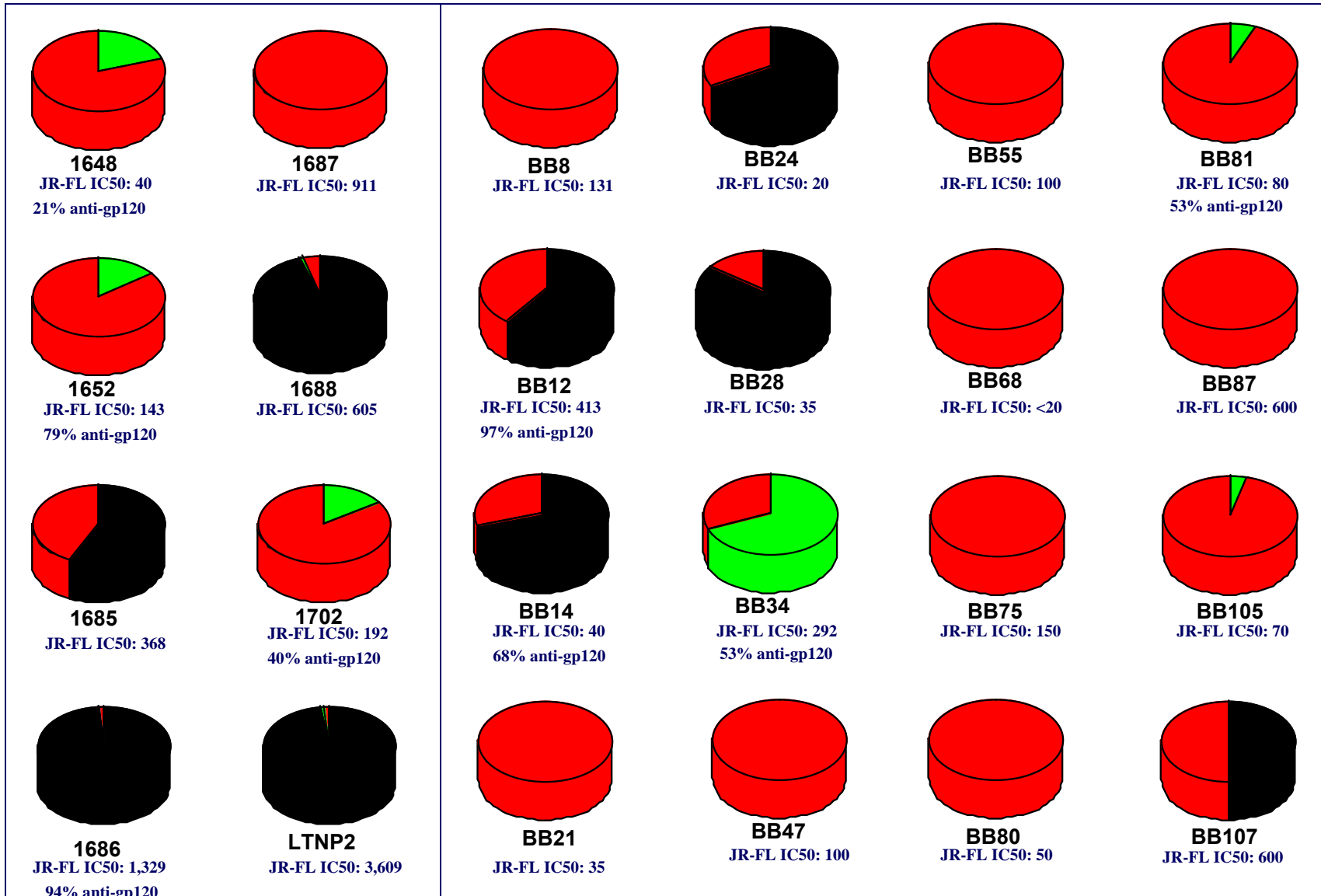
R T N N P R T

C H A Q R D V I G D
A T N D V I G D
R I G P G Q A F Y

clade C
plasma

C H A Q R D V I G D
A T N D V I G D
R I G P G Q A F Y
K S V R I G P G Q A F Y
R T N N P R T

Summary of mapping of JR-FL neutralization activity



Potential impact of HIV+ plasma mapping

- define broad neutralization: (including mixed samples)
- identify novel epitopes
- assist in efforts to isolate new nAbs
- map any newly emerging neutralizing mAbs
- may allow *rational* improvement of promising vaccines,
e.g. if we know the basis of type-specific neutralization activity,
we may be able to make targeted adjustments to broaden it.

What's next?...

Attempt to identify the “other” broad neutralizing activity in HIV+ plasmas:

- 1) Additional point mutants for gp120 fractionations and BN-PAGE
- 2) Combined approaches: e.g. gp120 fractionation and BN-PAGE
- 3) Improved epitope mimics for interference assays

Increase mapping capabilities:

- 4) Adapt other Env clones for mapping:
e.g. B and C panels and transmitted Env isolates to link up with parent vaccine strains and neutralization assay data.

New plasmas and sera:

- 5) Clinical trial sera
- 6) Animal vaccine sera

Thanks...



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the
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