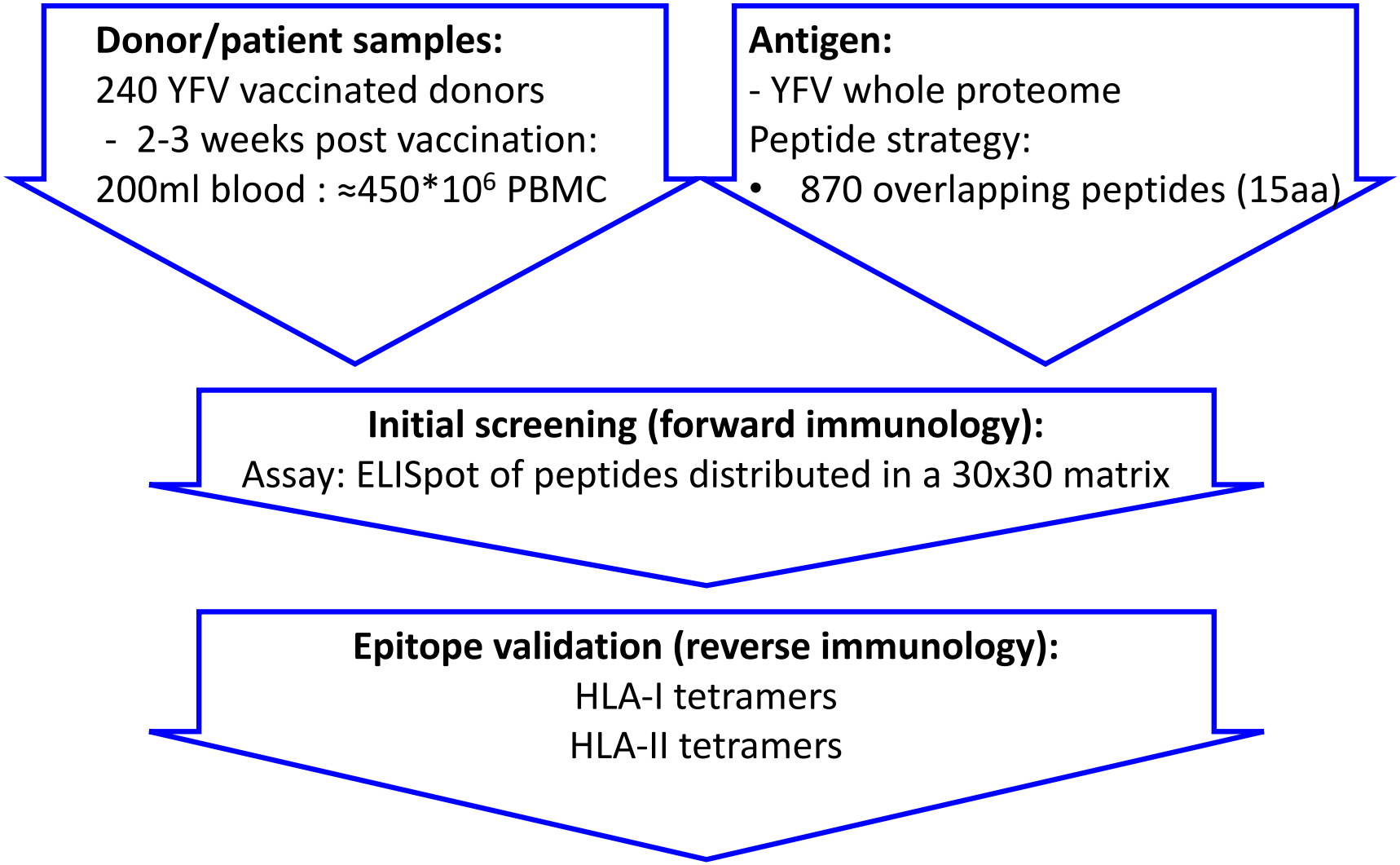
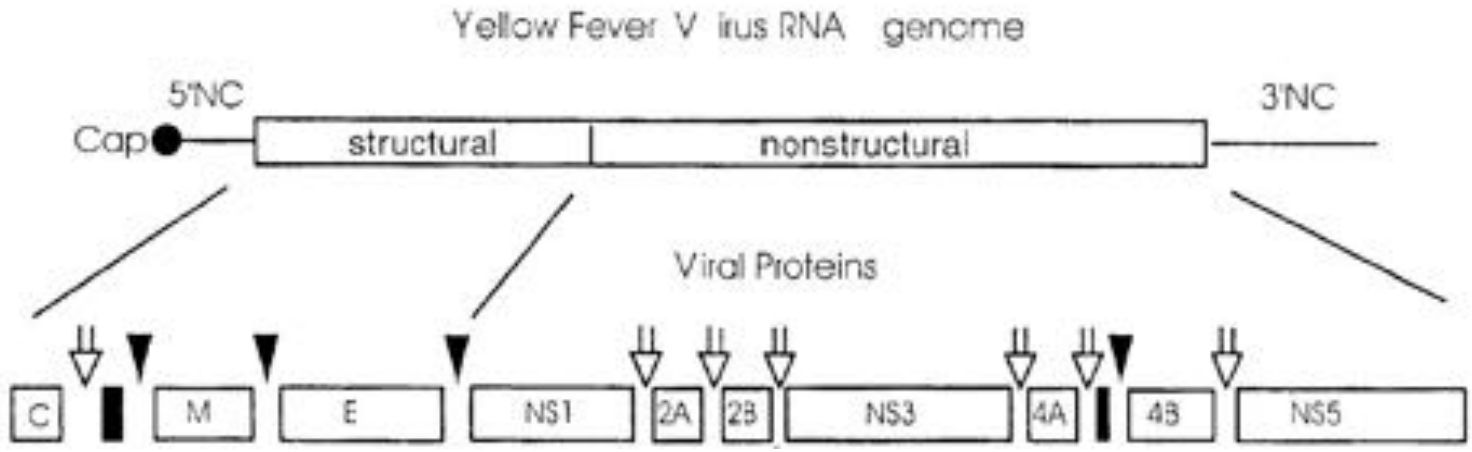
Large-scale T cell epitope discovery in Yellow Fever

YFV T cell epitope discovery strategy



Overlapping peptide representation of antigens



3411 aa long YF precursor protein

MSGRKAQGKTLGVNMVRRGVRSLSNKIKQKTKQIGNRPGPSRGVQGFIFFFLFNILTGKKITAHLKRLWKMLDPRQGLAVLRKVKRVVASLMRGLSSRKRRSHDVLTVQFLILGMLLMTG

MSGRKAQGKTLGVNM
KAQGKTLGVNMVRRG
KTLGVNMVRRGVRSL
VNMVRRGVRSLSNKI
RRGVRSLSNKIKQKT
RSLSNKIKQKTKQIG
NKIKQKTKQIGNRPG
QKTKQIGNRPGPSRG
QIGNRPGPSRGVQGF
RPGPSRGVQGFIFFF
SRGVQGFIFFFLFNI
QGFIFFFLFNILTGK
FFFLFNILTGKKITA
FNILTGKKITAHLKR

≈ 900 YF peptides, 15 amino acids long with 11 amino acid overlap, potentially representing all CD4 and CD8 T cell epitopes

One 30X30 matrix analyzed by ELISpot

Donor YF10-1067

24 positive columns x 27 positive rows ≈ 635 intersections

Matrix Columns

Matrix Rows	SFU/10^6	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30
		261	236	414	340	241	185	236	383	302	302	1685	561	0	0	0	211	409	178	0	0	482	746	426	213	317	576	279	325	0	340
R1	147	25220	25221	25222	25223	25224	25225	25226	25227	25228	25229	25230	25231	25232	25233	25234	25235	25236	25237	25238	25239	25240	25241	25242	25243	25244		25245		25248	15920
R2	414	25249	25250	25251	25252	25253	25254	25255	25256	25257	25258	25259	25260	25261	25262	25263	25264	25265	25266	25267	25268	25269	25270	25271	25272	25273	25274	25275	25276	25277	27463
R3	683	25278	25279	25280	25281	25282	25283	25285	25286	25288	25289	25290	25291	25292	25293	25295	25296	25297	25298	25299	25300	25301	25302	25303	25304	25305	25306	25307	25308	25309	27464
R4	358	25310	25311	25312	25313	25314	25315	25316	25317	25318	25319	25320	25321	25322	25323	25324	25325	25326	25327	25328	25329	25330	25331	25332	25333	25334	25335	25336	25337	25338	27465
R5	353	25339	25340	25341	25342	25343	25344	25345	25346	25347	25348	25349	25350	25351	25352	25353	25354	25355	25356	25357	25358	25359	25360	25361	25362	25363	25364	25365	25366		27466
R6	152	25367	25368	25369	25370	25371	25372	25373	25374	25375	25376	25377	25378	25379	25380	25381	25382	25383	25384	25385	25386	25387	25388	25389	25390	25391	25392	25393	25394	25395	
R7	152	25396	25397	25398	25399	25400	25401	25402	25406	25407	25412	25413	25414	25415	25416	25417	25418	25419	25420	25421	25422	25423	25425	25426	25427	25428	25429	25430	25431	25432	15878
R8	536	25433	25434	25435	25436	25437	25438	25439	25440	25441	25442	25443	25444	25445	25446	25447	25448	25449	25450	25451	25452	25453	25456	25457	25458	25459	25460	25461	25462	25463	27468
R9	117	25464	25465	25466	25467	25468	25469	25470	25471	25472	25473	25474	25475	25476	25477	25478	25479	25480	25481	25482	25483	25484	25485	25486	25487	25488	25489		25490	25491	
R10	259	25492	25493	25494	25495	25496	25497	25498	25499	25500	25501	25502	25503	25505	25506	25507	25508	25509	25512	25513	25514	25515	25516	25517	25518	25519	25520	25521	25522	25523	
R11	371	25524	25525	25526	25527	25528	25529	25530	25531	25532	25533	25534	25536	25537	25538	25539	25540	25541	25542		25545	25546	25547	25548	25549	25550	25551	25552	25553	25554	
R12	91	25555	25556	25557	25558	25559	25560	25562	25563	25564	25565	25566	25568	25569	25570	25571	25572	25573	25574	25575	25576	25577	25578	25579	25580	25581	25582	25583	25584	25585	27475
R13	140	25586	25587		25588	25589	25590	25591	25592	25593	25594	25595	25596	25597	25598	25599	25600	25601	25602	25603	25604	25605	25606	25607	25608	25609	25610	25611	25612	25613	27473
R14	216	25614	25615	25616	25617	25618	25619	25620	25621	25622	25623	25624	25625	25626	25627	25628	25629	25630	25631	25632	25633	25634	25635	25636	25637	25638	25639	25640	25641	25642	
R15	393	25643	25644	25645	25646	25647	25648	25649	25650	25651	25652	25653	25654	25655	25656	25657	25658	25659	25660	25661	25662	25663	25664	25665	25666	25667	25668	25669	25670	25671	
R16	124	25672	25673	25674	25675	25676	25677	25678	25679	25680	25681	25682	25683	25684	25685	25686	25687	25688	25689	25690	25691	25692	25693	25694	25695	25696	25697	25698	25699	25700	
R17	119	25701	25702	25703	25704	25705	25706	25707	25708	25709	25710	25711	25712	25713	25714	25715	25716	25717	25718	25719	25720	25721	25722	25723	25724	25725	25726	25727	25728	25729	
R18	0	**25730**	**25731**	**25732**	**25733**	**25734**	**25735**	**25736**	**25737**	**25738**	**25739**	**25740**	**25741**	25742	25743	25744	**25745**	**25746**	**25747**	25748	25749	**25750**	**25751**	**25752**		**25753**	**25754**	**25755**	**25756**	25757	
R19	218		25762	25763	25764	25765	25766	25767	25768	25769	25770	25771	25772			25775	25776	25777	25778	25779	25782	25783	25784	25785	25786	25787	25788	25789	25790	25791	15872
R20	343	25792	25793	25794	25795	25796	25797	25798	25799	25801	25802	25803	25804	25805	25806		25808	25810	25811	25812	25813	25814	25815	25816	25817	25818	25819	25820	25821	25822	27476
R21	830	25823	25824	25825	25829	25830	25831	25832	25833	25834	25835	25837	25838	25839	25840	25841		25842	25843	25844	25845	25846	25847	25848	25849	25850	25851	25852	25853	25854	27477
R22	0	25855	25856	25857	25858	25859	25860	25861	25862	25863	25864	25865	25866	25867	25868	25869	25870	25871	25872	25873	25874	25875	25876	25877	25878	25879	25880	25881	25882	25883	
R23	99	25885	25886	25887	25888	25889	25890	25891	25892	25893	25894	25895	25896	25897	25898	25899	25900	25901	25902	25903	25904	25905	25906	25907	25908	25909	25910	25911	25912	25913	27479
R24	617	25914	25915	25916	25917	25918	25919	25920	25921	25922	25923	25924	25925	25926	25927	25928	25929	25930	25931	25932	25933	25934	25935	25936	25937	25938	25939	25940	25941	25942	27480
R25	206	25943	25944	25945	25946	25947	25948	25949	25950	25951	25952	25953	25954	25955	25956	25957	25958	25959	25960	25961	25962	25963	25964	25965	25966	25967	25968	25969	25970	25971	
R26	124	25972	25973	25974	25975	25976	25977	25978	25979	25980	25981	25982	25983	25984	25985	25986	25987	25988	25989	25990	25991	25992	25993	25994	25995	25996	25997	25998	25999	26000	27471
R27	289	26001	26002	26003	26004	26005	26006	26007	26008	26009	26010	26011	26012	26013	26014	26015	26016	26017	26018	26019	26020	26021	26022	26023	26024	26025	26026	26027	26028	26029	15887
R28	99	26030	26031	26032	26033	26035	26036	26037	26038	26039	26040	26041	26042	26043	26044	26045	26046	26047	26048	26049	26050	26051	26052	26053	26054	26055	26056	26057	26058	26059	15916
R29	325	26060	26061	26062	26063	26064	26065	26066	26067	26068	26069	26482	26483	26484	26486	26487	26488	26489	26490	26491	26492	26493	26494	26495	26496	26497	26498	26499	26500	26501	
R30	0	26502	26503	26504	26505	26506	26507	26508	26509	26510	26511	26512	26513	26514	26515	26516	26517	26518	26520	26521	26522	26523	26524	26525	26526	26527	26528	26529	26530	26531	

Four 15x15 matrices analyzed by ICS

Donor YF10-1067

Intersections

ELISpot 30x30: 635

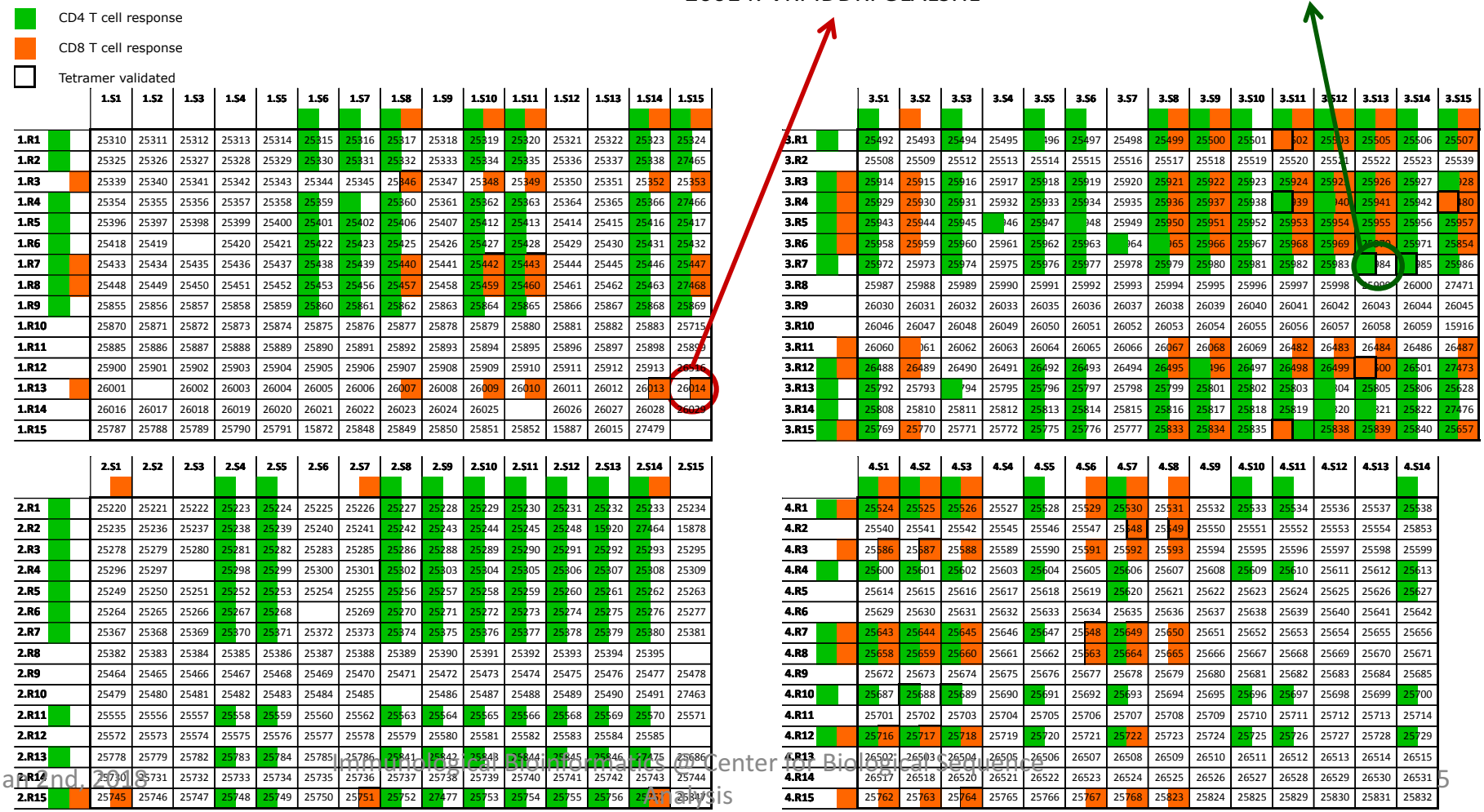
ICS four 15x15: 345

CD4 responses: 42 (7 tetramer validated)

CD8 responses: 23 (23 tetramer validated)

CD8 T cell response
26014: VRPIDDRFGLALSHL

CD4 T cell response
25984: EQEILNYMSPHHKKL



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1.R1	25310	25311	25312	25313	25314	25315	25316	25317	25318	25319	25320	25321	25322	25323	25324
1.R2	25325	25326	25327	25328	25329	25330	25331	25332	25333	25334	25335	25336	25337	25338	27465
1.R3	25339	25340	25341	25342	25343	25344	25345	25346	25347	25348	25349	25350	25351	25352	25353
1.R4	25354	25355	25356	25357	25358	25359		25360	25361	25362	25363	25364	25365	25366	27466
1.R5	25396	25397	25398	25399	25400	25401	25402	25406	25407	25412	25413	25414	25415	25416	25417
1.R6	25418	25419		25420	25421	25422	25423	25425	25426	25427	25428	25429	25430	25431	25432
1.R7	25433	25434	25435	25436	25437	25438	25439	25440	25441	25442	25443	25444	25445	25446	25447
1.R8	25448	25449	25450	25451	25452	25453	25456	25457	25458	25459	25460	25461	25462	25463	27468
1.R9	25855	25856	25857	25858	25859	25860	25861	25862	25863	25864	25865	25866	25867	25868	25869
1.R10	25870	25871	25872	25873	25874	25875	25876	25877	25878	25879	25880	25881	25882	25883	25715
1.R11	25885	25886	25887	25888	25889	25890	25891	25892	25893	25894	25895	25896	25897	25898	25899
1.R12	25900	25901	25902	25903	25904	25905	25906	25907	25908	25909	25910	25911	25912	25913	25916
1.R13	26001		26002	26003	26004	26005	26006	26007	26008	26009	26010	26011	26012	26013	26014
1.R14	26016	26017	26018	26019	26020	26021	26022	26023	26024	26025		26026	26027	26028	26029
1.R15	25787	25788	25789	25790	25791	15872	25848	25849	25850	25851	25852	15887	26015	27479	

	2.S1	2.S2	2.S3	2.S4	2.S5	2.S6	2.S7	2.S8	2.S9	2.S10	2.S11	2.S12	2.S13	2.S14	2.S15
2.R1	25220	25221	25222	25223	25224	25225	25226	25227	25228	25229	25230	25231	25232	25233	25234
2.R2	25235	25236	25237	25238	25239	25240	25241	25242	25243	25244	25245	25248	15920	27464	15878
2.R3	25278	25279	25280	25281	25282	25283	25285	25286	25288	25289	25290	25291	25292	25293	25295
2.R4	25296	25297		25298	25299	25300	25301	25302	25303	25304	25305	25306	25307	25308	25309
2.R5	25249	25250	25251	25252	25253	25254	25255	25256	25257	25258	25259	25260	25261	25262	25263
2.R6	25264	25265	25266	25267	25268		25269	25270	25271	25272	25273	25274	25275	25276	25277
2.R7	25367	25368	25369	25370	25371	25372	25373	25374	25375	25376	25377	25378	25379	25380	25381
2.R8	25382	25383	25384	25385	25386	25387	25388	25389	25390	25391	25392	25393	25394	25395	
2.R9	25464	25465	25466	25467	25468	25469	25470	25471	25472	25473	25474	25475	25476	25477	25478
2.R10	25479	25480	25481	25482	25483	25484	25485		25486	25487	25488	25489	25490	25491	27463
2.R11	25555	25556	25557	25558	25559	25560	25562	25563	25564	25565	25566	25568	25569	25570	25571
2.R12	25572	25573	25574	25575	25576	25577	25578	25579	25580	25581	25582	25583	25584	25585	
2.R13	25778	25779	25782	25783	25784	25785	25786	25841	25842	25843	25844	25845	25846	27475	25686
2.R14	25730	25731	25732	25733	25734	25735	25736	25737	25738	25739	25740	25741	25742	25743	25744
2.R15	25745	25746	25747	25748	25749	25750	25751	25752	27477	25753	25754	25755	25756	25757	25841

	3.S1	3.S2	3.S3	3.S4	3.S5	3.S6	3.S7	3.S8	3.S9	3.S10	3.S11	3.S12	3.S13	3.S14	3.S15
3.R1	25492	25493	25494	25495	496	25497	25498	25499	25500	25501	502	25503	25505	25506	25507
3.R2	25508	25509	25512	25513	25514	25515	25516	25517	25518	25519	25520	25521	25522	25523	25539
3.R3	25914	25915	25916	25917	25918	25919	25920	25921	25922	25923	25924	25925	25926	25927	928
3.R4	25929	25930	25931	25932	25933	25934	25935	25936	25937	25938	939	940	25941	25942	980
3.R5	25943	25944	25945	946	25947	948	25949	25950	25951	25952	25953	25954	25955	25956	25957
3.R6	25958	25959	25960	25961	25962	25963	964	965	25966	25967	25968	25969	25970	25971	25854
3.R7	25972	25973	25974	25975	25976	25977	25978	25979	25980	25981	25982	25983	984	985	25986
3.R8	25987	25988	25989	25990	25991	25992	25993	25994	25995	25996	25997	25998	25999	26000	27471
3.R9	26030	26031	26032	26033	26035	26036	26037	26038	26039	26040	26041	26042	26043	26044	26045
3.R10	26046	26047	26048	26049	26050	26051	26052	26053	26054	26055	26056	26057	26058	26059	15916
3.R11	26060	061	26062	26063	26064	26065	26066	26067	26068	26069	26482	26483	26484	26486	26487
3.R12	26488	26489	26490	26491	26492	26493	26494	26495	496	26497	26498	26499	500	26501	27473
3.R13	25792	25793	794	25795	25796	25797	25798	25799	25801	25802	25803	804	25805	25806	25628
3.R14	25808	25810	25811	25812	25813	25814	25815	25816	25817	25818	25819	820	821	25822	27476
3.R15	25769	25770	25771	25772	25775	25776	25777	25833	25834	25835		25838	25839	25840	25657

	4.S1	4.S2	4.S3	4.S4	4.S5	4.S6	4.S7	4.S8	4.S9	4.S10	4.S11	4.S12	4.S13	4.S14
4.R1	25524	25525	25526	25527	25528	25529	25530	25531	25532	25533	25534	25536	25537	25538
4.R2	25540	25541	25542	25545	25546	25547	25548	25549	25550	25551	25552	25553	25554	25853
4.R3	25586	25587	25588	25589	25590	25591	25592	25593	25594	25595	25596	25597	25598	25599
4.R4	25600	25601	25602	25603	25604	25605	25606	25607	25608	25609	25610	25611	25612	25613
4.R5	25614	25615	25616	25617	25618	25619	25620	25621	25622	25623	25624	25625	25626	25627
4.R6	25629	25630	25631	25632	25633	25634	25635	25636	25637	25638	25639	25640	25641	25642
4.R7	25643	25644	25645	25646	25647	25648	25649	25650	25651	25652	25653	25654	25655	25656
4.R8	25658	25659	25660	25661	25662	25663	25664	25665	25666	25667	25668	25669	25670	25671
4.R9	25672	25673	25674	25675	25676	25677	25678	25679	25680	25681	25682	25683	25684	25685
4.R10	25687	25688	25689	25690	25691	25692	25693	25694	25695	25696	25697	25698	25699	25700
4.R11	25701	25702	25703	25704	25705	25706	25707	25708	25709	25710	25711	25712	25713	25714
4.R12	25716	25717	25718	25719	25720	25721	25722	25723	25724	25725	25726	25727	25728	25729
4.R13	26502	26503	26504	26505	26506	26507	26508	26509	26510	26511	26512	26513	26514	26515
4.R14	26517	26518	26520	26521	26522	26523	26524	26525	26526	26527	26528	26529	26530	26531
4.R15	25762	25763	25764	25765	25766	25767	25768	25823	25824	25825	25829	25830	25831	25832

CD8 T cell epitope prediction; NetMHCpan

CD8 T cell response

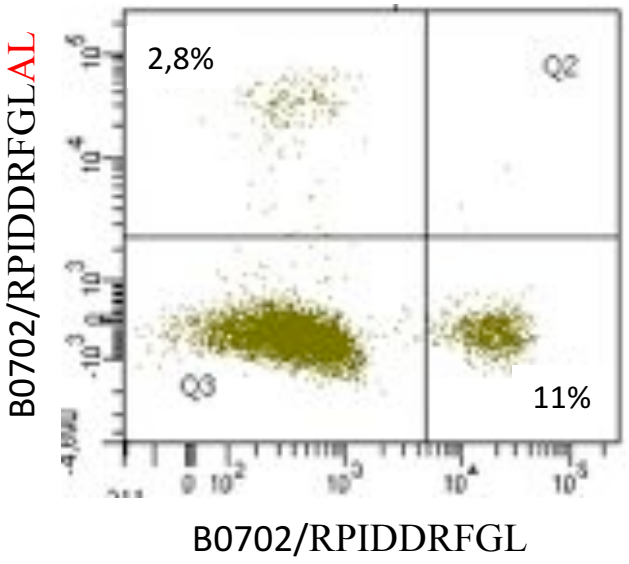
26014: VRPIDDRFGLALSHL

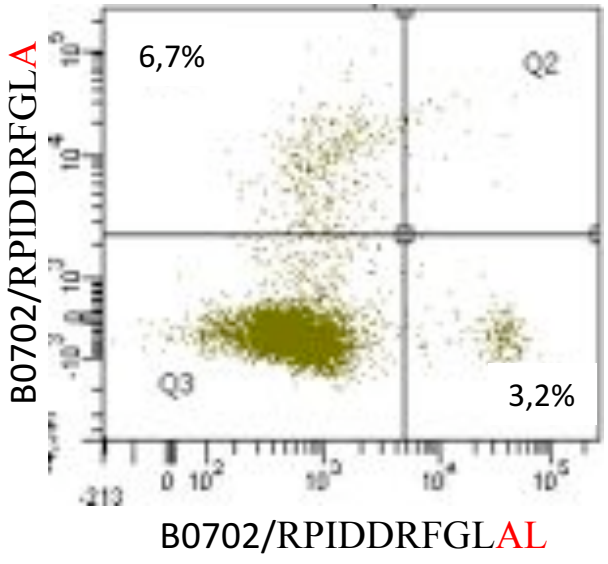
```
# HLArestrictor with NetMHCpan Inhouse version 2.5
# HLA types used:  HLA-A02:01,  HLA-A32:01,  HLA-B07:02,  HLA-B40:01,  HLA-C07:02,  HLA-C03:04
# Peptide lengths:  8,  9,  10,  11
# Sort-method: OR.  Sort-mode: HLA-oriented
# %rank threshold for strong binding peptides: 0.5%rank
# %rank threshold for weak binding peptides: 2.0%rank
# Affinity threshold for strong binding peptides: 50.0nM
# Affinity threshold for weak binding peptides: 500.0nM
# Number of predictions per peptide: Not specified
# Non-binders shown up to a prediction score of 1.0*(weak binding threshold)

Results for Peptide Sequence: VRPIDDRFGLALSHL
-----------------------------------------------------------------------------
Pos     Length  Peptide         HLA     1-log50k(aff)   Affinity(nM)    %Rank           Label           Estimated prediction accuracy

2       11      RPIDDRFGLAL     HLA-B07:02      0.827           7               0.05            Strong binder   0.853
2       9       RPIDDRFGL       HLA-B07:02      0.682           31              0.25            Strong binder   0.853
2       10      RPIDDRFGLA      HLA-B07:02      0.483           268             1.0             Weak binder     0.853

1       10      VRPIDDRFGL      HLA-C07:02      0.402           NA              2.0             Weak binder     0.853

8       8       FGLALSHL        HLA-C03:04      0.506           210             4.0             Combined binder 0.694
2       11      RPIDDRFGLAL     HLA-C03:04      0.505           212             4.0             Combined binder 0.694
```

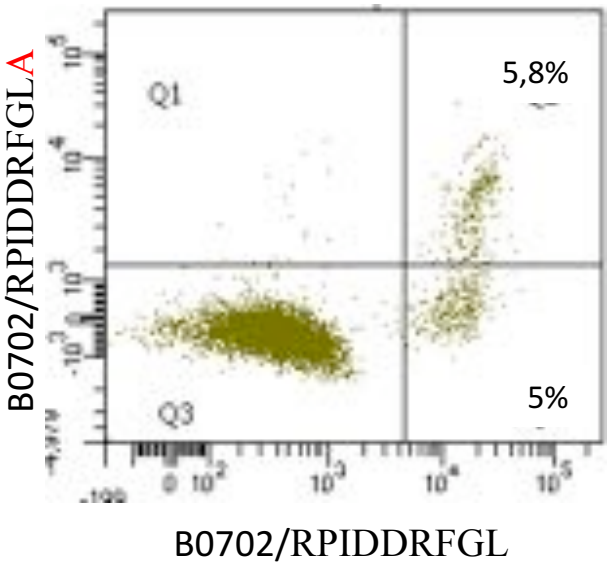
HLA-I tetramer validation

VRPIDDRFGLALSHL
RPIDDRFGL
RPIDDRFGLA
RPIDDRFGLAL

HLA-	Peptide	Prediction (nM)	Stability ($t_{½}$)	Ex Vivo Tetramer analysis TMR+ (% CD8+)	TMR+ (% CD8+, CD38+)
B07:02	RPIDDRFGL	31	6.3	0,3%	2.3%
B07:02	RPIDDRFGLA	268	3.1	0%	0%
B07:02	RPIDDRFGLAL	7	2.5	0.1%	1%





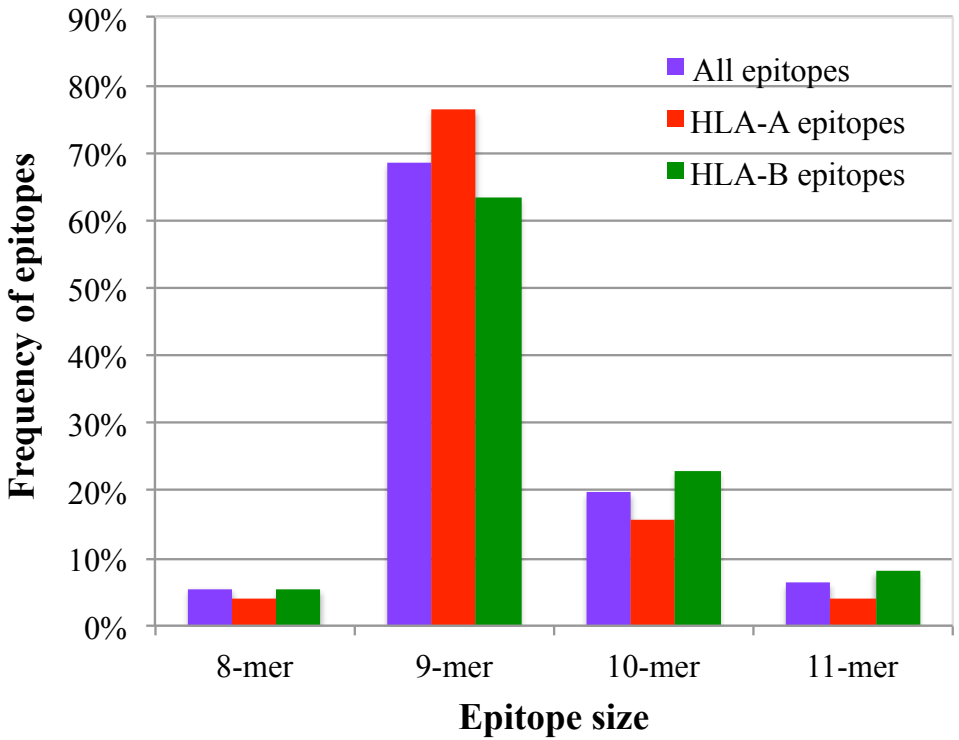


Identified YFV specific CD8+ T cell epitopes

Epitope screening and identification in 50 YFV vaccinated donors

Tetramer validated 127 epitopes

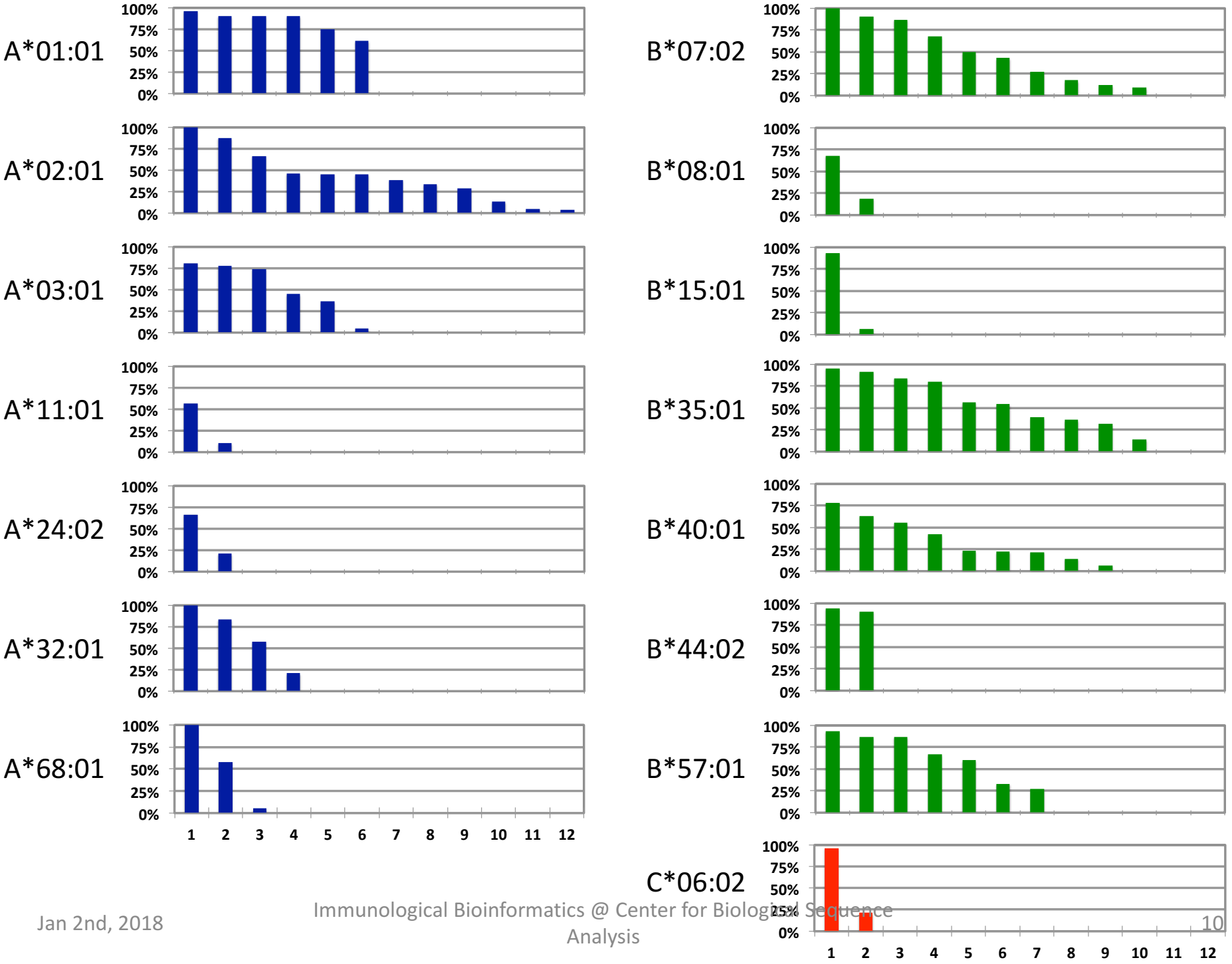
- 41 different HLA-I molecules
- 103 different peptides



$CD8^+$ T cell dominance

The 127 tetramer validated epitopes were Identified by screening of 50 YFV vaccinated donors

For dominance analysis the 127 epitopes were evaluated in 192 YFV vaccinated donors and whenever possible tested in at least 20 donors with the HLA-I allotype in question

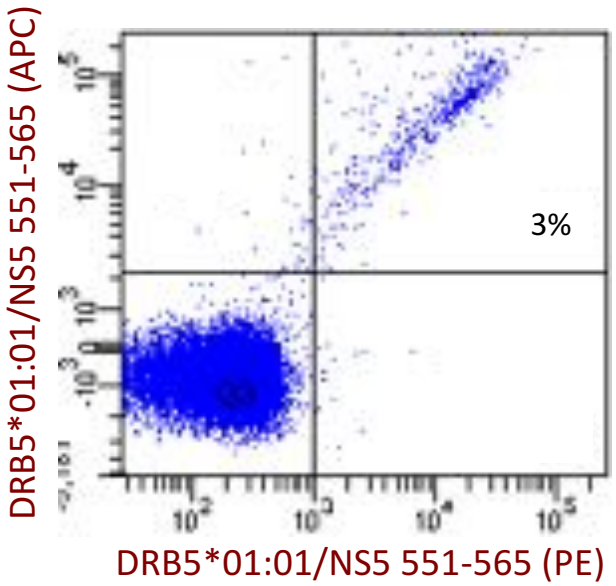

A*01:01
A*02:01
A*03:01
A*11:01
A*24:02
A*32:01
A*68:01
B*07:02
B*08:01
B*15:01
B*35:01
B*40:01
B*44:02
B*57:01
C*06:02
100%
75%
50%
25%
0%
1 2 3 4 5 6 7 8 9 10 11 12

CD4 T cell epitope prediction; NetMHCIIpan

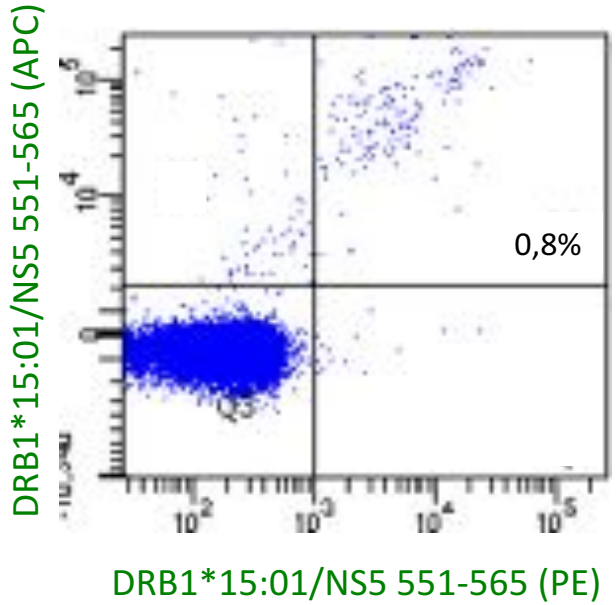
YF10-1067: DRB1*13:02; DRB1*15:01; DRB3*03:01; DRB5*01:01

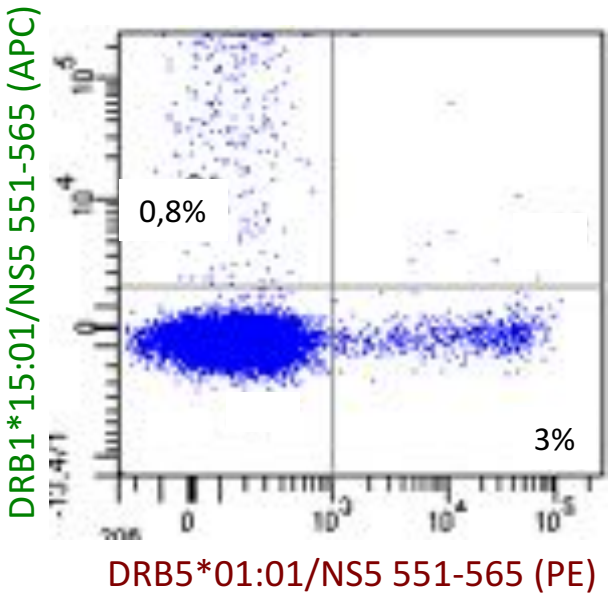
```
# NetMHCIIpan version 2.1

# Input is in FSA format
# Threshold for Strong binding peptides  50.000
# Threshold for Weak binding peptides 500.000
# Rank Threshold for Strong binding peptides   0.500
# Rank Threshold for Weak binding peptides   2.000
-----------------------------------------------------------------------------------------------------------
  pos       HLA            peptide        Identity Pos       Core 1-log15k(aff) Affinity(nM)    %Rank  BindLevel
-----------------------------------------------------------------------------------------------------------
    0 DRB1*1302    EQEILNYMSPHHKKL        Sequence   3  ILNYMSPHH         0.332       618.51    32.00
-----------------------------------------------------------------------------------------------------------

Protein Sequence. Allele DRB1*1302. Number of high binders 0. Number of weak binders 0. Number of peptides 1

-----------------------------------------------------------------------------------------------------------
# Threshold for Strong binding peptides  50.000
# Threshold for Weak binding peptides 500.000
# Rank Threshold for Strong binding peptides   0.500
# Rank Threshold for Weak binding peptides   2.000
-----------------------------------------------------------------------------------------------------------
  pos       HLA            peptide        Identity Pos       Core 1-log15k(aff) Affinity(nM)    %Rank  BindLevel
-----------------------------------------------------------------------------------------------------------
    0 DRB1*1501    EQEILNYMSPHHKKL        Sequence   3  ILNYMSPHH         0.680        21.68     0.70 <= SB
-----------------------------------------------------------------------------------------------------------

Protein Sequence. Allele DRB1*1501. Number of high binders 1. Number of weak binders 0. Number of peptides 1

-----------------------------------------------------------------------------------------------------------
# Threshold for Strong binding peptides  50.000
# Threshold for Weak binding peptides 500.000
# Rank Threshold for Strong binding peptides   0.500
# Rank Threshold for Weak binding peptides   2.000
-----------------------------------------------------------------------------------------------------------
  pos       HLA            peptide        Identity Pos       Core 1-log15k(aff) Affinity(nM)    %Rank  BindLevel
-----------------------------------------------------------------------------------------------------------
    0 DRB3*0301    EQEILNYMSPHHKKL        Sequence   3  ILNYMSPHH         0.507       114.90    16.00 <= WB
-----------------------------------------------------------------------------------------------------------

Protein Sequence. Allele DRB3*0301. Number of high binders 0. Number of weak binders 1. Number of peptides 1

-----------------------------------------------------------------------------------------------------------
# Threshold for Strong binding peptides  50.000
# Threshold for Weak binding peptides 500.000
# Rank Threshold for Strong binding peptides   0.500
# Rank Threshold for Weak binding peptides   2.000
-----------------------------------------------------------------------------------------------------------
  pos       HLA            peptide        Identity Pos       Core 1-log15k(aff) Affinity(nM)    %Rank  BindLevel
-----------------------------------------------------------------------------------------------------------
    0 DRB5*0101    EQEILNYMSPHHKKL        Sequence   4  LNYMSPHHK         0.858         3.91     0.30 <= SB
-----------------------------------------------------------------------------------------------------------
```

HLA-II tetramer analysis



HLA-	Protein	Sequence	Binding (nM)
DRB1*15:01	NS5 551-565	EQEILNYMSPHHKKL	29
DRB5*01:01	NS5 551-565	EQEILNYMSPHHKKL	2

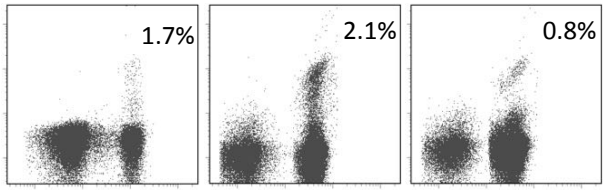




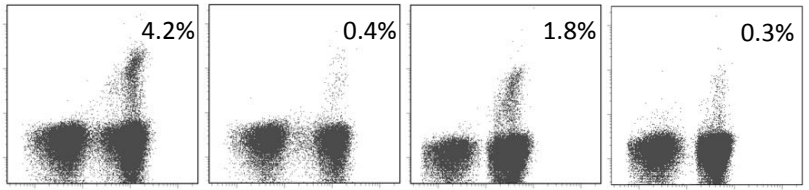
Tetramer validated CD4⁺ T cell epitopes; donor YF1067

Donor YF1067	CD4⁺ T cell responses
15-mer peptides recognized	**36**
Tetramer validated epitopes	**22 out of 36**

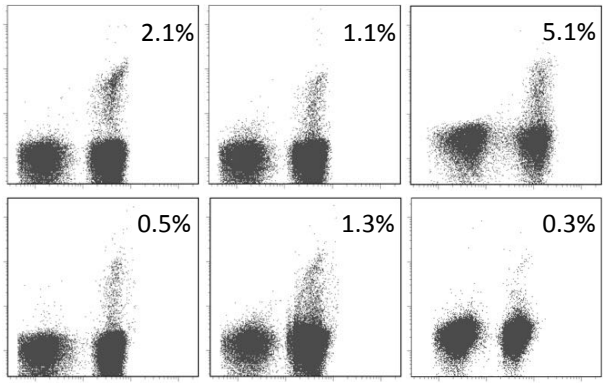
DRB1*13:02



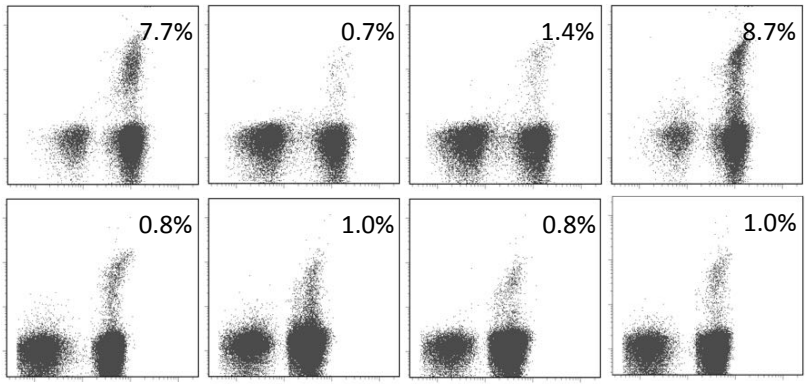
DRB1*15:01



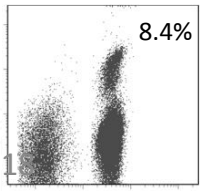
DRB3*03:01



DRB5*01:01



DPA01:03/DPB04:01



Tetramer validated CD4$^+$ T cell epitopes

CD4+ T cell epitopes identified:

119 different 15-mer peptide epitopes

Analyzed 142 HLA-II tetramers

- 14 different HLA-II molecules
- 63 different peptides

Tetramer validated 51 epitopes

- 13 different HLA-II molecules
- 39 different peptides

HLA-	Number of epitopes
DRB1*01:01	5
DRB1*0301	6
DRB1*0401	2
DRB1*0404	6
DRB1*07:01	2
DRB1*11:01	1
DRB1*1301	2
DRB1*1302	6
DRB1*1501	3
DRB3*0101	2
DRB3*0301	7
DRB5*0101	9
DPA1*01:03/DPB1*04:01	1
Total	**51**

Modes of T cell Epitope Discovery

”Forward Immunology”

Have a T cell response -> search experimentally for epitope specificity & restriction

”Reverse Immunology”

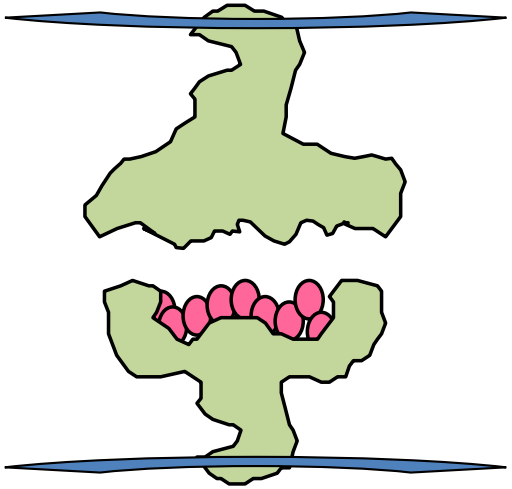
Predict epitope specificity and restriction -> search experimentally for responding T cells

”Forward & Reverse” – HLA tetramer empowered

Have a T cell response -> predict epitope specificity and restriction -> validate experimentally

Cancer immunotherapy against cancer mutations

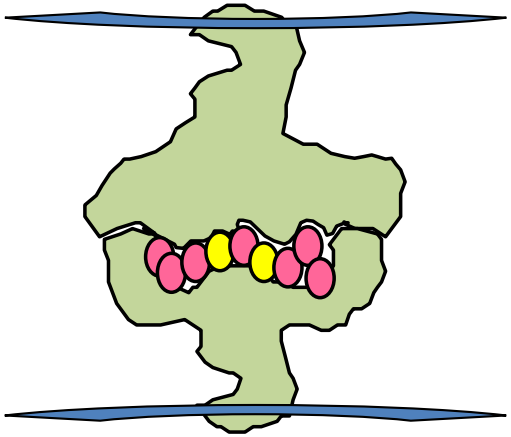
Self tolerant TcR



Self-peptide
APC

Auto tolerance

Cencer-specific TcR



Cancer mutation
APC

Cancer Immunotherapy

Cancer immunotherapy against cancer mutations

- Identify cancer mutation by
  - exome sequencing
  - “elute and sequence” (nLC-MS)
  - bioinformatics-driven epitope identification
- Generate immunotherapy
  - Immune check-point inhibitors
  - Vaccination
  - Adoptive T cell transfer

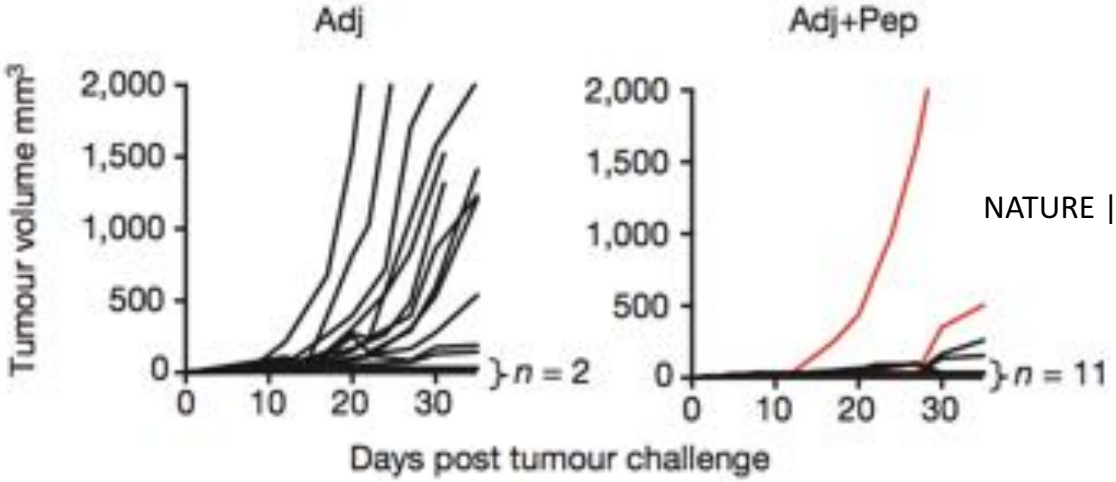
Cancer immunotherapy against cancer mutations

LETTER

doi:10.1038/nature14001

Predicting immunogenic tumour mutations by combining mass spectrometry and exome sequencing

Mahesh Yadav[1]*, Suchit Jhunjhunwala[1]*, Qui T. Phung[1], Patrick Lupardus[1], Joshua Tanguay[1], Stephanie Bumbaca[1], Christian Franci[1], Tommy K. Cheung[2], Jens Fritsche[2], Toni Weinschenk[2], Zora Modrusan[1], Ira Mellman[1], Jennie R. Lill[1]§ & Lélia Delamarre[1]§



NATURE | VOL 515 | 27 NOVEMBER 2014

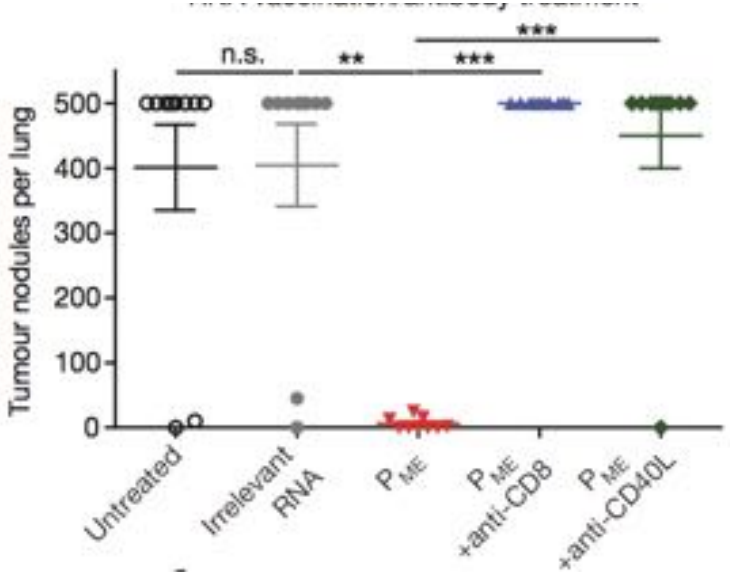
Cancer immunotherapy against cancer mutations

LETTER

doi:10.1038/nature14426

Mutant MHC class II epitopes drive therapeutic immune responses to cancer

Sebastian Kreiter[2], Mathias Vormehr[2]*, Niels van de Roemer[2]*, Mustafa Diken[2], Martin Löwer[1], Jan Diekmann[1,3], Sebastian Boegel[1], Barbara Schrörs[2], Fulvia Vascotto[1], John C. Castle[1], Arbel D. Tadmor[1], Stephen P. Schoenberger[4], Christoph Huber[2], Özlem Türeci[1]§ & Ugur Sahin[1,2,3]§



NATURE | VOL 520 | 30 APRIL 2015

Cancer immunotherapy against cancer mutations

- Yadav et al
  - *"to be fully useful in the clinic, it will probably be necessary … to rely entirely on computational predictions of peptide binding*"
- Kreiter et al
  - T cell epitopes "*selected as vaccine targets solely through bioinformatic prioritization on the basis of their expression levels and HLA-II-binding capacity … may exert their antitumour function by augmentation of CTL responses through epitope spreading*"

Cancer immunotherapy against cancer mutations

LETTER

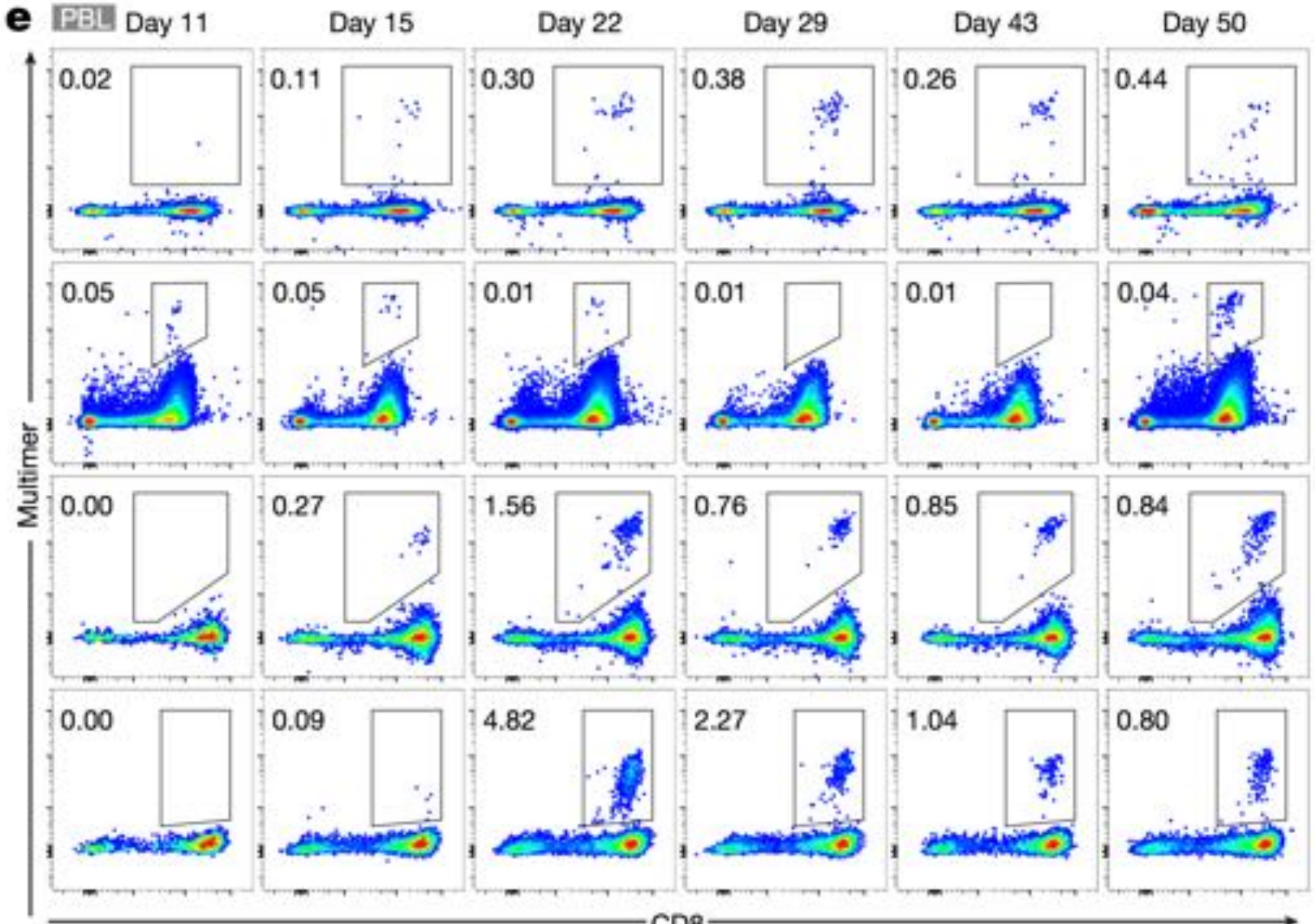
doi:10.1038/nature23003

Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer

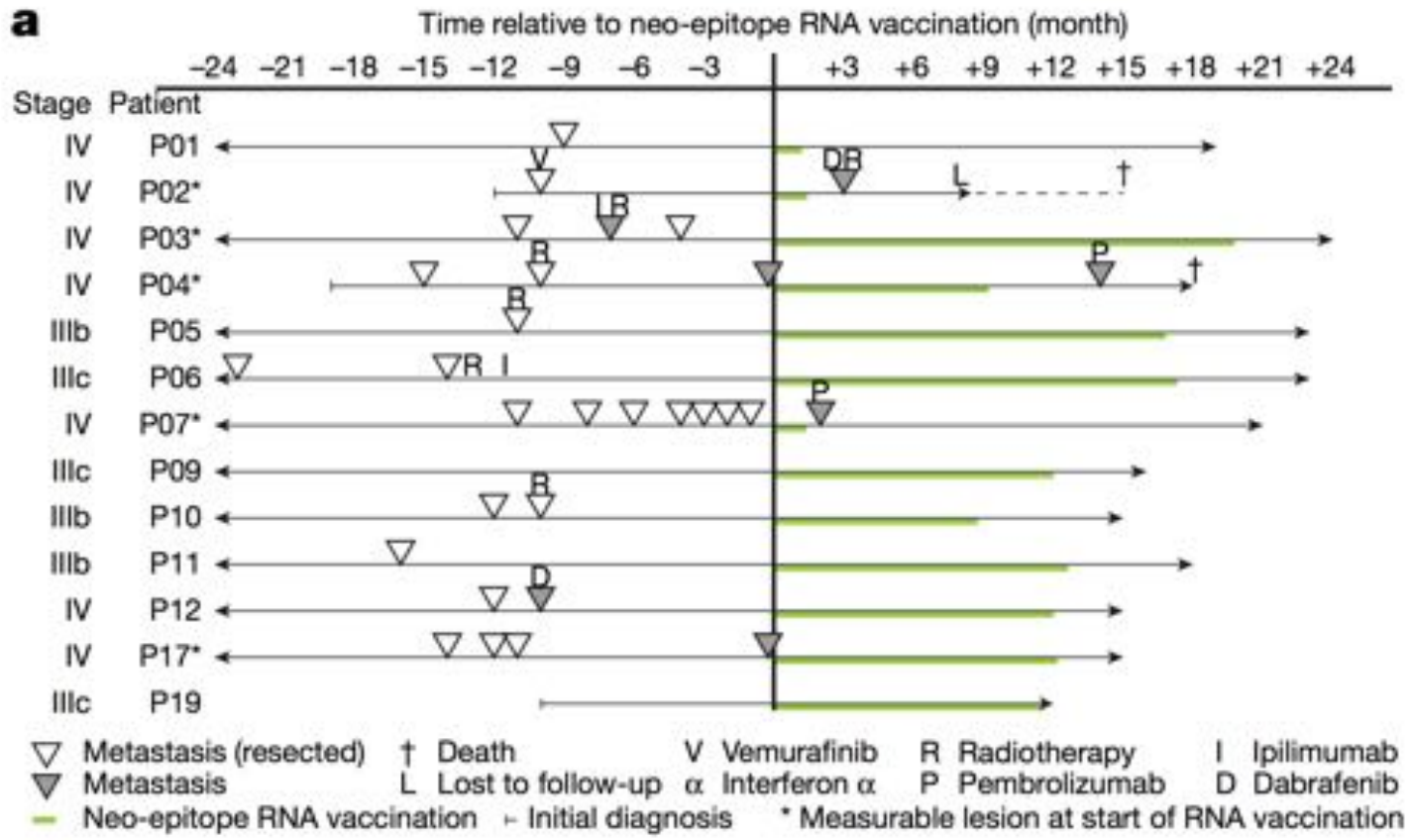
Ugur Sahin[1,2,3], Evelyna Derhovanessian[1], Matthias Miller[1], Björn-Philipp Kloke[1], Petra Simon[1], Martin Löwer[2], Valesca Bukur[1,2], Arbel D. Tadmor[2], Ulrich Luxemburger[1], Barbara Schrörs[2], Tana Omokoko[1], Mathias Vormehr[1,3], Christian Albrecht[2], Anna Paruzynski[1], Andreas N. Kuhn[1], Janina Buck[1], Sandra Heesch[1], Katharina H. Schreeb[1], Felicitas Müller[1], Inga Ortseifer[1], Isabel Vogler[1], Eva Godehardt[1], Sebastian Attig[2,3], Richard Rae[2], Andrea Breitkreuz[1], Claudia Tolliver[1], Martin Suchan[2], Goran Martic[2], Alexander Hohberger[3], Patrick Sorn[2], Jan Diekmann[1], Janko Ciesla[4], Olga Waksmann[4], Alexandra-Kemmer Brück[1], Meike Witt[1], Martina Zillgen[1], Andree Rothermel[2], Barbara Kasemann[2], David Langer[1], Stefanie Bolte[1], Mustafa Diken[1,2], Sebastian Kreiter[1,2], Romina Nemecek[5], Christoffer Gebhardt[6,7], Stephan Grabbe[3], Christoph Höller[5], Jochen Utikal[6,7], Christoph Huber[1,2,3], Carmen Loquai[3]* & Özlem Türeci[8]*

NATURE|VOL 547|13 JULY 2017

Cancer immunotherapy against cancer mutations



Cancer immunotherapy against cancer mutations



13 late stage melanoma ptt received RNA mutanome vaccines

8 were tumor free at vaccination. CD4 & CD8 T cell responses were induced. No relapse

5 had relapse before vaccination

2 developed clinical response

1 stable, 1 slowly progressing

1 progressive, terminated, received ICI, complete response

Cancer immunotherapy against cancer mutations

An immunogenic personal neoantigen vaccine for patients with melanoma

Patrick A. Ott[1,2,3*], Zhuting Hu[1*], Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4†], Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]

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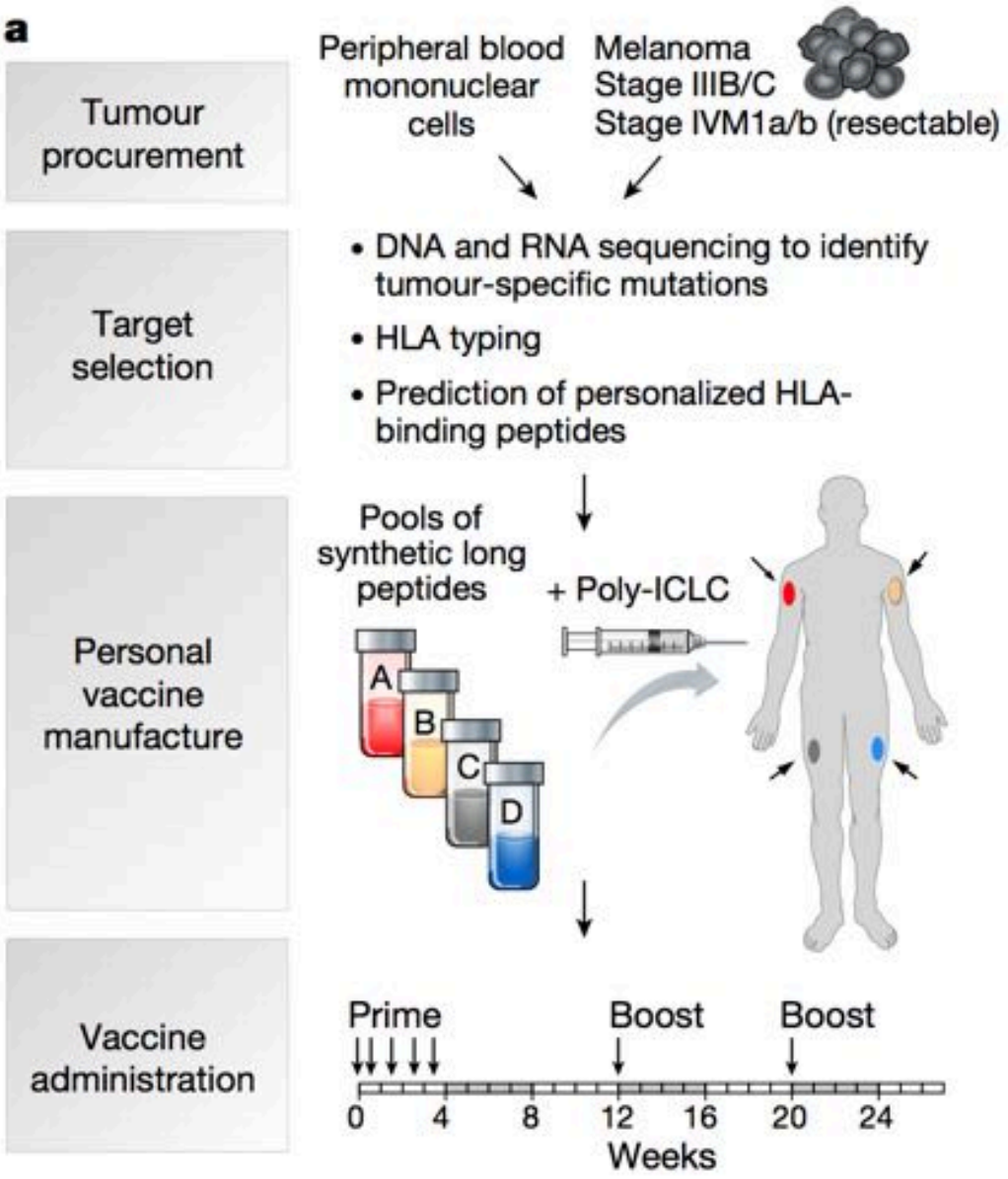
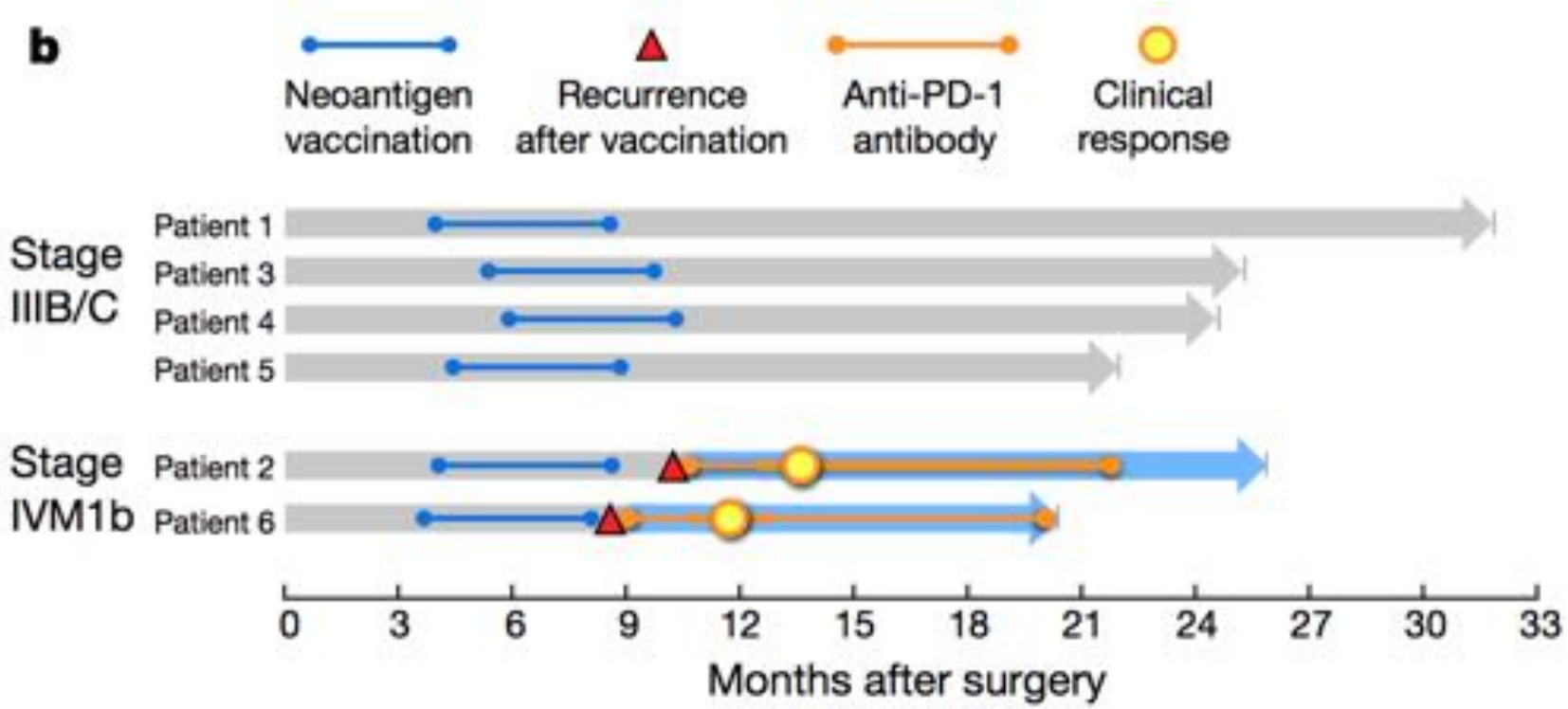
Cancer immunotherapy against cancer mutations



Cancer immunotherapy against cancer mutations



The MHC project

- Department of Immunology and Medical Microbiology, University of Copenhagen
  - Anette Stryhn, Thomas Østerbye, Daniel Jensen, Kasper Lamberth, Mikkel Harndahl, Sune Justesen, Michael Madsen, Gustav Roder, Lotte Nielsen, & Soren Buus
- Center for Biological Sequence Analysis, Technical University of Denmark
  - Morten Nielsen, Claus Lundegaard, Thomas Blicher, Soren Brunak, & Ole Lund
- La Jolla Institute
  - Alessandro Sette, Björn Peters

- Funding:
  - NIAID x 2
  - EU commission x 3
  - Danish MRC, Novo Nordisk Foundation, Lundbeck Foundation

- Monomers or tetramers for your research?
  - Contact info@immunaware.com