

Table 3. Peptide library scanned for HLA-A3 binding

Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD
Tyrosinase-related protein 2	TLLGGRPYR	84	1	Tyrosinase	VSSKNLMEK	74	3	GPI00	<u>L</u> YRRRLMK	90	2
	GTYEGLLR	83	2		YMVPFIPLYR	72	2		IALNFFGSOK	86	1
	VLLAFLQYR	74	11		SLLCRHKRK	70	2		GATLRLVK	84	2
	RMVNMVPPF	69	6		GLVSLCRHK	64	5		ALLAVGATK	83	1
	LMETHLSSK	62	2		SIFEQWLQR	63	4		<u>ALNFFGSOK</u>	80	2
	VMSLHNLVH	59	8		KSYLEQASR	60	0		QLRALDGGNK	79	0
	VLLAFLQYRR	55	4		KFFAYLTLAK	51	4		QLVLHQILK	72	3
	LLRRNQMG	48	1		MVPFIPLYR	43	7		GVSRLRTK	72	2
	FLLSCLGCK	47	5		TQYESGMDK	36	3		QLRTKAWNR	62	1
	GLLRNQMG	46	2		FMGFNCNCK	36	3		QVWGGQPVY	59	4
	MTVDSLUNK	42	1		CVSSKNLMEK	27	4		VSCQGGLPK	58	10
	FQNSTFSFR	41	8		LVSLCRHK	26	7		VLASLIYRR	54	2
	AFDEWMKR	39	1		FFAYLTLAK	22	1		TVSCQGGLPK	49	1
	VVISNRLLY	36	11		VSLCRHKRK	19	1		VVLASLIYR	45	3
	ILEHVRKEK	30	1		LLRWEQEIQK	14	2		GALLAVGATK	38	1
	FLGALDLAK	27	0		ALLGGSEIWR	2	0		HTMEVTVYH	35	4
	HVRKEKATK	27	3		SMHNALHIYM	0	2		ILKGGSGTY	30	2
	PLMETHLSSK	27	3		YMVPFIPLY	0	2		SLIYRRRLMK	30	7
	QVMSLHNLVH	21	6		NIFDLSAPEK	-1	1		LVLKRCLLH	29	7
	FLGALDLAKK	19	1		IPIGTYGQMK	-3	1		SSHWLRLPR	20	1
	NSMKLPTLK	14	1		QLSGRGSCQ	-4	2		TVYHRRGSR	18	6
	SLVKECCPR	7	1		DLSAPEKDK	-4	2		RQLRTKAWNR	15	3
	TLLGGRPY	3	1		HIYMNGTMSQ	-4	3		QKRSFVYVVK	14	4
	LLAFLQYRR	3	4		HFPRACVSSK	-4	1		LGVSRLRTK	11	1
	CMTVDSLUNK	2	1		PLRRNPGNH	-4	1		LIVRRRLMKQ	9	3
	GFLSCLGCK	1	1		ALLAGLVS	-5	2		QILKGGSGTY	7	1

Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD
Tyrosinase-related protein 1	SLSPQEREQF	0	3	Nodal	RLPSSADVEF	-5	2	Mart1	CWRGGQVSLK	6	4
	DIRDCLSLQK	0	1		PIGTYGQMK	-5	0		AESTGMTPEK	5	2
	FLNGTNALPH	0	1		WLLGAAMVG	-5	2		CQLVLHQILK	3	3
	TTNILEHVRK	-1	1		VLYCLLWSFQ	-5	1		YLAEADLSY	2	1
	AFLQYRRLRK	-1	1		LLAGLVSLIC	-6	1		AVVLASLYR	1	1
	RLRKGYTPLM	-1	3		SYMVPFIPLY	-8	1		GLSIGTGRAM	0	3
	RKFFHRTCK	-1	0		RLLVRRNIFD	-8	1		ILLVLMVVL	-1	2
	QFLGALDLAK	-1	2		YDLFVWMHYI	-8	0		ALQLHDPSGY	-1	1
	RLGAESANVC	-2	1		PLYRNGDFF	-9	1		AVGATKVPR	-2	1
	LVTLNNGTY	-3	1		NLLSPASFF	-9	2		AVVLASLYI	-2	3
	ILPGAQQQF	-4	1		RLLYRRNIF	-9	2		TLIGANASF	-4	1
	FLQYRRLRK	-4	1		HAYIQSLK	90	1		AVIGALLAVG	-4	1
	FVLLAFLQY	-4	1		SLYRDPLPR	88	1		AVIGALLAV	-4	1
	RLIGNESFA	-4	1		KTKPLSMLY	74	2		VLYRYGSFS	-4	1
	ALVGLFVLLA	-5	3		RVAGECWPR	67	3		YLEFGPVTA	-5	1
	KILPGAQQQF	-5	1		VTRPLSKWLK	57	3		LISRALVVTH	-5	1
	ALDLAKKRVH	-6	1		ATVATALLR	56	1		LLVLMVVL	-5	1
	TLISRSRF	-7	1		STCCAPVTKT	54	3		TLISRALVV	-6	0
	SVYDFFVWLH	-7	0		ATNVLLMLY	44	9		QVLGGPVSG	-6	1
	RMYNMVPFFP	-7	1		LAYMLSLYR	38	8		QVPLDCVLY	-6	2
	NILEHVRKEK	-7	2		WLKRPGALEK	36	8		PLLDGTATL	-7	1
	RLLYNATTN	-8	1		HAYIQSLKR	24	5		FLRNQPLTF	-7	2
	RLLYNATTNI	-8	1		IYPKQYNAY	14	2		KVLPDGGQVIW	-7	2
	FVWLHYYSVR	-8	2		AIEIFHPK	12	10		KVLPDGOVI	-8	2
	ASVLRARR	83	2		VLEVTRPLSK	8	1		HVGTQCALTR	0	1
	SLPYWNFATR	77	4		LKRPGALEK	-1	1		AHFTYGYPKK	-3	2
	GLFDTPPFY	60	3		ALLQAGAAT	-3	2		VLLLIGCWY	-4	2
	LTWHRYHLRL	55	10		LLQAGAATV	-4	1		GVLLLIGCWY	-4	1

Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD	Protein	Sequence	Inhibition	SD
	AVFDEWLRR	53	4		GWGSIHYPK	-4	2		RDSKVSLEK	-4	0
	WTHYYSVKK	47	4		MLYSNLSQE	-5	1		DAHFIYGYPK	-5	1
	FVWTHYYSVK	42	6		ALLQAGATV	-6	2		LLIGCWYCR	-8	3
	LPYWNFAFRK	18	7		SPLAYMLSLY	-6	3		LLIGCWYCR	-8	2
	NVAPMVQR	17	2		AVDGGQWTF	-6	0				
	YSNSTNSFR	9	4		ALLRTRGQP	-6	2				
	ALIFGTASY	8	2		TVTLSQVTF	-7	1				
	KLLSLGCI	2	1		LLMYSNLSQ	-7	1				
	EIIAIAVVG	-1	4		NHAYIQSLK	-8	2				
	FVRALDMAK	-3	1		NLIGGWSWI	-8	1				
	ALLVAFJG	-3	0		QLGGSTLLW	-8	2				
	TLISPSNVF	-3	2		PLAYMLSLY	-8	1				
	FLNGTGGQTH	-3	2		PLAYMLSLYR	-10	1				
	ALLVALIF	-3	1								
	ILGPDGNT	-4	1								
	AVRSLHNLAH	-4	3								
	NLLDLSKEEK	-4	1								
	AVGALLVA	-5	2								
	ALDMAKRTH	-5	1								
	FVRALDMAKR	-5	1								
	HRYHLLRLEK	-6	1								
	CIFFPLLFQ	-7	0								
	ALIFGTASYL	-7	2								
	SIYNYFVWTH	-8	1								

Two hundred three predicted HLA-A3 ligands from six melanoma-associated proteins were selected based on three binding prediction algorithms. In an initial screen, peptides were screened at 5 μ M in a 45-hour FP competition assay format. Peptides in bold were chosen for further analysis to determine IC₅₀ values. Underlined peptides represent

previously described HLA-A3 ligands. Inhibition reflects the percentage reduction in polarization of the tracer peptide.

Values represent mean \pm SD of triplicate samples.