



Supplementary Materials

T Cells Expressing a TCR-Like Antibody Selected Against the Heteroclitic Variant of a Shared MAGE-A Epitope Do Not Recognise the Cognate Epitope

Mesha Saeed, Erik Schooten, Mandy van Brakel, David K. Cole, Timo L. M. ten Hagen* and Reno Debets

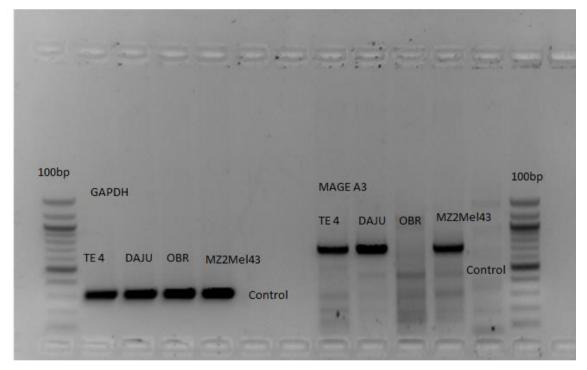


Figure S1. Complete PCR Gel showing the expression of MAGE A3 on tumor cell lines. GAPDH taken along as a control.

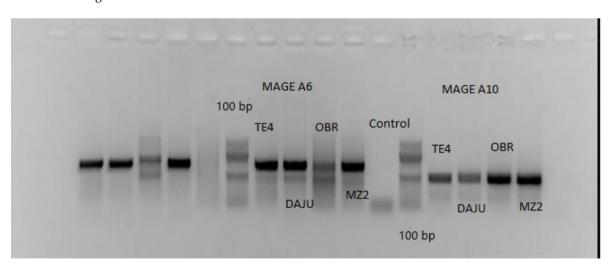


Figure S2. Complete PCR Gel showing the expression of MAGE A6 and MAGE A10 on tumor cell lines.

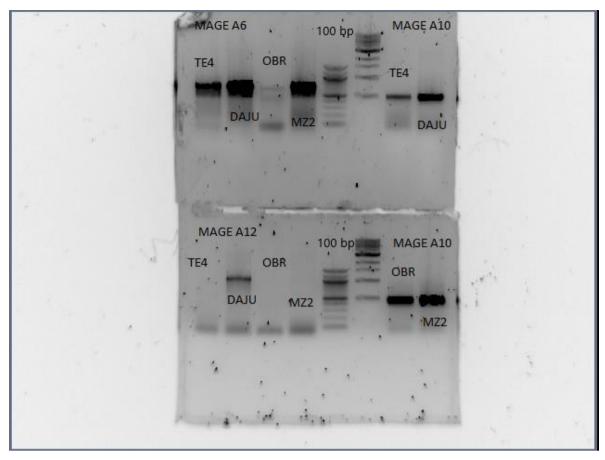


Figure S3. Complete PCR Gel showing the expression of MAGE A6, MAGE A10 and MAGE A12 on tumor cell lines.

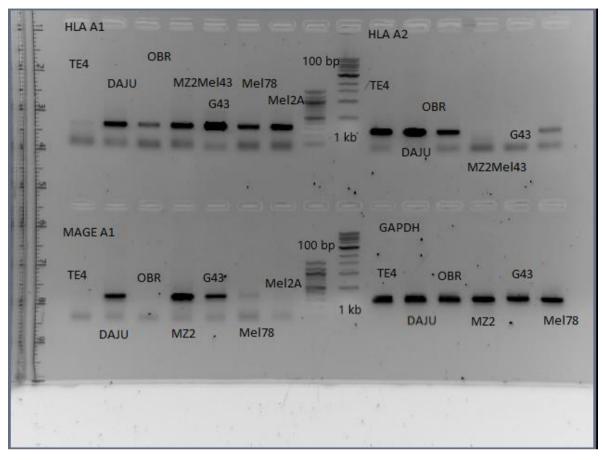


Figure S4. Complete PCR Gel showing the expression of MAGE A1, HLA A1, HLA A2 and GAPDH on tumor cell lines.

Table S1. Sizes and compositions of the phage-Fab libraries.

Antibody source ^a	Library	Insert (%) ^b	Size (cfu) ^c
VH or VL libraries PBL	VH Vκ Vλ	60 95 90	5.6x10 ⁸ 2.0x10 ⁸ 2.9x10 ⁸
Spleen	VH Vκ Vλ	99 100 90	1.2x10 ⁷ 4.0x10 ⁷ 5.2x10 ⁷
Fab libraries			
PBL	VH PBL VL PBL	80	1.4x10 ⁹
Spleen	VH ^{Spleen} VL ^{Spleen}	75	4.2x10 ⁹
combi Fab libraries			
PBL/ Spleen	VH PBL VL ^{Spleen}	78	2.3x10 ⁹
Spleen/ PBL	VH ^{Spleen} VL PBL	95	6.5x10 ⁸
Total size ^d		82	8.6x10 ⁹

^aSee the MM section for a description of the different (combinatorial) libraries. ^bPercentages of inserts determined by PCR using a minimum of 20 clones per library. ^cSizes of libraries given in clone forming units (cfu) and not corrected for clones without a Fab insert. ^dSum of cfu's of both Fab libraries and combinatorial libraries.

Table S2. Validation of phage-Fab library following selections with a set of control proteins.

Antigen	Selection round	Binders (ELISA)	Unique clones (restriction patterns)
NEF ^a LMP1 ^b	2 3	71/94 72/94	nt 6/72
PSMA ^c (EKM)	3	39/93	6/39

 $[^]a$ NEF₅₆₋₂₀₅ (CAA13474.1): negative regulatory factor; nt is not tested. b LMP1374-386 (QAR15098.1): latent membrane protein 1. c PMSA (EKM)307-750 (AAA60209.1): prostate specific membrane antigen.

Table S3. Sequences of control proteins used for validation of phage-Fab library.

NEF ₅₆₋₂₀₅	AWLEAQEEEGDVGFPVRPQVPLRPMTYKGAFDLGFFLKEK
(NCBI Genbank acc. number: CAA13474.1)	GGLDGLVYSKKRQEILDLWVYHTQGFFPDWQCYTPGPGVR
(YPLTFGWCYKLVPLDPTEVEEANKGENNSLLHPICQHGMDD
	EDGEVLVWRFDSSLARRHVARELHPEFYK
	EDGEVLVWNFD35LARNHVARELHFEFTK
LMP1 ₃₇₄₋₃₈₆	DDPHGPVQLSYYD
(NCBI Genbank acc. number: QAR15098.1)	
Donas	EVANCES A DRDESS MID COLVEY (DVANVED CET CALEST OVE WAARIN
PSMA ₃₀₇₋₇₅₀	EKMGGSAPPDSSWRGSLKVPYNVGPGFTGNFSTQKVKMHI
(NCBI Genbank acc. number: AAA60209.1)	HSTNEVTRIYNVIGTLRGAVEPDRYVILGGHRDSWVFGGIDP
	QSGAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLG
	STEWAEENSRLLQERGVAYINADSSIEGNYTLRVDCTPLMYSL
	VHNLTKELKSPDEGFEGKSLYESWTKKSPSPEFSGMPRISKLGS
	GNDFEVFFQRLGIASGRARYTKNWETNKFSGYPLYHSVYETYE
	LVEKFYDPMFKYHLTVAQVRGGMVFELANSIVLPFDCRDYAV
	VLRKYADKIYSISMKHPQEMKTYSVSFDSLFSAVKNFTEIASKF
	SERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYR
	HVIYAPSSHNKYAGESFPGIYDALFDIESKVDPSKAWGEVKRQ
	IYVAAFTVQAAAETLSEVA

NEF: negative regulatory factor; LMP1: latent membrane protein 1; PMSA (EKM): prostate specific membrane antigen.