Printed peptide arrays identify prognostic TNC serumantibodies in glioblastoma patients

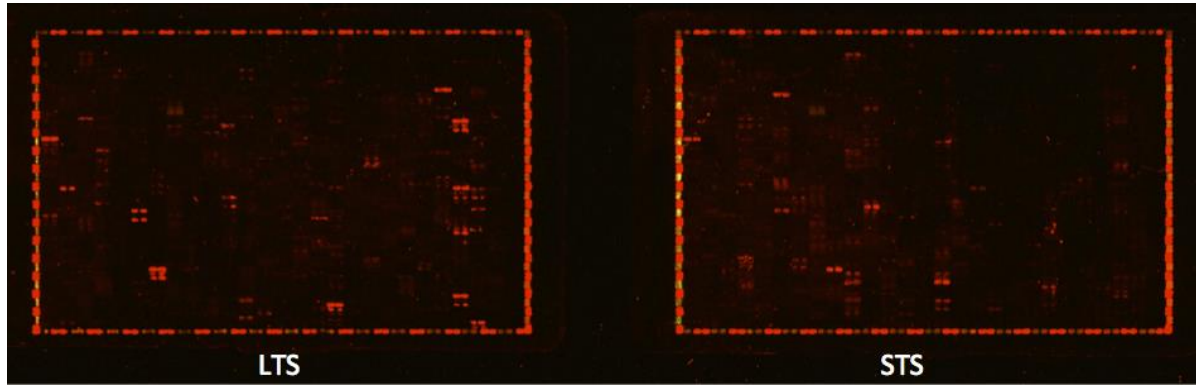## Supplementary Material

Supplemental Table 1: Clinicopathological characteristics of screening set consisting of 10 long-term and 14 short-term surviving GBM patients. All sera were obtained preoperatively from therapy-naïve patients. Board-certified neuropathologists verified diagnosis of all tumor specimens. The majority of patients received adjuvant radio-chemotherapy. *p<0.05, **p<0.01, ***p<0.001.

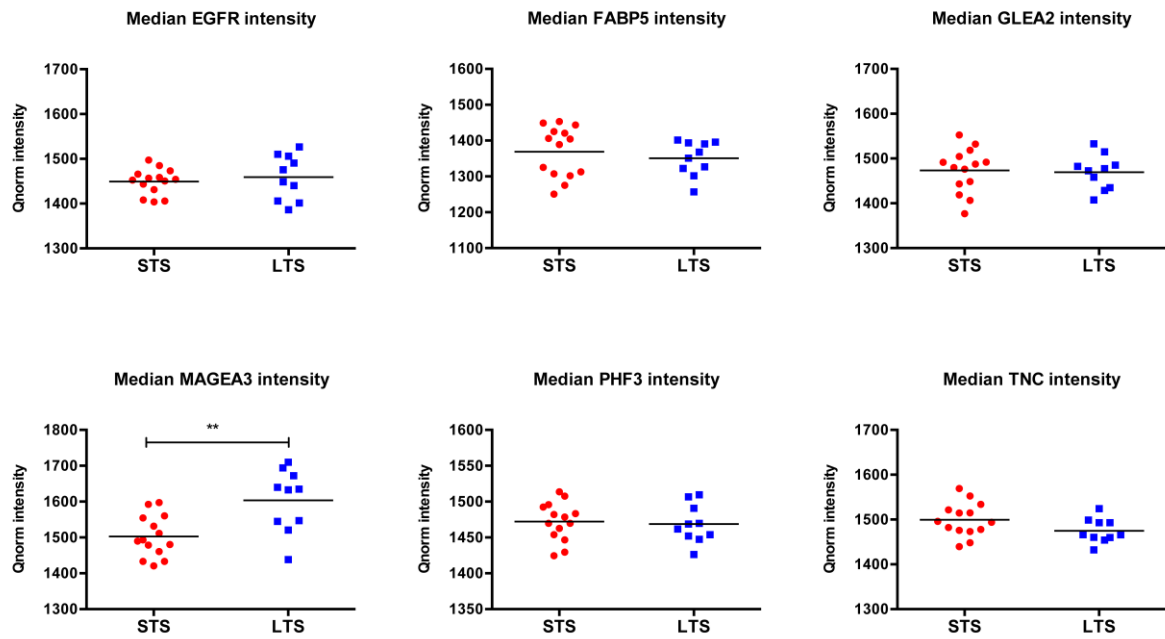| Characteristic	Long-term survivor (LTS)		Short-term survivor (STS)		
	No.	%	No.	%	p-value
Case number	10		14		
Histological diagnosis					
Glioblastoma	10	100	14	100	
Age (months)					
Median	59		63		
Range	36-67		33-81		0.371
Gender					
Male	5	50	10	71	
Female	5	50	4	29	0.403
Treatment					
Surgery	10	100	14	100	
No radio- or chemotherapy	0	0	1	7	
Chemotherapy only	0	0	2	14.3	
Radiotherapy only	1	10	2	14.3	
Radio-chemotherapy	9	90	9	64.3	
Overall survival (months)					
Median	58		8		
Range	42-185		6-10		1.88E-05***
MGMT					
Hypermethylated	7	70	8	57	
Unmethylated	2	20	4	27	
Unknown	1	10	2	16	0.659
IDH1					
wild-type	9	90	14	100	
mutant (132H)	1	10	0	0	0.417
KPI					
100%-90%	6	60	12	86	
80%-70%	3	30	2	14	
Unknown	1	10	0	0	0.343
Study Center					
Heidelberg	10	100	14	100	

Abbreviations: KPI = preoperative Karnofsky Performance Index; LTS = long-term survivor; STS = short-term survivor; IDH1 = mutation in isocitrate dehydrogenase 1 (R132H); MGMT = promotor methylation of O-6-methylguanine-DNA methyltransferase. Statistical tests: Student's t-test (age, overall survival), fisher's exact test (gender, MGMT, IDH1, KPI), No. = number of cases

Supplemental Table 2 : Differential antibody abundance for the Top30 peptides in LTS and STS patients. Peptides are assigned to their corresponding antigen and ordered according to their p-value. A positive log2 fold change (log2 FC) and difference in signal intensity equals a higher titer in LTS patients.
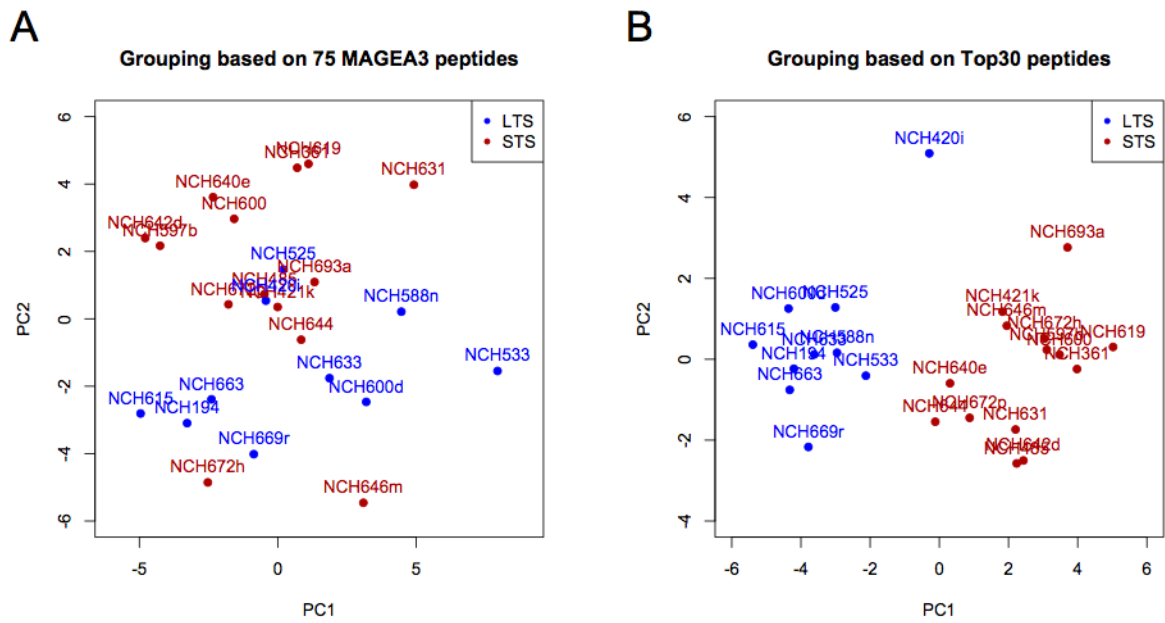
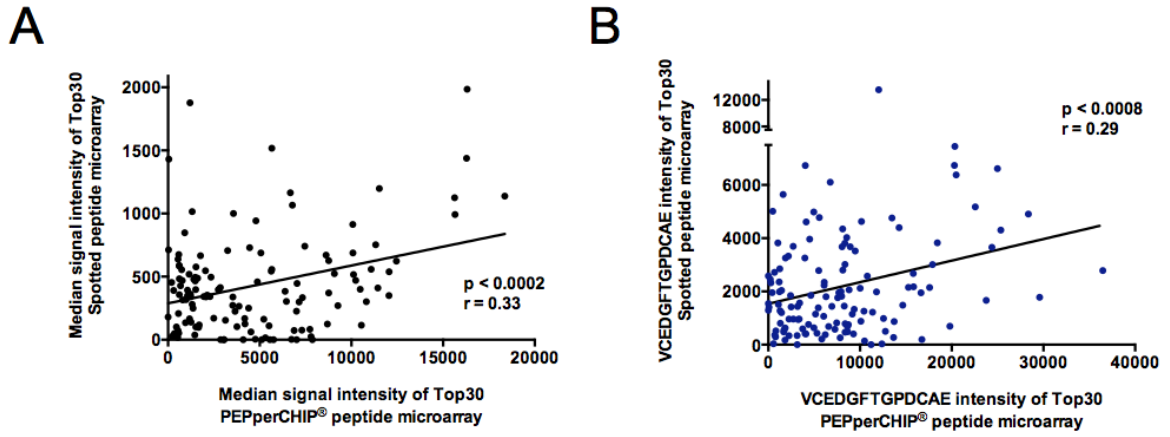| Peptide	Antigen	P-Value	Intensity difference	log2 FC
VCEDGFTGPDCAE	TNC	0.000633244	1570.269	0.8745
KLICSEKGKVSEK	GLEA2	0.00065385	-611.7965	-0.578
KETVKLSHEDDHI	PHF3	0.001009832	692.643	0.5615
VSGNTVEYALTDL	TNC	0.001521112	1303.234	0.7635
QAHSVLKKTLQDQ	PHF3	0.003270692	566.2445	0.4935
TGFLLIQAWPENR	EGFR	0.003687734	-713.4405	-0.6515
KKLFGTSGQKTKI	EGFR	0.004568125	-1066.191	-0.9985
KVVDCLKFFRKAK	GLEA2	0.004801176	-1280.414	-0.8375
EGKYSFGATCVKK	EGFR	0.00611762	-1535.3945	-1.0485
KLSHEDDHILEDA	PHF3	0.006157174	1806.9675	0.8915
KDKEKNKEKKFKE	GLEA2	0.006621059	-1308.243	-0.9535
RAGTPYTVTLHGE	TNC	0.006866234	888.161	0.697
ILKETEFKKIKVL	EGFR	0.007445671	-903.569	-0.9175
GEVVVAEVGWDAL	TNC	0.007588535	847.007	0.5785
LFMRRRHIVRKRT	EGFR	0.007926637	-3424.9665	-1.707
ETVNLAWDNEMRV	TNC	0.008115363	534.7985	0.374
APSQIEVKDVTDT	TNC	0.008284273	708.921	0.5435
SDFLSNMSMDFQN	EGFR	0.009048923	-999.5805	-0.809
NKVEAAQNLTLPG	TNC	0.009790343	-1240.1265	-1.0125
GKVKALEEDNLSE	GLEA2	0.010438833	1041.3535	0.673
NLKDPKNNILFKK	PHF3	0.011974617	-904.3435	-0.5075
KSPQENLREPKRK	GLEA2	0.011985668	-924.8655	-0.778
ALEEDNLSESSSE	GLEA2	0.01338913	1218.787	0.6815
PKNNILFKKVLKG	PHF3	0.014111071	-878.718	-0.7685
EGGVLKKVIRHKR	TNC	0.014459747	-1546.8	-0.813
YRALMDEEDMDDV	EGFR	0.014666953	2564.201	0.764
QIVFTHRINIPRR	TNC	0.015208285	-755.187	-0.6435
KFSDKEEHEQNDS	PHF3	0.015536046	1202.837	0.719
ENLQIIRGNMYYE	EGFR	0.0157327	-1586.7025	-0.6345
NFYQVKDIRRPER	PHF3	0.016741913	-1120.506	-0.7945

Supplemental Figure 1: Representative scans of screening PEPperCHIP® microarray (1745 peptides) for a long-term (LTS) and a short-term surviving (STS) patient. A red fluorescent labeled secondary antibody binding to the human heavy chain visualized patient antibodies specifically bound to spotted peptides on the array. The red spots on the border of the array denote control spots. Duplicate spots within this border depict antibodies against the candidate antigens.

Supplemental Figure 2 : Median intensity for all peptides of an antigen on the 1745 PEPperCHIP® screening array. Every scatter plot illustrates the median centered, quantile normalized signal intensity for all peptides of an antigen per patient. Differences between STS and LTS patients are shown. P-values were calculated employing Student's t-test. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 3 : Correlation heatmap between MAGEA3 peptides on the PEPperCHIP® screening array. The signal of each MAGEA3 peptide across all patients is correlated with all other 74 MAGEA3 peptides on the screening array, clustered (hierarchical clustering) and illustrated in a heatmap. Blue color denotes a high correlation.

Supplemental Figure 4. Principal component analysis visualizing the grouping of LTS and STS patients according to the first and second principal component (PC1, PC2) for (A) all 75 MAGEA3 peptides on the screening array and (B) the Top30 peptides of the comparative analysis. Principal component analysis was performed on median centered, quantile normalized signal intensities of screening arrays.

Supplemental Figure 5: Correlation between the signal intensities of the Top30 PEPperCHIP® peptide microarray and the Top30 spotted array (pre-synthesized peptides). Correlation of (A) median signal intensities and (B) VCEDGFTGPDCAE intensity for validation set 2 (n=129) are shown. P-values and Pearson's r were calculated using the Pearson correlation coefficient.

Supplemental Material & Methods

FASTA sequences of glioblastoma-associated antigens

Top 30 peptides are color-coded in the FASTA sequence. Two pairs of PHF3 peptides were overlapping (yellow and red highlight). The prognostic anti-TNC peptide (VCEDGFTGPDCAE) is highlighted in green.

>sp|Q92576|PHF3_HUMAN **PHF3** OS=Homo sapiens GN=PHF3 PE=1 SV=3

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