

Supplementary Data

Intravenous Infusion of Phage-displayed Antibody Library in Human Cancer Patients: Enrichment and Cancer-Specificity of Tumor-Homing Phage-Antibodies

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Supplementary Table S1: Schematic outline of the study and follow-up monitoring

Task and parameters including blood analysis	Before infusion	Every 15 min during infusion	2 hrs after infusion	1-Week follow-up	1-Month follow-up	2-Month follow-up
Informed consent	X (Prior to any protocol event)					
Meeting with ombudsman	X					
Pre-medications	X					
Physical examination	X			X	X	X
Blood pressure	X	X	X		X	X
Pulse	X	X	X		X	X
Temperature	X	X	X		X	X
Respiratory rate	X	X	X		X	X
Heart rate	X	X	X		X	X
Weight	X				X	X
Complete blood count	X				X	X
Creatinine	X				X	X
Albumin	X				X	X
Alkaline phosphatase	X				X	X
AST*	X				X	X
ALT**	X				X	X
Direct bilirubin	X				X	X
Total bilirubin	X				X	X
Total Protein	X				X	X
Anti-filamentous phage antibody	X					
Pregnancy testing as needed	X					
Pulmonary testing as needed	X					
Neurological testing as needed	X					
General level of consciousness	X	X	X	X	X	X
Toxicity monitoring for adverse events		X	X (All times throughout)	X	X	X

*AST=Aspartate aminotransferase, **ALT=Alanine aminotransferase

Supplementary Table S2:

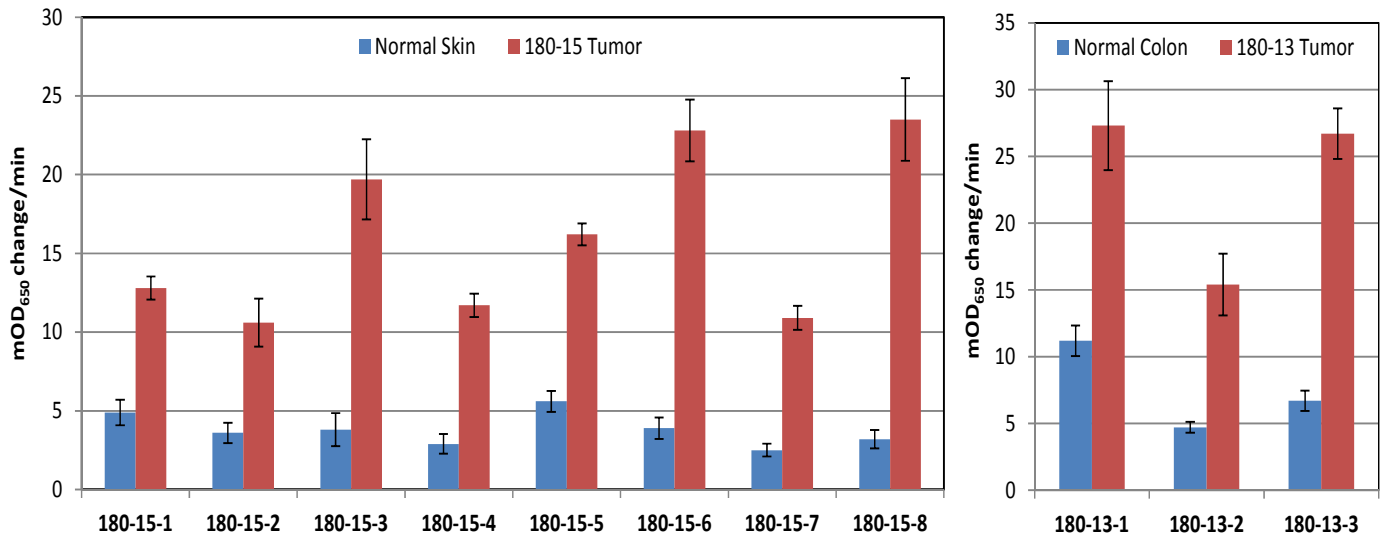
Examples of matches by the tumor-binding scFv CDRH2 amino acid sequences to the variable regions of several interesting antibodies.

Clone number and Protein ID	Alignment of clones with human proteins	Name of aligning proteins	Comment
09-8083 08-0289 GB: ABD59021.1	1 SAIGAYCNYTAV 12 1 SAISSTCASTTY 12 51 SGISSTCGATTY 62	Antibody to TREM-like transcript 1 (TLT1).	TLT1 is a costimulatory ITIM immunoreceptor in platelets similar to platelet endothelial cell adhesion molecule-1 and a mediator of metastasis [15].
08-2081 07-0299 GB: AAV66329.1	1 SDIACYGYTTSY 12 1 SSING-CNTTSYA 12 49 SGINGTGYTTSYA 61	Human Ig C1 HC variable region to asialoglycoprotein receptor.	Asialoglycoprotein is involved in cell adhesion and cancer metastasis and used for hepatocellular carcinoma drug targeting [16].
07-0365 GB: AAC26479.1	1 SSTA-TGYTTYA 12 49 SAISGSGYTTYA 61	Human IgM HC to GM2 ganglioside.	The ganglioside antigens act as immunosuppressors of cytotoxic T cells and dendritic cells, promote tumor-associated angiogenesis, and regulate cell adhesion, motility and metastasis. They are directly connected with transducer molecules to initiate adhesion coupled with signaling, and are modulators of signal transduction through tyrosine kinases associated with growth factor receptors or other protein kinases [17].
08-2035 GB: ACH41918.1	1 SGISDDGNSTAVTD 14 49 SGIDDDCGSTNAD 62	Single chain variable fragment to human VEGFR2.	Neovascularization in tumor tissue (unpublished).
07-2355 PDB: 2VXS K	1 SAISGSGGSTYY 12 49 SAISGSGGSTYY 60	Human neutralizing antibody to IL-17A .	IL-17 induces MMP-9 expression via p38 MAPK and ERK1/2 dependent NF-kappaB and AP-1 activation [18]. Overexpressed in a variety of cancers [24,25].
09-8033 GB: AAY15114.1	1 SDIGTSGYDTYY 12 49 SAIGTSG-DTYY 59	MOG-specific immunoglobulin heavy chain Fab fragment.	(MOG) responsible for development of neurodegenerative disorders, such as MS and EAE [19].

The motifs are shown in grey highlighting. Exact matches of amino acids are shown in white; conservative substitutions are shown in black.
GB = Gene bank ID; PDB = Protein data bank ID.

References:

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Supplementary Figure 1: The results of immunofluorescence microscopy for tumor-binding were confirmed by tissue lysate ELISA. The tumor-specific phage-antibodies from patient 180-15 that showed binding to one normal human organ tissue were analyzed for their binding to 180-15 tumor and normal human skin lysates. The tumor-specific clones from patient 180-13 that showed binding to a maximum of two normal human organ tissues were analyzed for their binding to 180-13 tumor and normal human colon lysates. The bars represent the arithmetic mean \pm SE (n=3) of the experiment done in triplicate. For all the clones, the tumor lysate bindings were significantly ($p < 0.001$; Student t-test) higher in comparison to corresponding control normal tissue lysate.