***Supplementary Methods***

Library design

*AL-1013-04:* the germ-line hot spot residues (see LeFranc et al, IMGT/VQEST) were identified by nucleotide sequence analysis (1). The variability in each of the germline hot spot mutations was restricted to 8-9 selected residues to reduce the complexity, generating a highly functional library. The theoretical variability of this library was 1.9 x 107 individual variants. *AL-1013-05: A*ll surface-exposed CDR-residues identified based on the structure model of B44 were varied, restricting the variability to one or two homologous residues, except in H3 where additional variability was introduced. The theoretical variability of this library was 1.9 x 107 individual variants. *AL-1013-06: 11 r*esidues in the central part of CDRL3 and in the structurally adjacent CDRH2 were randomized. The residues were identified from the structure model of B44. The theoretical variability of this library was 1.6 x 107 unique variants. For all the libraries above, the residues that were varied were selected to represent the physiochemical properties of all of the naturally occurring amino acids (2;3). *AL-1013-07*: The B44 scFv sequence was used as template for three subsequent rounds of random mutagenesis using GeneMorphII PCR Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer’s instructions. Each of the four libraries were cloned into a phagemid vector (pAB1) and transformed into the F+ strain TG1, generating individual colonies covering the theoretical size of the libraries, except for the random library, where the library was limited to 6.3 x 108 individual colonies.

Selection strategy

Phage selection using biotinylated CD40-Fc (R&D Systems, Minneapolis, MN, USA) and magnetic beads has been described previously (4), and the details of the selection rounds are described in Supplementary table S1. In the last two rounds of selection, non-biotinylated CD40-Fc (R&D Systems) was added for 10 min before incubation on the magnetic beads.

Biacore analysis

The affinity measures of purified antibodies by surface plasmon resonance using the Biacore 3000 instrument were performed according to manufacturer’s protocols. The CD40-Fc (R&D Systems) was immobilized to the BIAcore sensorchip, CM5, using conventional amine coupling. The anti-CD40 antibodies diluted 1/3 from 1 to 0.012 nM were analyzed for binding in HBS-P (GE Healthcare, Little Chalfont, UK) at a flow rate of 30 μl/min at 25°C and 37°C and at two different pH (Supplementary Table 1).

Screening of individual clones

The high-throughput expression of scFv was run in 384-format in 2xYT medium with 0.1 mM IPTG present from start, at 37°C. The HTS assay was based on a sandwich ELISA measuring binding of scFv-his fragment in crude E. coli supernatants to CD40 (R&D Systems) coated in microtiter plates. The samples were incubated for 1h at RT and then washed using either a normal wash (3 wash cycles) or harsh wash (3 wash cycles followed by incubation with PBST for 30 min followed by 3 wash cycles). Detection Ab, Penta-His-HRP was added and the plates were subsequently developed using SuperSignal Pico Chemiluminescent substrate and detected with Envision reader (Perkin Elmer, Waltham, MA, USA).

FIND™ (Fragment Induced Diversity)

Two rounds of FIND™were performed, where the output from round 1 was used as template for round 2. Plasmid DNA was prepared from the clones obtained from the initial selection round using NucleoBond Xtra Midi Plus Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions.

*ELISA used for measuring IL-12p40 from bone marrow derived dendritic cells*

High binding 96 well plates (Corning Inc., Amsterdam, The Netherlands) were coated with an IL-12p40 capture antibody (Biolegend, San Diego, CA, USA; Cat no. 505202), which was diluted to a final concentration of 1µg/ml in 0.05M bicarbonate buffer (C3041-50CAP, Sigma-Aldrich). After washing (PBS with 0.05% Tween) and blocking (PBS with 1% BSA), the standard (Biolegend, Cat no. 563801) and supernatants were diluted in PBST (PBS with 1%BSA and 0.05%Tween) and incubated for 2 hours at 37°C. After washing, the plates were incubated with an IL-12p40 detection antibody (Biolegend, Cat no. 505202) which was diluted to 1µg/ml in PBST. After 1 hour of incubation at 37°C (and washing) the plates were incubated with Avidin/HRP (Dako, Stockholm, Sweden; Cat no. P0347) diluted 1:4000 in PBST, for 1 hour at RT and developed with TMB (Dako, Cat no.S1599). The absorbance was read at 450nm using Emax precision microplate reader (Molecular Devices, Sunnyvale, CA, USA). The results were analyzed using SoftMax version 2.35 (Molecular Devices).

Measurements of IL-12p70The levels of IL-12p70 was measured from was measured using ELISA (eBioscience, San Diego, CA, USA, and R&D Systems Minneapolis, MN, USA). The average IL-12p70 levels at plateu level (1-10µg/ml) was measured from 7 donors. Values below the LOD was replaced with the LOD divided by the square root of 2 as outlined by Hornug et al (1990, Applied Occupational and Environmental Hygiene). The isotype control and medium samples generated values below LOD in all cases but one. The levels of IL-12p70 obtained by stimulation of ADC-1013 was significant compared to isotype control (Wilcoxon matched-pairs signed rank test, Graph Pad Prism 6.0).

*Generation of hCD40tg mice*

The sequence encoding the murine CD40 promotor was fused upstream to the human CD40 sequence and cloned into the pDL29 vector. A 3.2 kbp DNA fragment of the resulting plasmid was excised using HindIII and XbaI restriction endonucleases and the DNA was injected in three sessions into C57BL/6N-derived zygotes. The C57BL/6N female mice were superovulated at 28-34 days and mated in the PolyGene (PolyGen AG, Rümlang, Switzerland) mouse facility to C57BL/6N breeder males. Injected zygotes were cultivated overnight and transferred into pseudopregnant CB6F1 females from CBA and C57Bl/6J mice. hCD40tg founders were identified by PCR. Mice with a single hCD40 integration site was identified.

Adoptive cell transfer

Splenocytes from complete responders were isolated from 4 mice and spleenocytes from naïve mice were used as control. Splenocytes (10 x 106) was injected at day 7 intravenously. The mice were injected with MB49 tumors on the right flank on day 0. The tumor growth was followed over time.

**References**

 (1) Ho M, Pastan I. In vitro antibody affinity maturation targeting germline hotspots. Methods Mol Biol 2009;525:293-308, xiv.

 (2) Koide A, Tereshko V, Uysal S, Margalef K, Kossiakoff AA, Koide S. Exploring the capacity of minimalist protein interfaces: interface energetics and affinity maturation to picomolar KD of a single-domain antibody with a flat paratope. J Mol Biol 2007 Nov 2;373(4):941-53.

 (3) Fellouse FA, Esaki K, Birtalan S, Raptis D, Cancasci VJ, Koide A, et al. High-throughput generation of synthetic antibodies from highly functional minimalist phage-displayed libraries. J Mol Biol 2007 Nov 2;373(4):924-40.

 (4) Ellmark P, Ottosson C, Borrebaeck CA, Malmborg Hager AC, Furebring C. Modulation of the CD40-CD40 ligand interaction using human anti-CD40 single-chain antibody fragments obtained from the n-CoDeR phage display library. Immunology 2002 Aug;106(4):456-63.