Supplementary Materials and Methods

Generation of a yeast-display recombinant antibodies (scFv) library derived from B cells of ovarian cancer patient ascites.

B cells used for V_L/V_H amplification were isolated from ascites (n=10) and PBMCs (n=1) of ovarian cancer patients (stages III or IV) after Ficoll gradient and purification using CD19 magnetic beads (Miltenyi). Total RNA was extracted (1.25 mg) and twenty-two µg of mRNA (1.7%) were isolated (mRNA purification kit, Qiagen), consistent with the fact that mRNA normally accounts for 1-5% of total RNA. Fifty reverse transcription reactions were performed to permit the PCR amplification of the V_H and V_L gene fragments. A set of primers was designed to amplify human subfamilies of V_H and V_L gene fragments based on the MIGT data base (49). Annealing sequences were added to primers amplifying V_H and V_L fragments to enable gap repair with pAGA2 vector and the generation of a long-linker between V_L and V_H [GGSSRSSSSGGGGSGGGG] (32). The long-linker DNA sequence was modified to encode yeast-optimized codons (5'-ggcggatcctctaggtcaagttccagcggcggcggtggcagcggaggcggcggt -3').

The following annealing sequences were added to the primers: 5'-ggtggtggaggttctggtggtggtggatctgtc-3' to forward V_L primers (annealing with 5' end of linearized Nhe I-Xho I pAGA2 vector); 5'-cgctgccaccgccgccgctggaacttgacctagaggatccgcc-3' to reverse V_L primers (annealing with long-linker); 5'-ctaggtcaagttccagcggcggcggtggcagcggaggcggcggt-3' to forward V_H primers (annealing with long-linker); and 3'-gtcttcttcagaaataagcttttgttcggatccctcgaa-'5 to reverse V_H primers (annealing with 3' end of linearized Nhe I-Xho I pAGA2

vector). PCR amplification were performed using a hot start of 5 min at 94ºC, followed by 25 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min, with a final step of extension of 7 min at 72°C. The pAGA2 yeast display vector was linearized by Nhe I and Xho I. Linearized vector and PCR products were separated by electrophoresis, purified using a gel extraction kit (Invitrogen, Carlsbad, CA), and transfected into EBY100 yeast cells at a 1/3/3 ratio. Gap repair efficiency was evaluated by sequencing of fifty clones, as previously described.

Cloning of recombinant folate receptor alpha (FOLR1), protein expression and purification.

The extracellular domain of FOLR1 was amplified from cDNA of human ovarian cancer cell line SKOV3 using FOLR1 forward primer 5'-ctgaaggctagcattgcatgggccaggactg-3' and FOLR1 reverse primer 5'-cagtctagatctactcatggctgcagcatagaac-3'. FOLR1 cDNA was verified by sequencing and cloned into a mammalian expression vector (pCDNA3, Invitrogen) for mammalian expression fused to 6XHIS Tag in 293-F mammalian cells. Recombinant FOLR1 was purified with Nickel sepharose beads (Sigma).

Cloning of recombinant B7-H4, protein expression and purification.

The extracellular domain of B7-H4 (IgC+IgV) was amplified from cDNA of human macrophages after *in vitro* tumor-polarization (8), using B7-H4 forward primer 5'-ggttctggtggtggaggttctggtggtggtggatctgagtttggtatttcagggagacactccatca-3' and

B7-H4 reverse primer 5'-agaccgaggagagggttagggataggcttaccgtcgacagaagcctttgagtttagcagctgtag-3'. B7-H4 cDNA was verified by sequencing and cloned into a mammalian expression vector (pTT28, kind gift from Yves Durocher, National Research Council of Canada) for mammalian expression fused to 6XHIS Tag in 293-F mammalian cells. Recombinant B7-H4 (rB7-H4) was purified with Nickel sepharose beads (Sigma) and detectable by Western Blot as a 75Kb fragment with an anti-B7-H4 polyclonal antibody (Suppl. Figure 1).

Construction of pELNS-B7-H4 lentivirus

For the production of cDNA encoding full B7-H4, RNA was isolated from OVCAR-3 ovarian tumor cells and reverse transcript with the kit "ready-to-go you-prime First-Strand Beads" (GE Healthcare, Piscataway, NY, USA). Resulting cDNA was used as template for PCR amplification of B7-H4 cDNA fragment of 795-bp with the primers B7-H4-F 5'-ACGCTCTAGAATGGCTTCCCTGGGGCAGATCCTCT-3' and

B7-H4-R: 5'-ACGCGTCGACTTATTTTAGCATCAGGTAAGGGCTG-3'. The resulting PCR products contained an XbaI site (B7-H4-F) and a SalI site (B7-H4-R) was and were digested for cloning into a third generation self-inactivating lentiviral expression vector (pELNS, (29)) in which the transgene expression is driven by the EF-1a promoter, to obtain pELNS-B7-H4.

Recombinant lentivirus production.

High-titer replication-defective lentiviral vectors were produced and concentrated as previously described (29). 293T human embryonic kidney cells were seeded at 10 x 10^6 per T-150 tissue culture flask 24h before transfection. All plasmid DNA were purified using the QIAGEN Endo-free Maxi prep kit. Cells were transfected with 7 μg pVSV-G (VSV glycoprotein expression plasmid), 18 μg of pRSV.REV (Rev expression plasmid), 18 μg of pMDLg/p.RRE (Gag/Pol expression plasmid), and 15 μg of pELNS transfer plasmid using Express Inn (Open Biosytems). Viral supernatant was harvested at 24 and 48h post-transfection. Viral particles were concentrated by ultracentrifugation for 3h at 25,000 rpm with a Beckman SW28 rotor (Beckman Coulter, Fullerton, CA) and resuspended in 4ml of RPMI full medium.

Lentiviral transduction of cancer cell lines and T2 cells with pELNS-B7-H4

For the transduction of the cancer cell lines C30 , MDA231 and 624 with pELNS-B7-H4 lentiviral particles 1.5 x 10^5 tumor cells were seeded in a six-well plate one day prior their transduction. Next day, the medium was replaced with 1ml of lentivirus when the cells reached a confluence of about 30%. Medium was replaced twenty-four hours after transduction with fresh RPMI medium (C30, 624) or DMEM medium (MDA231). For the transduction of T2 cells, 1ml of lentivirus was applied to 3 X 10^5 cells in a 24 well plate. The expression of B7-H4 was assessed at day 5 post-transduction.

Production of retroviral particles by transient transfection of 293 GP cells

The HER-2 TCR (Lanitis E. et al., manuscript in preparation) and the MART-1 TCR (DF5a) (38) used in this study were in the pMSGV1 vector backbone, which is a derivative of the vector pMSGV [murine stem cell virus (MSCV)-based splice-gag vector] and utilizes a MSCV long terminal repeat (LTR) (50). To produce retroviral supernatants, $1x10^6$ of 293-GP cells (transient viral producer cells) in a 6-well plate were co-transfected with 1.5 μg of retroviral vector DNA from each of the constructs and 0.5 μg of envelope DNA (RD114) using the Lipofectamine 2000 reagent (Invitrogen) and Optimem medium (BD Biosciences). Media was changed to DMEM with 10% FBS after 18 hrs., and viral supernatants were harvested at the 48 hrs. time point. These supernatants were then used to transduce T cells for expression of a TCR that targets either HER-2- or MART-1-derived peptide.

Retroviral Human T cell transduction

Primary human T cells were purchased from the Human Immunology Core at University of Pennsylvania and were isolated from healthy volunteer donors following leukapheresis by negative selection. All specimens were collected under a University Institutional Review Board-approved protocol, and written informed consent was obtained from each donor. T cells were plated at a concentration of 1×10^6/ml in 24-well plates (Costar) in complete media (RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, 10 mM HEPES), and stimulated with anti-CD3 and anti-CD28 mAbs coated beads as described

by manufacturer (Invitrogen) (37) for 18-24 hrs. prior to transduction. Non-tissue culture-treated 12-well plates (Becton Dickinson Labware, Franklin Lakes, NJ) were treated with 25 μg/ml of recombinant retronectin fragment at 4°C, as directed by the manufacturer (RetroNectin, Takara, Otsu, Japan). After an overnight incubation, the retronectin was removed and the plate was blocked with 2% BSA in PBS at RT for 30 minutes. The retroviral vector supernatant (2-3 ml) was then applied by centrifugation (2000 X g for 2 hrs.), and after discarding the supernatant 5 x 10^5 of stimulated T cells were added to each well in a final volume of 1 ml RMPI growth medium per well. Plates were centrifuged for 10 min at 1000 X g and incubated overnight. The transduction process was repeated the following day. After transduction, the cells were grown in the RMPI with 10% FBS and human recombinant interleukin-2 (Novartis) was added every other day to a 100 IU/ml final concentration. Cell density of 0.5-1x10^6 cells/ml was maintained. Expression of the exogenous HER-2 and MART-1 TCR was validated 5 days after transduction using APC-conjugated MHC-peptide tetramers (Becton Dickinson, San Jose, CA) with specificities for HLA-A2–$HER2_{369-377}$ and HLA-A2-$MART\text{-}1_{26–35}$.

Flow Cytometry

Flow cytometry was performed as described (8). Before labelling cells were incubated with murine IgG (Jackson Laboratories) to block non-specific binding of Fcγ receptors. 7-AAD (BD Biosciences) was used to exclude dead cells.

Monoclonal antibodies: CD14-PE-CY7 and isotype PE were purchased from E-Biosciences. Anti-B7-H4 mAb PE was purchased from Serotec. CD206-APC and CD45-APC-CY7 were obtained from BD Biosciences. Epcam was obtained from DAKO (clone Ber-Ep4).

ELISA

IFN-γ ELISA was performed as indicated by manufacturer (Biolegend).

Western Blotting

Western blotting was performed as described (8). Briefly, 10 µg of total cell lysates were loaded on gradient polyacrylamide gel (Biorad). B7-H4 was detected with polyclonal goat anti-human B7-H4 antibody (1 µg/ml) (Abcam) followed by HRP-conjugated rabbit anti-goat polyclonal antibody (1 µg/ml) (Abcam).

Xenograft models of ovarian cancer

Balb/c nude mice were obtained from Charles River Laboratories. Mice of 6-8 weeks were injected intraperitoneally with $3x10^6$ of OVCAR5 ovarian cancer cells. Mice were sacrificed at 6-9 weeks after tumor implantation.

For the purpose of human immune reconstitution, adult NOD-Scid-IL-2Rgc null (NSG) mice (Jackson Laboratory) were sub-lethally irradiated (275 cGy) twenty four hours prior to intravenous injection of cord blood-derived CD34+ cells (1.5 to 2 $x10^5$/mouse). Between 14 and 16 weeks after CD34 cell injection, mouse peripheral blood was analysed by flow cytometry to determine the percentage of human human CD45+, CD3+, CD19+, and

CD33+ cells. Mice were randomized to experimental groups based on the level of human chimerism (CD45+) and the percentage of human CD3+ cells. Mice were then injected subcutaneously with $1x10^6$ OVCAR5 cells. Once tumor volumes reached ~100mm^2, mice were treated with 100 µg of scFv intraperitoneally. Treatments were performed four times within 2 weeks and tumor development was measured by calliper. Volumes were calculated using the formula V = ½ (length × width2), where length is the greatest longitudinal diameter and width is the greatest transverse diameter. Mice were sacrificed 8 weeks after tumor implantation and tumor samples were collected.