Supplement: Material and Methods

Construction of retrovirus vectors: The anti-CTLA-4 scFv was cloned from total RNA isolated from UC10-F10-11 hybridoma cells (ATCC) by the method of Gilliland and colleagues (48). Leucine residues at positions 43 and 89 in the 4F10 variable region light chain sequence were mutated to methionine and glutamine, respectively, to increase scFv expression (31). The anti-phOx scFv in pHook (Invitrogen) was removed by digestion with Sfi I and Sal I restriction enzymes. The anti-CTLA-4 scFv was inserted into the same sites, immediately after the Vκ leader sequence and HA epitope tag and before the myc epitope and platelet-derived growth factor receptor transmembrane domain in pHook. A stop codon was introduced immediately after the myc epitope to allow secretion of the antibody. The entire cassette was transferred to the retrovial vector pLHCX (Clontech, Mountain View, CA) to create pLHCX-αCTLA-4. pLHCX-αCTLA-4-γ1 was generated by inserting the cDNA fragment coding the hinge-CH_2-CH_3 of human IgG_1 (generously provided by Dr. Shie-Liang Hsieh, National Yang-Ming University, Taipei, Taiwan) into the unique Sal I site of pLHCX-αCTLA-4 between the scFv and myc epitope. pLHCX-αCTLA-4-mγ1 was generated in a similar fashion by inserting the cDNA fragment coding the CH_2-CH_3 of human IgG_1 into the Sal I site in pLHCX-αCTLA-4.

Western Blot: TC-1 and derivative cells ($1.5x10^5$) were boiled in 20 µl reducing or nonreducing SDS-PAGE buffer as indicated in the figure legends. Three or seventeen µl of the samples were resolved by SDS-PAGE, immunoblotted with rat anti-HA (Roche) and HRP-conjugated goat anti-rat IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) and visualized by ECL detection (Pierce, Rockford, IL).

Flow cytometry: 10^5 splenocytes or TIL were suspended in 120 µl WB and incubated for 45 min at 4ºC with the following monoclonal antibodies (final concentration 5µg/ml): anti-FoxP3-PE (clone FHK16s, eBiosciences, San Diego, CA), anti-CD4-PE, anti-CD8-PE, anti-CD8-FITC, anti-CD11b-PE, anti-CD19-PE, anti-CD25-FITC (clone 7D4) (all

BD Biosciences) and anti-CD25-FITC (clone PC61.5; eBiosciences). Subsequently cells were washed with WB two times and 10^4 cells were analyzed by flow cytometry using the two-laser flow cytometer FacsCalibur and analyzed using Cellquest software (Becton Dickinson).

Immunohistochemistry for human cancer tissue sections: Paraffin sections of cervical and breast cancer tissues were analyzed using mouse anti-FoxP3 antibody (clone 236A/E7) and rabbit anti-CD8 antibody (both Abcam, Cambridge, MA). Antigen retrieval was performed on deparaffinized sections by boiling for 5 minutes in Tris-EDTA buffer (10 nmol:1 nmol, pH 9.0) for FoxP3 staining or 2 minutes in Antigen Unmasking Solution (Vector Laboratories, Burlingame, CA) for CD8 staining. Endogenous peroxidase was blocked with 3% hydrogen peroxide in PBS for 10 minutes at room temperature. Sections were incubated with anti-FoxP3 (1:40) overnight at 4ºC or with anti-CD8 (1:100) for 30 minutes at RT, followed by staining with the 2-step plus poly-HRP anti-mouse/rabbit IgG detection system (Golden Bridge International, Everett, WA). Sections were counterstained with hematoxylin, dehydrated and mounted.

Immunohistochemistry for mouse tissue and organs: For histological assessment of autoimmune disease, mouse tissues and organs (heart, lung, brain, stomach, mesenterium, liver, kidney, muscle, skin) were fixed in 10% formalin and processed for hematoxilin and eosin staining. All samples were examined by two experienced pathologists for typical inflammation signs in a blinded fashion. Immunohistochemistry for IgG on kidney sections was performed as described for tumor sections using a polyclonal, HRP-labeled, anti mouse IgG antibody (eBiosciences).