**Supplementary figures legends**

**Suppl. Fig. 1. *Flow-cytometric characterization of genetically modified MSCs* A)** Flow cytometry analysis demonstrates that murine MSCs express CD29, CD44 and Sca-1, while being negative for the CD45 and CD11b markers. **B)** Human MSCs were defined by positivity for CD73, CD90 and CD105, while negative for CD45, CD34, CD19, CD3 and CD14.

**Suppl. Fig. 2. *Cytokine production by MSCs and PBMCs during co-culture and toxicity screening* A)** IL-7 and IL-12 cytokine production after 48h *in vitro* culture of human MSCs transduced with IL-7 and IL-12 encoding vectors. **B)** *In vitro* production of murine IL-7 and IL-12 by genetically modified murine MSCs after 48h, measured by ELISA. **C)** hMSC and PBMC co-culture at different ratio of together with stimulatory anti-CD3/CD28 beads. Interferon-γ (IFNγ) production after 72 hours as readout. **D)** Mouse weight monitoring during tumor- and MSC/control injection does not show display systemic toxicity of MSCIL7/12. **E)** Representative H&E stainings of peripheral organs from MSCIL7/12-treated mice does not show histopathological alterations in the treatment cohort.

**Suppl. Fig. 3. *Flow cytometry analysis of T-cell surface marker expression* A)** Gating strategy for differentiation of T cells, defined by CD3, CD4, CD8, CD44 and CD62L marker expression. Naïve (nv, CD44-, CD62L+), effector (eff, CD44-, CD62-), memory (mem, CD44+ CD62L-) and central memory (cm, CD44+, CD62L+). **B)** Gating strategy for analysis of CD69, PD-L1, CTLA, TIGIT, PD-1 and KLRG1 marker expression on CD4 and CD8 T cells.

**Suppl. Fig. 4. *Flow cytometry analysis of immune populations*** Gating strategy for the analysis of immune cell populations in peripheral blood and tumor-infiltrating immune cells after exclusion of doublets and dead cells.

**Suppl. Fig. 5. *Spleen weight* A)** Evaluation of the spleen size of mice treated on day 10 and killed on day 15. **B)** Endpoint analysis of spleen weight of mice treated on day 10. T-test for comparisons, *p*-values are defined as \* < 0.05, \*\* < 0.01, \*\*\* < 0.001 and \*\*\*\* < 0.0001.

Supplementary Materials

**Cytokine ELISA**

After thawing, MSCs were cultivated for a few days, harvested and then seeded at a defined density in Bio-1 (human MSCs) or alphaMEM (murine MSCs) and cultured for 48h-72h. Supernatants were harvested, freed from residual cells via centrifugation and analyzed via commercially available ELISAs (R&D systems) for either human or murine IL-7 and IL-12 as appropriate.

**Human PBMC/MSC coculture assay**

Human peripheral blood mononuclear cells (PBMC) were derived from leukapheresis products by the Department of transfusion medicine, cell therapeutics and haemostaseology (University Clinic Munich). They were either used fresh or cryopreserved for future use. In case frozen PBMCs were used they were rested overnight in RPMI (Lonza, BE12-918F) containing 10% FCS (Biochrome, S0115) and 1% Glutamax (Gibco, 35050-038) and then seeded into vials in the indicated MSC to PBMC ratios keeping the PBMCs at a constant number of 400.000. Cells were cultured in RPMI (+10% FCS + Glutamax) with or without human MSCs in the indicated ratios for 3 days under stimulation with 1 µg/ml anti-CD3 (BD Biosciences) and 5 µg/ml anti-CD28 (BD Biosciences) antibodies. INFγ and TNFα content of supernatants was determined by ELISA.

**Human surface marker analysis**

hMSCs were cultivated for a few days, harvested, split into three vials and stained with three antibody combinations: (1) isotypes; (2) CD34, CD45, CD73, CD90, CD105; (3) CD3, CD14, CD19, CD41, CD235a antibodies listed below. FACS analysis was performed with a MACSQuant system (Miltenyi biotec).

|  |  |  |  |
| --- | --- | --- | --- |
| **Antigen** | **Fluorochrome** | **Clone** | **Manufacturer** |
| CD3 | PE-Cy7 | SK7 | Becton Dickinson |
| CD14 | APC | 61D3 | eBioscience |
| CD19 | APC-Vio770 | LT19 | Miltenyi Biotec |
| CD34 | PE-Cy7 | 8G12 | Becton Dickinson |
| CD41a | PE | HIP8 | Becton Dickinson |
| CD45 | FITCH | 2D1 | Becton Dickinson |
| CD73 | APC | AD2 | Becton Dickinson |
| CD90 | APC-Vio770 | DG3 | Miltenyi Biotec |
| CD105 | PE | 266 | Becton Dickinson |
| Glycophorin A (CD235a) | PE | IS5-21F5 | R&D Systems |
| IgG1 | APC | MOPC-21 | Becton Dickinson |
| IgG1 | APC-Vio770 | IS5-21F5 | Miltenyi Biotec |
| IgG1 | FITC | MOPC-21 | Becton Dickinson |
| IgG1 | PE | MOPC-21 | Becton Dickinson |
| IgG1 | PE-Cy7 | MOPC-21 | Becton Dickinson |
| Live/Dead | 7-AAD | - | Becton Dickinson |
| Hoechst 33342 | - | - | Becton Dickinson |

**Murine surface marker analysis**

Murine MSCs (mMSCs) were cultivated for a few days, harvested, split into eight vials and stained with the antibodies listed below (one antibody per vial). Flow-cytometry analysis was performed with a MACSQuant system (Miltenyi).

|  |  |  |  |
| --- | --- | --- | --- |
| **Antigen** | **Fluorochrome** | **Clone** | **Manufacturer** |
| CD11b | APC | M1/70 | eBioscience |
| CD45 | APC | 30-F11 | eBioscience |
| CD29 | Pacific Blue | HMβ1-1 | BioLegend |
| CD44 | APC | IM7 | BioLegend |
| Sca-1 | Pacific Blue | D7 | BioLegend |
| IgG2a,k | Pacific Blue | RTK2758 | BioLegend |
| IgG | Pacific Blue | HTK888 | BioLegend |
| IgG2b,k | APC | RTK4530 | BioLegend |

**Supplemental Tables**

**Table S1:** List PCR primers for murine *Tcrb* sequencing

|  |  |  |
| --- | --- | --- |
|  | **Primer name** | **Sequence** |
| **Forward primer** | **M-Tcrbv1-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTctggTACCACGTGGTCAAGCTG |
| **M-Tcrbv2-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTcgaaCAGTATCTAGGCCACAATGC |
| **M-Tcrbv3-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTggaCCCAAAGTCTTACAGATCCC |
| **M-Tcrbv4-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTttggaGACGGCTGTTTTCCAGAC |
| **M-Tcrbv5-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTtactGGTATAAACAGAGCGCTGAG |
| **M-Tcrbv12-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTattctGGGGTTGTCCAGTCTCCa |
| **M-Tcrbv13-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTtggagGCTGCAGTCACCCAAAG |
| **M-Tcrbv14-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTgatatGCAGTCCTACAGGAAGGG |
| **M-Tcrbv15-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTctgGAGTTACCCAGACACCCAG |
| **M-Tcrbv16-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTaacaCCTAGGCACAAGGTGACAG |
| **M-Tcrbv17-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTaccaaaaGAAGCCAAACCAAGCAC |
| **M-Tcrbv19-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTattcctGATTGGTCAGGAAGGGC |
| **M-Tcrbv20-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTcatgaGGATGGAGTGTCAAGCTG |
| **M-Tcrbv21-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTCCATGGACTCTGGGGTTGT |
| **M-Tcrbv23-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTgatgCTGCAGTTACACAGAAGCC |
| **M-Tcrbv24-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTaccCAGACTCCACGATACCTGG |
| **M-Tcrbv26-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTgatatctGGTGAAAGGGCAAGGAC |
| **M-Tcrbv29-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTatgttttGCTGGAATGTGGACAGG |
| **M-Tcrbv30-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTagtgtCCTCCTCTACCAAAAGCC |
| **M-Tcrbv31-FW** | ACACTCTTTCCCTACACGACGCTCTTCCGATCTCTAACCTCTACTGGTACTGGCAG |
| **Reverse primer** | **M-Tcrbj1-1-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTaACTGTGAGTCTGGTTCCTTTACC |
| **M-Tcrbj1-2-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCTGGTCCCTGAGCCGAAG |
| **M-Tcrbj1-3-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcggCTTCCTTCTCCAAAATAGAGC |
| **M-Tcrbj1-4-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcaGACAGCTTGGTTCCATGACCG |
| **M-Tcrbj1-5-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcGAGTCCCCTCTCCAAAAAGCG |
| **M-Tcrbj1-6-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGTGAGCCGGGTGCCTGC |
| **M-Tcrbj2-1-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCGTGTCCCTGGTCCGAAG |
| **M-Tcrbj2-2-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcCCAGCACTGTCAGCTTTGAGC |
| **M-Tcrbj2-3-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgGTTCCTGAGCCAAAATACAGCG |
| **M-Tcrbj2-4-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgGTGCCCGCACCAAAGTACAAG |
| **M-Tcrbj2-5-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTaGTGCCTGGCCCAAAGTACTGG |
| **M-Tcrbj2-7-RV** | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcCTAAAACCGTGAGCCTGGTGC |

Green letters indicate the forward (FW) and reverse (RV) RV Illumina adapter sequence. Black letters indicate previously published FW(66) and RV(67) primer sequences and red letters changes to the sequences. Note that the sequence for the M-Tcrb21-FW primer is not published elsewhere.