Retroviral vectors and vector production

The retroviral SFFV-derived vectors SF11tCD34, SF11flCD34 or SF11dLNGFR encode human truncated or full-length forms of CD34 or cytoplasmically deleted form of the low-affinity nerve growth factor receptor, respectively.1,2 SF91PdsRED2 expresses the dsRED2 fluorescent protein. The SF91 backbone contains the SFFV LTR and modifications of RNA processing elements as described.3 SF91.IRES-EGFP.WPRE has also been described elsewhere.4 XRCC4 was cloned into SF91.IRES-EGFP.WPRE to obtain SF91.XRCC4.IRES-EGFP.WPRE. The retroviral SFFV-derived vectors SFα11tCD34, SFα11flCD34 and SFα11EGFP encode mouse truncated or full-length forms of CD34 and the enhanced green fluorescent protein, respectively. The retroviral vector HaMDR1 and the HaMDR1 GP+E86 producer cells were generated as described.5 The HaMDR1 vector backbone was derived from the Harvey murine sarcoma retrovirus. SF91TAg (long name FMEV-lox-EGFP2A-SV40LT-tCD34) contains sequences of a loxP-site flanked cDNA of SV40 TAg antigen, coexpressed with EGFP as a self-cleaving EGFP2A-TAg fusion protein.6 Ecotropic cell-free virus supernatants were generated by transient transfection of Phoenix-gp packaging cells (kindly provided by G. Nolan).7 Titers of ecotropic supernatants were determined on SC1 fibroblasts.

Bone marrow transduction and transplantation

Lineage negative (Lin−) BM cells were transduced as previously described.8 Briefly, lineage negative cells were isolated from complete BM by magnetic sorting using lineage specific antibodies (Gr1, CD11b, CD45R/B220, CD3e, TER-119; Pharmingen, Hamburg, Germany). The Lin− cell population was then cultured in StemSpan HS2000 medium (CellSystems, St. Katharinen, Germany), containing 50 ng/ml mSCF, 100 ng/ml hFlt-3 ligand, 100 ng/ml hIL-11, 20 ng/ml mIL-3, 1% penicillin/streptomycin, 2 mM glutamine. Retroviral transduction was done using Retronectin (TaKaRa, Otsu, Japan) preloaded plates with different multiplicity of infection (MOI) according to experimental design.8 5 x 10^5 up to 2 x 10^6 cells were transplanted into lethally irradiated mice, as described.2,5 For HaMDR1 transduction, unseparated BM cells were seeded on irradiated (15 Gy) virus producing GP+E86 cells as described.5 For transduction of SF91.XRCC4.IRES.EGFP and SF91.IRES.EGFP, BM was harvested from tibia and femurs of 8- to 12-weeks-old C57BL/6J mice (Jackson Laboratories) 48-60 h after treatment with 5-fluorouracil (50 mg/kg; SoloPak Laboratories, Franklin Park, IL). Low density BM cells were isolated and prestimulated for 48 h at 37°C in 5% CO2 with 100 ng/ml of recombinant rat stem cell factor, 100 ng/ml of megakaryocyte growth and development factor and 100 ng/ml
of granulocyte colony-stimulating factor (Peprotech, Rocky Hill, NJ) in Iscove's modified Dulbecco's medium supplemented with 10% FBS (HyClone, Logan, UT) and 2% penicillin-streptomycin (Invitrogen, Carlsbad, California). Retroviral transduction was performed on fibronectin fragment CH296 (RetroNectin; Takara Shuzo, Biotechnology Group, Otsu, Japan) at a concentration of 8 µg/cm² with a multiplicity of infection of ranging from 1-4 in the presence of cytokines. Over 48 h, the cells were incubated with viral supernatant twice for 15h each. After transduction, hematopoietic cells were harvested using cell dissociation buffer (Invitrogen, Carlsbad, Ca). The recipient mice for BM transplantation (B6-CD45.1) were irradiated with 7 + 4.75 Gy (137Cs source, Nordion International, Kanata, Canada]. Then 1x10⁶ cells were injected either retroorbitally or via the tail into the recipients Three weeks after transplantation, half of the animals were randomly selected and subjected to 4 Gy sub-lethal irradiation. After 8 weeks the primary recipients were sacrificed to obtain BM. Harvested cells were washed and 2x10⁶ BM cells were transplanted into lethally irradiated secondary recipients.

References