

Vector construction and production

T9PD was derived from the previously described TNS9¹ by addition of the central polypurine tract (cPPT) element^{2,3} (nucleotides 4781-4898 HIV-1 NCBI Accession number EF36312), and the hPGK-DHFR (human phosphoglycerate kinase promoter driving the dihydrofolate reductase cDNA) cassette⁴, and deletion of the 3'LTR U3 region. The globin transcription unit in T9PD and TNS9.3 (which lacks hPGK-DHFR) is identical to that in TNS9.¹ S9, which encode the shorter 265bp⁵ globin promoter, was derived from T9PD by removal of nucleotides 69933 to 70282 (XbaI to SnaBI site) (NCBI Accession number NG_000007). The HS1 element of LCR was amplified from human genomic DNA (nucleotides 20900 – 21973 NCBI Accession number NG_000007) (Forward primer: 5'-AAACACCTCTAGGCTATAAGGCAACAGAGC-3' and Reverse primer 5'-AAGTAAACTTCCACAACCGCAAGC-3'). HS1 was inserted in T9PD at the *XbaI* site lying between the β -globin promoter and HS2. T12PD was derived from T9PD by truncating the 5'flanking region of HS4 from position 8516 to 9135 (NCBI Accession number NG_000007). The pCL⁶ plasmid encoding the human IFN- β S/MAR (Scaffold/Matrix Attachment Region) was kindly provided by Dr. J. Bode and the 800bp element inserted into T9PD vector to replace 5'flanking region of HS4, in both orientations (T12SAR1PD and T12SAR2PD). The T12HS3PD construct was obtained by insertion of a 620bp fragment of the 5' flanking region of HS3 into T12PD vector in the same orientation. PCR amplified fragments of human glucose-6-phosphate dehydrogenase (G6PD) cDNA were used as spacer sequence. Viral stocks were generated by triple transfection of the recombinant vectors, pCMVR8.9⁷, and pMD.G⁸ into 293T cells as described.^{1,9} The pseudotyped virions were concentrated by ultracentrifugation and titrated in NIH3T3 cells as described.^{1,10,11} All vectors used in this study were produced at high titer. Sample unconcentrated titer in 3T3NIH cells (n=3) and provirus size for the corresponding vectors is shown in table below:

Vector name	Provirus size[bp]	Titer [TU/ml]	SD
T9PD	8452	7.58E+05	8.11E+04
T12PD	9275	5.17E+06	4.66E+05
S9PD	9482	2.33E+06	7.85E+05
S10PD	10568	9.58E+05	1.43E+05
T10PD	10966	1.45E+06	2.89E+05

Vector copy number quantification

Vector copy numbers obtained by Southern blot were corroborated by TaqMan analysis using the following primers and probes: (Gag-specific: 5'-GGAGCTAGAACGATTTCGCAGTT-3'; 5'-GTTGTAGCTGTCCCAGTATTTGTC-3'; probe: 5'-ACAGCCTTCTGATGTTTCTAACAGGCCAGG-3', mouse β -actin specific: 5'-TCACCCACACTGTGCCCAT-3'; 5'-AGCCAGGTCCAGACGCAG-3'; probe: 5'-TACGAGGGCTATGCTCTCCCTCACGC-3').

Bone marrow chimeras

Animal work was approved by the Institutional Animal Care and Use Committee at MSKCC. Bone marrow (BM) was flushed from the femurs and tibias of 8- to 16-week-old male Hbb^{th3/+} mice 4 days after iv of 5-fluorouracil (5-FU, Pharmacia; 150mgxkg⁻¹ body weight). Cells were prestimulated for 12hrs in X-VIVO15 media (BioWhittaker, Cambrex Bio Sci) supplemented with β -mercaptoethanol (0.5mM) (GIBCO-Invitrogen, Carlsbad, CA), L-glutamine (2mM), penicillin (100IU/ml) and streptomycin (100ug/ml), recombinant mouse stem cell factor (rmSCF) (100ng/ml) and recombinant mouse thrombopoietin (rmTPO) (100ng/ml) (R&D Systems). 1×10^6 BM cells / ml / well were transduced, in supplemented X-VIVO15 media, at MOI 20-35 on RetroNectin-coated 6-well plates (15ug/ml, TAKARA Shuzo) for 8hrs. Recipient mice (11- to 18-week old Hbb^{th3/+} mice) were irradiated with 10.5Gy (split dose 2×5.25 Gy 6hrs apart) on the day of transplantation. $5 \times 10^5 - 1 \times 10^6$ cells were administered by intravenous injection.

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