Systematic comparison of culture media and transduction enhancers for optimised CD34⁺-cell-based retroviral gene therapy protocols

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INTRODUCTION

Ex-vivo retrovirus-based cell modification has demonstrated considerable success in treating monogenetic haematological diseases. However, current gene therapy protocols may profit from further optimisation of CD34⁺ HSPC culture and infection to improve cell quality, increase efficiency and reduce cost, and to allow the application of difficult-to-transduce cells or vectors.
We compared different culture media for CD34⁺ HSPC expansion and preservation of stemness, as well as tested different transduction enhancers (TE) and their combination for their ability to increase HSPC transduction with lentiviral or alpharetroviral vectors in order to optimise current retroviral vector-based gene therapy protocols.

RESULTS

COMPARISON OF HSPC CULTURE MEDIA

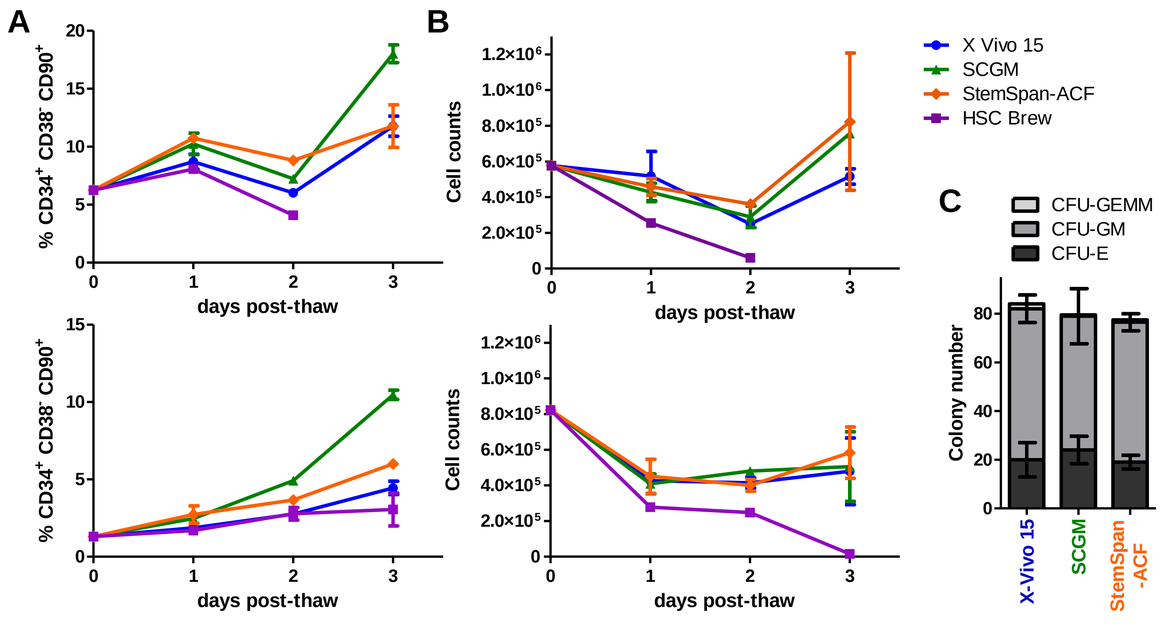

Figure 1. *[**A**,**B**] Three different GMP-grade and one animal-component-free (ACF) media were compared, all supplemented with hSCF (300ng/mL), hFLT3-L (300ng/mL) and hTPO (100ng/mL). Cells from n = 2 healthy donors were thawed, seeded in the different media and analysed daily by FACS determining [A] HSPC marker expression and [B] cell counts. [**C**] CFU assay. Cell plating in MethoCult was performed on day 3 post-thaw and of culture in the different media. Colonies were manually counted 2 weeks post-plating.*

TRANSDUCTION ENHANCERS (TE) INCREASE HSPC GENE TRANSFER RATES

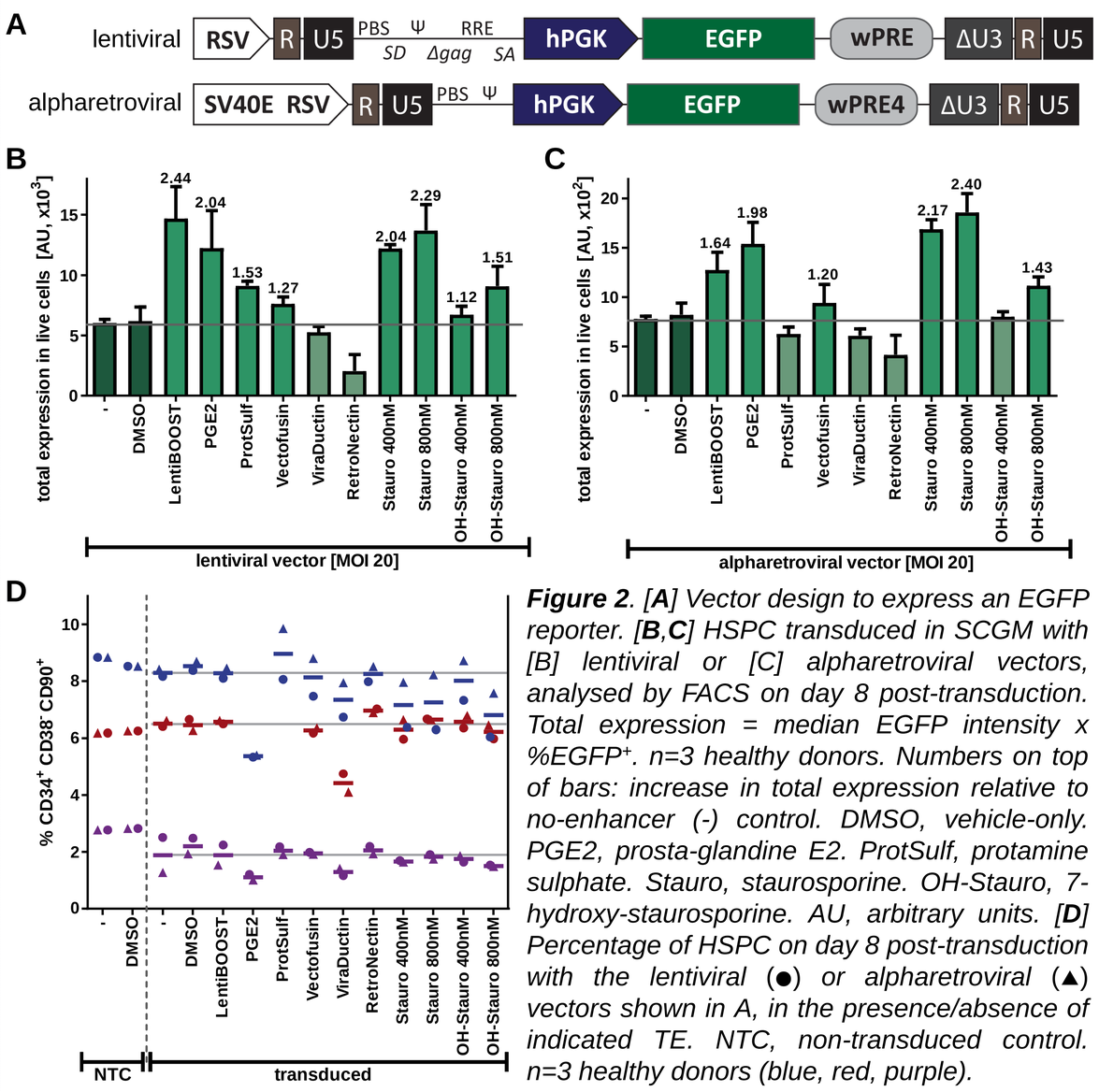

Figure 2. *[**A**] Vector design to express an EGFP reporter. [**B**,**C**] HSPC transduced in SCGM with [B] lentiviral or [C] alpharetroviral vectors, analysed by FACS on day 8 post-transduction. Total expression = median EGFP intensity x %EGFP⁺. n=3 healthy donors. Numbers on top of bars: increase in total expression relative to no-enhancer (-) control. DMSO, vehicle-only. PGE2, prosta-glandine E2. ProtSulf, protamine sulphate. Stauro, staurosporine. OH-Stauro, 7-hydroxy-staurosporine. AU, arbitrary units. [**D**] Percentage of HSPC on day 8 post-transduction with the lentiviral (●) or alpharetroviral (▲) vectors shown in A, in the presence/absence of indicated TE. NTC, non-transduced control. n=3 healthy donors (blue, red, purple).*

TE COMBINATION FURTHER INCREASES LENTIVIRAL HSPC GENE TRANSFER RATES

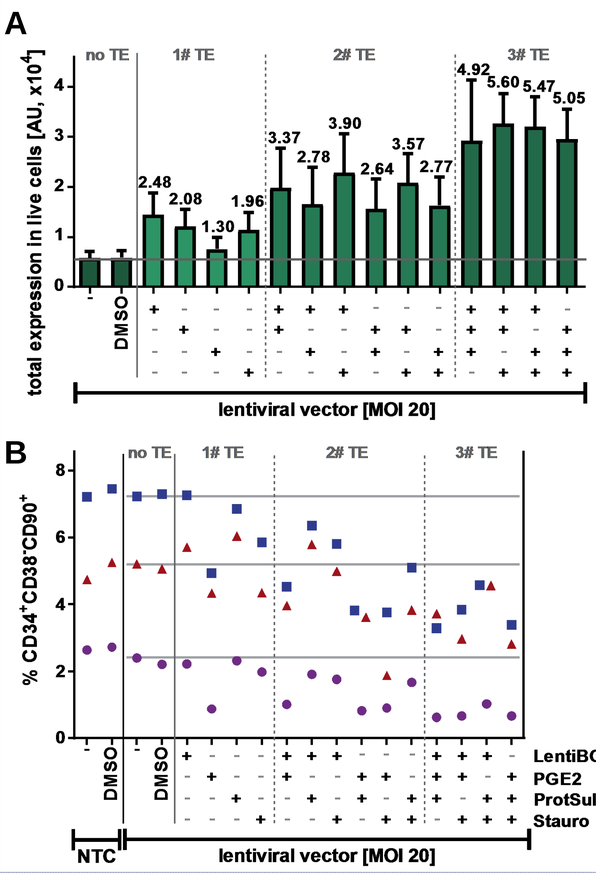

Figure 3. *Selected TE were combined for HSPC transduction with a lentiviral EGFP vector (Fig. 2A) in SCGM. Cells were analysed by FACS on day 8 post-transduction for [**A**] total expression (= median EGFP intensity x %EGFP⁺) and [**B**] percentage of HSPC. n=3 healthy donors. [**C**] Transduced cell populations were additionally analysed for cell viability (% DAPI⁻) the day after transduction with a lentiviral vector (Fig. 2A) at MOI 20 (●, ▲) or MOI 10 (■). n = 3 healthy donors.*

TE INCREASE TRANSDUCTION RATES WITH A CLINICAL-GRADE LENTIVIRAL VECTOR

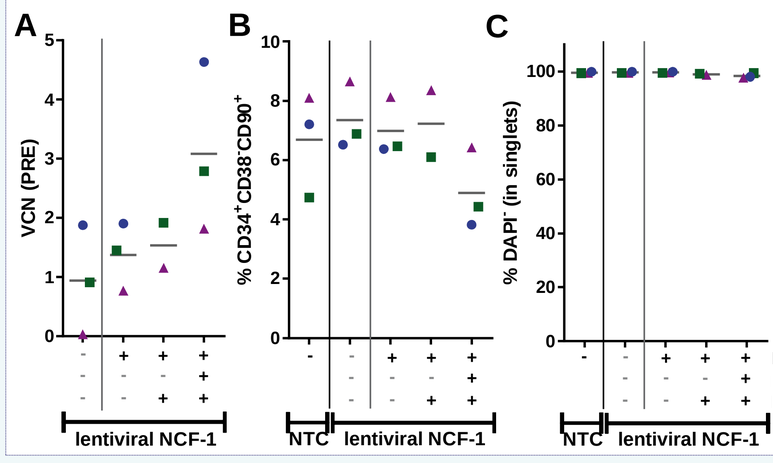

Figure 4. *Selected TE were combined to transduce HSPC with a clinical-grade lentiviral NCF-1 vector at MOI 20 (●, ■) or MOI 10 (▲). [**A**] Vector copy numbers (VCN, assessed through qPCR on PRE) and [**B**] percentage of HSPC on day 8 post-transduction. n=2/3 healthy donors. [**C**] Viability (% DAPI⁻) on day 1 post-transduction.*

LENTIBOOST PLUS PROTSULF INCREASE THE VCN OF AN HSPC ATIMP FOR X1-SCID

		w/o TE	+ TE	fold increase
Viability after transduction		94.47	87,90	0,93
VCN	Liquid culture (7 days of cuture)	0.71	4,53	6,38
	Individual CFUs (Mean ± SD; n)	0.77 ± 0.22; 71	2.43 ± 2.23; 71	3,16
	Individual CFUs (BFU-E) (Mean ± SD; n)	0.70 ± 0.22; 35	2.61 ± 2.49; 36	3,73
	Individual CFUs (non BFU-E) (Mean ± SD; n)	0.87 ± 0.34; 36	2.24 ± 1.96; 35	2,57
% of vector⁺ CFUs	Total	25.35	83,10	3,28
	BFU-E	31.43	83.33	2,65
	Non BFU-E	19.44	82.86	4,26

Figure 5. *Healthy donor HSPC were modified with a lentiviral vector in a GMP manufacturing process to generate an ATIMP for X1-SCID. LentiBOOST and ProtSulf were combined (+TE) and tested against no-enhancer (w/o TE) transduction conditions. Cells were transduced at a density of 1.5x10⁶ cells/mL and a vector concentration of 1x10⁸ IG/mL (MOI 66.66), with 2 hits of transduction (days 1 and 2 post-thaw). Transduced cell populations were analysed for viability (Trypan blue) and vector copy numbers (VCN, via qPCR) in liquid culture and individual CFUs. Fold increase = "+TE" divided by "w/o TE".*

CONCLUSION

- SCGM presented superior in terms of cell expansion and preservation of HSPC stemness
- 6/8 (lentiviral) and 5/8 (alpharetroviral) TE increased HSPC transduction
- the four best performing TE yielded an even higher increase in transduction rates upon their various combination
- LentiBOOST plus protamine sulphate increased the VCN of a clinical-grade lentiviral (NCF-1) vector
- LentiBOOST plus protamine sulphate also led to an increase in VCN in a GMP manufacturing process of an HSPC ATIMP for X1-SCID

→ SCGM plus TE may be used to improve current gene therapy protocols

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