New modified poloxamers enhancing virus efficiency for cell therapy engineering

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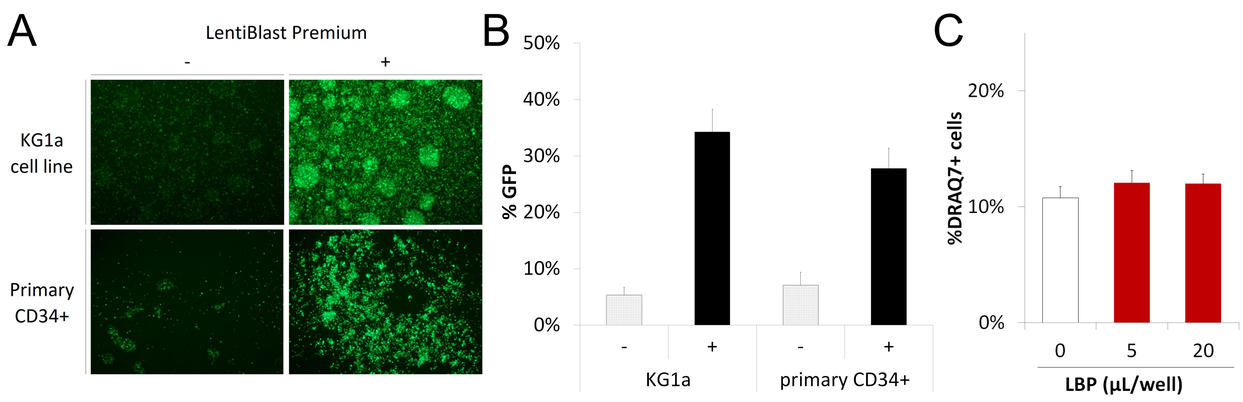
INTRODUCTION

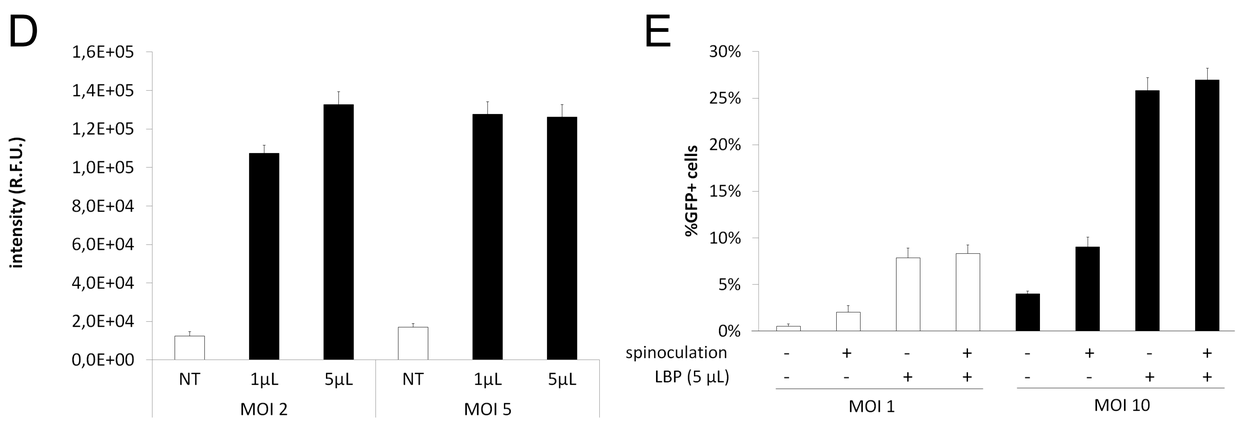
The capacity to genetically modify Hematopoietic Stem Cells (HSC) with the help of lentiviral vectors (LV) for gene therapy in the context of autologous hematopoietic stem cell transplantation (HSCT) has been clearly demonstrated for genetic diseases. Today, multiple HSCT gene therapy trials using lentivirus to treat genetic disorders are ongoing and even completed. However, **transduction of HSC is still challenging since high loads of viral vector need to be applied** to overcome existing barriers and achieve clinically relevant transduction levels. Thus **there is a need to increase LV efficiency without inducing non-desired effects**.

In this work we report the **development of LentiBalst Premium (LBP), a totally new patented cationic block-copolymer designed to enhance lentiviral transduction** and to stabilize magnetic nanoparticles.

We demonstrate that **this new modified poloxamer LBP yields high transduction efficiencies of HSC without affecting their viability** and that it stabilizes and potentiates magnetic nanoparticles to enhance transduction of CD34+ HSC directly on immunomagnetic cell sorting devices. This innovative application brings LBP one step away from automatic cell manufacturing process integrating transduction of CD34+ HSC in close system, a major improvement in the genetic modification of HSC for personalized cell therapeutic strategies.

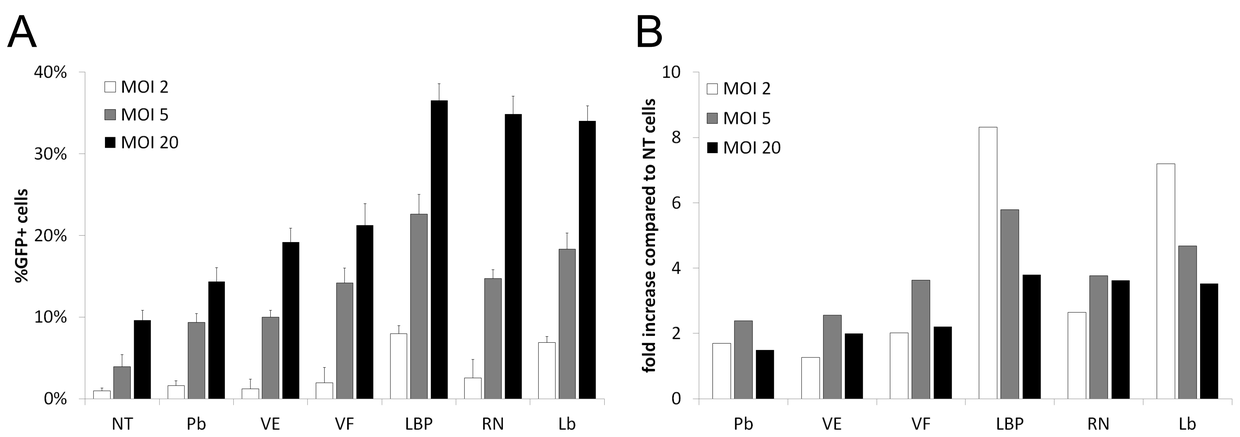
Efficiency on CD34+ cells and Toxicity





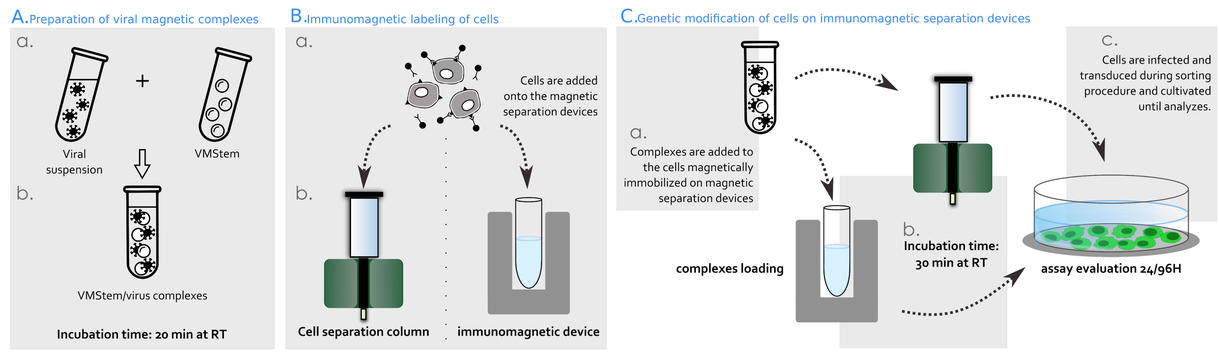
KG1a and CD34+ primary stem cells were infected with GFP-encoding Lentivirus using a M.O.I. of 5 in presence or not of 5µL LentiBlast Premium. 72H after, fluorescence microscopy (A) and cytometer analysis (B) demonstrated **the efficiency of LBP to increase viral mediated transduction** while DRAQ7 DNA binding dye staining and prove the total innocuousness of LentiBlast Premium even at high dose (C). In primary CD34+, LBP increased the protein production level in a dose-dependent way when cells were infected at two M.O.I (D). When using LBP, no spinoculation step is needed when transducing CD34+ cells and moreover lowering time and handling.

Comparison with competitors

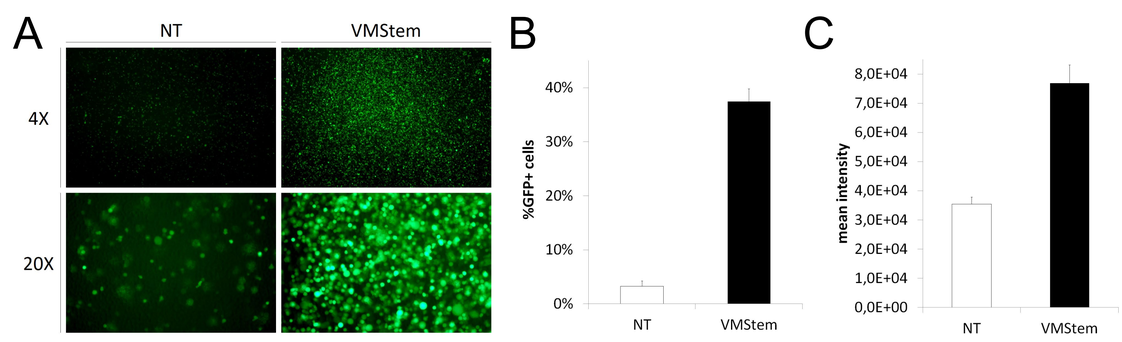


KG1a were infected with GFP encoding lentivirus at M.O.I. of 2, 5 and 20 in presence or not of transduction enhancers. (A) Percentage of GFP+ cells was measured 72H after by flow cytometry and fold increase in transduction was calculated in comparison to non treated cells (B). NT, non treated infected cells; Pb, polybrene; VE, ViralEntry; VF, Vectofusin; LBP, LentiBlast Premium; RN, RetroNectin; Lb, LentiBOOST. Results are given as the mean of samples (n=3) ± SD.

Effeciency on cell sorting devices

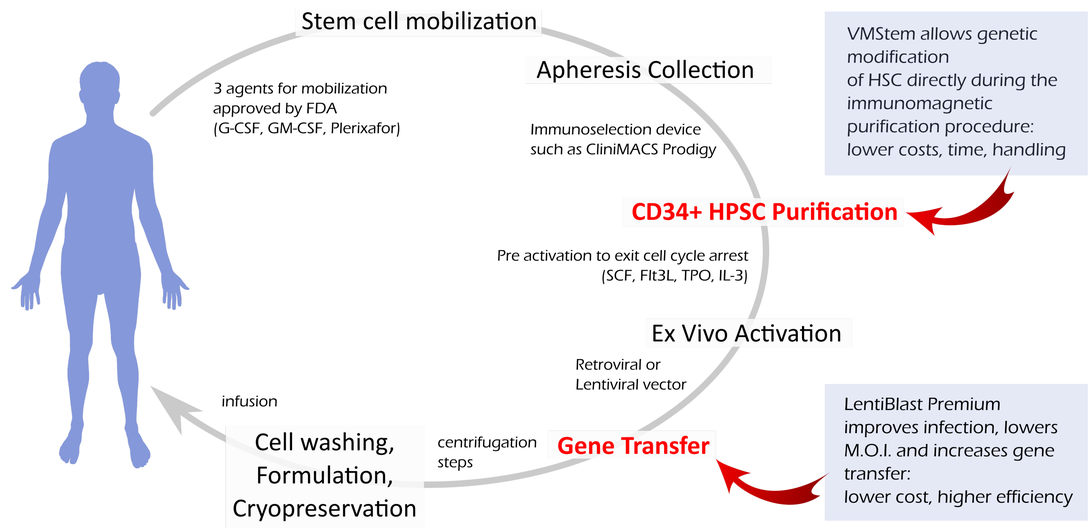


(A) Lentiviral vectors are associated with ViroMag Stem (VMStem) magnetic nanoparticles and incubated at RT for 20 min. (B) Stem cells are immunomagnetically labeled and loaded cell separation devices. (C) Complexes are then added to the separation magnets and incubated for 30 min at RT. **During this procedure, the target cells are immobilized by the magnets and get in close contact with magnetized virus that favors their genetic modification.** Finally, cells are flushed from the device and cultivated until evaluation of the experiment.



The capactity of **VMStem to increase LV-mediated transduction** on cell sorting device was monitored by fluorescence microscopy (A) and FACS analysis with %GFP+ cell (B) and fluorescence intensity (C) measurements.

LentiBlast Premium and Cell Therapy



ViroMag Stem and LentiBlast Premium fit perfectly during the major steps in viral vector genetically modified HSC cell manufacturing process. VMStem empowers the purification process with the capacity to genetically modify CD34+ stem cells directly on cell sorting device such as the CliniMACS prodigy® while LBP improves transduction after sorting and/or activation. Both reduce viral dose, time and handling and consequently the cost of experiment while increasing gene transfer and overall efficiency.

(figure adapted from Wang X. et al, Methods & Clinical Dev. 2017).

CONCLUSION

The vast majority of *ex-vivo* modified gene therapy products have been generated using retroviruses and lentiviruses, however their capacity to genetically modify HSC remains low even using high M.O.I. In order to reduce the consequent host innate immunity response and dramatically lower cost linked to viral vector manufacturing, we have developed **LentiBlast Premium (LBP), a new class of cationic poloxamer based on cationic block-copolymer** and the resulting magnetic nanoparticles **ViroMag Stem (VMStem)** for genetic modification on cell sorting devices.

All the experiments demonstrated that LBP was able to increase lentivirus-mediated transduction of CD34+ Stem cell line or primary cells without impairing their survival both in terms of gene expression and protein production. Its formulation enhanced with magnetic nanoparticles allows to integrate lentiviral mediated genetically modified HSC cell manufacturing process using immunomagnetic cell sorting devices.

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