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ENGINEERING HUMAN HEMATOPOIETIC STEM CELLS FOR *EX VIVO* NEUTROPHIL PRODUCTION

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Introduction. Neutrophils are the most abundant type of leukocytes in human blood. As the first line of the immune response, neutrophils play an important role in containing and treating infections. Neutrophils are used in specific cases as therapeutic option in neutropenic patients, but are also investigated as applications in immunotherapy (1). However, *in vitro* studies of human peripheral blood neutrophils are hampered by their short half-life, susceptibility for priming and activation during standard enrichment procedures, and impossible storage. We now differentiate neutrophil-like cells *ex vivo* from human hematopoietic stem and progenitor cells (HSPCs), however the initial yield of HSPC remain an issue as these cells are extremely rare and expensive to obtain (2). A conditionally immortalized HSPC progenitor cell line would solve this problem by supplying a virtually unlimited source of human neutrophil progenitors to produce and study mature human neutrophils *ex vivo*.
Therefore, in this work, we aim to establish a conditionally immortalized HSPC line through the time-and drug-restricted expression of HPV E6 and E7 oncoproteins.

Methodology. Briefly, CD34$^+$ HSPCs were collected from umbilical cord blood (UCB) and transduced using a third-generation lentiviral system delivering an HPV-E6/E7 Tet-inducible system and a fluorescent tracker gene (Fig 1.), as previously reported (3). Transduced and untransduced cells were differentiated into neutrophils in StemLine II, with SCF, G-CSF and TPO, as previously reported (2). After 15-days of differentiation, the surface markers phenotype (CD11b and CD15), ROS production and phagocytosis was analyzed by flow cytometry.

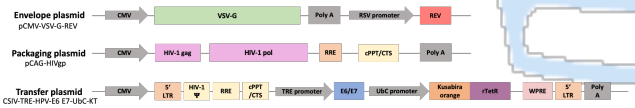

Fig. 1. Immortalization system

Results. We produced high titers of virus able to transduce adherent and suspension cells. We are currently optimizing conditions to transduce and expand HSPCs. Untransduced control CD34$^+$ HSPCs could be differentiated into neutrophil-like cells with >70% of cells exhibiting a CD15$^+$/CD11b$^+$ phenotype after 15 days of differentiation. Morphological heterogeneity of differentiated cultures was consistent with the various late stages of differentiation and mature neutrophils observed in flow cytometry (Fig. 2). In addition, differentiated neutrophil-like cells showed antimicrobial functions such as ROS production and *S. aureus*-BioParticles phagocytosis rates comparable to that of peripheral blood (PB) neutrophils (Fig. 3).

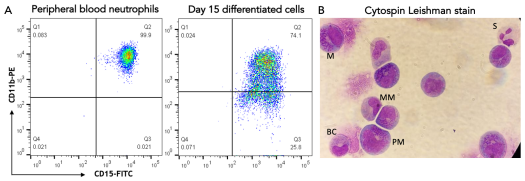

Fig. 2. Representative dot plots from CD11b$^+$CD15$^+$ cells from PB neutrophils and *ex vivo* differentiated cells from HPSCs (A) and Cytospin Leishman stain showing morphological heterogeneity (B).

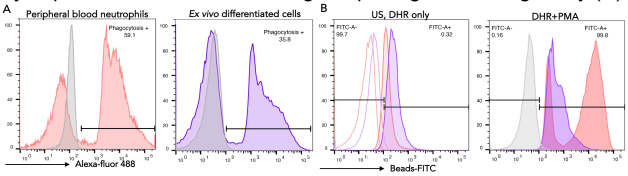

Fig. 3. Representative histograms of *S. aureus*-BioParticles phagocytic capacity (A) and ROS production (B) of PB neutrophils and *ex vivo* differentiated cells from HPSCs.

Conclusions. We differentiate CD34$^+$ HSPCs into neutrophil-like cells using growth factors-supplemented chemically defined media. Day 15 differentiated neutrophil-like cells perform neutrophil functions like phagocytosis and ROS production. We have developed a pseudovirus able to transduce adherent and suspension cell lines.

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