High-throughput FACS-purification of Transduced Progenitors Expressing Defined VEGF Levels Induces Controlled and Safe Angiogenesis in Chronic Ischemia

Andrea Banfi1, Thomas Wolff2, Heidi Misteli3, Philipp Fueglistaler2, Roberto Gianni-Barrera1, Lorenz Guerke2, Michael Heberer2
Corresponding Author: Andrea Banfi (abanfi@uhbs.ch)

1Department of Biomedicine and 2Department of Surgery, Basel University Hospital, Basel, Switzerland.

Introduction
Vascular Endothelial Growth Factor (VEGF) gene delivery is a powerful strategy to restore flow to ischemic tissue or to vascularize critical-size tissue-engineered constructs. However, we have previously shown that VEGF can induce normal or aberrant angiogenesis depending exclusively on the amount secreted in the microenvironment, and not on its total dose, as it remains localized in the matrix around each producing cell1,2. This study aims to: 1) develop a clinically applicable method to achieve controlled angiogenesis in vivo by cell-based gene delivery; and 2) test its safety in chronic ischemia.

Materials and Methods
We developed a FACS-based method to rapidly purify genetically engineered progenitors producing desired levels of VEGF. Primary mouse myoblasts were transduced with a retroviral construct expressing VEGF164 linked quantitatively to a non-functional, truncated form of syngenic CD8a. Differences in VEGF expression were reflected by the cell-surface amount of CD8a, detected and quantified on live cells by FACS. FACS-purified populations were implanted in non-ischemic (mouse) and chronically ischemic (rat) skeletal muscles.

Results
VEGF expression was linearly correlated (R²=0.89) with that of CD8a at the single cell level analyzing individual clones isolated across the whole range of expression in the heterogeneous primary population (2 to 142 ng/10⁶ cells/day). Angiogenesis induced by these clones was analyzed in vivo and a clone expressing the highest VEGF level (34.0±1.7 ng/10⁶ cells/day) that induced only normal angiogenesis was selected as a reference. Cells expressing similar CD8a levels (and therefore of VEGF) were rapidly purified from the primary population (Fig. 1). VEGF and CD8a expression of the purified cells was stable during in vitro expansion over 23 doublings. In non-ischemic muscle, the heterogeneous population always caused angiomas. However, purified cells completely prevented angioma growth and induced robust normal angiogenesis, which was stable after 4 months.

Discussion and Conclusions
This clinically applicable, high-throughput technique significantly improved safety of VEGF expression without compromising efficacy. It is readily applicable to other progenitor populations (e.g. MSC) and it could help develop safe and efficacious vascularization strategies in regenerative medicine.

Fig. 1. FACS-purification of myoblasts expressing specific VEGF levels (blue tinted curves in right panels) from a heterogeneous transduced population (black empty curve) with 2 gates (red and black segments in left panel) based on the level of CD8a expression of a reference clone (blue empty curve).

Also after implantation in chronically ischemic muscle, the FACS-purified population induced only normal angiogenesis around the fibers where myoblasts had engrafted, leading to a doubling of vessel length density compared to control cells, and completely avoided aberrant vascular structures. Long-term safety was verified up to 3 months after implantation.

References
2. von Degenfeld G. et al. (2006) FASEB J.

Acknowledgments
Financial support by the Swiss Heart Foundation and the EU FP7 (ANGIOSCAFF).

Disclosures
Authors have nothing to disclose.