

**Transplantation Publish Ahead of Print**

**DOI: 10.1097/TP.0000000000002986**

**First Report on Ex vivo Delivery of Paracrine Active Human Mesenchymal Stromal Cells to Liver Grafts During Machine Perfusion**

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**Authorship**

MMAV participated in research design, writing of the paper, performance of the research and data analysis; LM participated in research design, writing of the paper, performance of the research and data analysis; RYR participated in performance of the research; JdH participated in performance of the research; KW participated in performance of the research; IJS participated in performance of the research; JMSP participated in performance of the research; MH participated

in research design; BMK contributed analytic tools (LC/MS); HH participated in performance of the research; SRRH contributed cells (hMSC); JNM participated in research design; CWGML participated in research design and contributed new reagents; LJWvdL participated in research design, writing of the paper, and data analysis; JdJ participated in research design, writing of the paper, performance of the research, and data analysis.

Conflict of Interest (COI) statement: The authors declare no conflicts of interest.

Financial Disclosure statement: The authors declare no funding was received for this study.

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Machine perfusion is rapidly becoming the standard for graft preservation and provide new opportunities for organ salvage, reconditioning and repair. Based on their immune modulatory and regenerate properties,<sup>1</sup> arguable mesenchymal stromal cells (MSCs) are the most promising cell therapy for graft repair “on the pump”, though proof of concept is still lacking.<sup>2</sup> Here we show the feasibility of delivering clinically relevant numbers of human MSCs (hMSC;  $5 \times 10^6/\text{kg}$ ) during 30 min hypothermic oxygenated machine perfusion (HOPE) in porcine liver grafts (n=8)(Figure 1). Full methods are described in Supplemental Materials and Methods (SDC, <http://links.lww.com/TP/B821>). To track the biodistribution of hMSC, cells were genetically labeled with click beetle red luciferase (CBred2).<sup>3</sup> As shown in Figure 2, bioluminescent imaging of both the anterior and posterior side of the liver showed a wide range and patchy distribution of hMSC which retain throughout the liver after 30 minutes of perfusion. No significant difference between arterial (n=4) or venous infusion (n=4) was observed. Histological and RNA-expression analysis confirmed the delivery of the hMSC throughout the liver grafts. About  $10^4$ - $10^5$  hMSC were estimated to be still present per kg liver graft. Importantly, the hMSC retained their paracrine activity after infusion. Using both Luminex and LC-MS/MS analysis, increasing levels of human-specific IL-6 and IL-8 released in porcine blood were shown during 4 hour normothermic perfusion. In conclusion, this is the first report showing effective delivery of hMSC in a liver machine perfusion model. Evidence of paracrine activity of hMSC after delivery indicate regenerative and immune modulatory effects during normothermic graft perfusion, though this needs further research in a transplantation model.

## References

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## Figure Legends

### Figure 1. Graphical abstract of the procedure.

The time line indicates the details of each time point. Pig livers are procured after a warm ischemia period of 30 min and infused with  $5 \times 10^6 - 10^7$  CBred2-labeled hMSC either via the portal vein or hepatic artery. The distribution of hMSC is visualized by bioluminescence imaging (BLI) immediately (time point 0) after infusion using an IVIS Imaging System 200. The liver is then placed on a purpose-made perfusion device in which it is perfused with cold ( $10^\circ\text{C}$ ) oxygenated UW-organ preservation fluid. After 30 min of perfusion, the distribution of the hMSC is again imaged using BLI. After imaging, the livers were perfused for 4 hours with their own, heparinized, full blood at normothermic temperature ( $37^\circ\text{C}$ ) by ex vivo machine perfusion after which biopsies were collected for further analysis. The BLI signal is quantified, presence of hMSC was assessed in biopsies taken from left and right periphery and center of the liver using human-specific antibodies, proteomic and genetic analysis.

**Figure 2. Effective delivery of paracrine active hMSCs during normothermic hepatic machine perfusion.** Live imaging of the livers (n=8) infused with CBred2-transduced hMSC in the hepatic artery (n=4, A, B, E, F) or portal vein (n=4, C, D, G, H) using the IVIS Imaging System 200, directly after infusion (directly after infusion, A, B, C, D) and after 30 min of perfusion (E, F, G, H). Images are representative images showing bright luciferin-based luminescence at the posterior (A, C, E, G) and anterior side (B, D, F, H) of the liver, demonstrating whole organ coverage of the infused hMSC. The presence of hMSC was confirmed by immunohistochemical staining for copGFP (I arrows; 40x objective) at end of perfusion whereas no staining was observed in liver biopsies prior to infusion (J). Representative pictures are shown. Human-specific B2M gene expression was determined by nested qPCR to

confirm the presence of human MSC in the pig liver at the genetic level (M). Shown are the raw Ct-values of B2M expression of three pigs. B2M expression was measured in the pig samples that were perfused with hMSC, (pig 1-3) and in nonperfused pig livers (n=3). nd: not detectable. Luminex-based analysis using human specific cytokines showed that IL-6 (O) and IL-8 (P) was secreted in the liver perfusate in 2 of the 4 pigs that were infused via the hepatic artery (filled symbols). In pig livers that were not infused with hMSC no IL-6 or IL-8 was detected (open symbols). Human-specific IL-6 and IL-8 protein release was confirmed in the same two pig livers by LC/MS analysis (data not shown).

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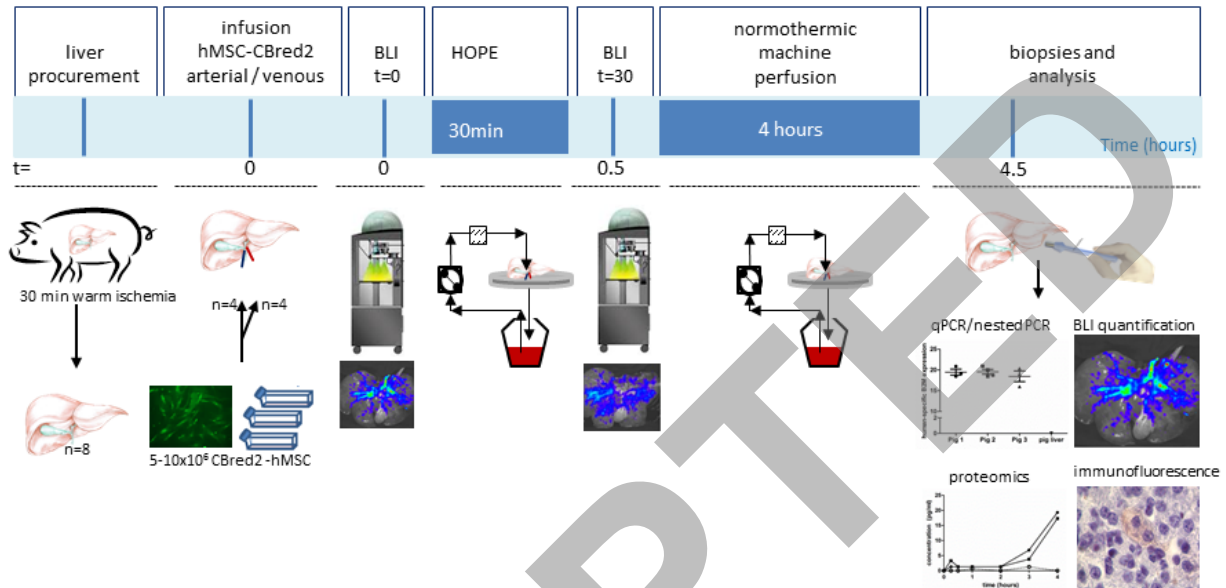


Figure 1.

**Figure 2**

