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## Abstract 26

# The Role of Oxygen in Cord Blood Hematopoietic Stem and Progenitor Cell Expansion and Engraftment

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**Introduction:** Hematopoietic stem (HSC) and progenitor cells (HPCs) are exposed to differing oxygen tensions ranging from <1% to 21% as they reside in/move through different tissues or are harvested for clinical utility. Functional changes in HSCs/HPCs are induced by acute changes in oxygen tension (e.g., a change in percent of cells in cycle).

**Objectives:** We sought to determine if variable oxygen levels affect expansion and/or functional properties of cord blood (CB) HSCs/HPCs *ex vivo* and in vivo.

**Methods:** Human CB CD34+ cells were grown in expansion culture +/-UM171, an agonist of HSC self-renewal that expands transplantable CB HSCs, in five oxygen tensions: 1%$O_2$, 3%$O_2$, 5%$O_2$, 14%$O_2$, and 21%$O_2$. HSCs/HPCs were enumerated by flow cytometry. Functional HPCs were enumerated by plating in semi solid media for colony forming unit assays (CFU). Cell cycle and reactive oxygen species (ROS) were measured by flow cytometry. Ability of expanded cells to engraft was determined by transplantation in non-lethally irradiated NSG mice.

**Results:** Immunophenotypic HPCs and functional HPC CFUs expanded significantly more after 7 days of growth in higher oxygen tensions (5%$O_2$-21%$O_2$) compared to lower (1%$O_2$-3%$O_2$), while immunophenotypic HSCs expanded best at 5%$O_2$. HSCs/HPCs grown in low oxygen tensions had significantly lower ROS levels, significantly higher percentage of cells in $G_0$, and were slightly but reproducibly smaller/less granular than those grown in high oxygen levels. HSC/HPC numbers were reduced in high oxygen tensions 1-2 days after plating but were better maintained in low, suggesting cells undergo a culture shock/stress after plating that is mitigated by reduced oxygen. In the presence of UM171, HSCs expanded significantly better at higher oxygen levels, but HPCs are better maintained in 5%$O_2$. *Ex vivo* CD34+ expansions maintained under physiological $O_2$ levels (1-14%$O_2$) demonstrated significantly better/faster neutrophil recovery following transplantation compared to cells expanded at 21%$O_2$ or input.

**Discussion:** HSCs/HPCs proliferate rapidly in high oxygen but have fewer quiescent cells, higher ROS, and are larger and more granular which are all characteristics associated with exhaustion. While high oxygen allows for faster growth, low tensions may mitigate cell stress and allow for prolonged growth (i.e., HSC/HPC expansion) while maintaining functional properties.