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EX VIVO EXPANSION OF HUMAN HAEMOPOIETIC PROGENITOR CELLS

by

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ABSTRACT

The studies described within this thesis focus on the *ex vivo* growth of human haemopoietic progenitor cells (HPC) with the objective of defining culture conditions for generating myeloid post-progenitor cells for therapy.

Initial studies demonstrated that a combination of six recombinant human HGF including interleukin-1 (IL-1), IL-3, IL-6, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage-CSF (GM-CSF) and stem cell factor (SCF) (136GGMS) provide a potent stimulus for *de novo* generation of myeloid cells and progenitors from mobilised blood $CD34^+$ cells. Subsequently, the four-factor combination of IL-3, IL-6, G-CSF and SCF (36GS) was shown to stimulate equivalent myeloid cell production from $CD34^+$ cells to that observed with 136GGMS. These studies demonstrated that sufficient myeloid post progenitor cells for clinical transplantation could be generated from $CD34^+$ cells cultured in 36GS. In addition, it is shown that generation of nucleated cells and nascent committed myeloid progenitor cells from $CD34^+$ cells during the first 14 days of *ex vivo* culture is mainly attributed to committed HPC with $CD34^+HLA\text{-}DR^+$ and $CD34^+CD38^+$ phenotypes. Analysis of HGF-receptor expression indicated that $CD34^+$ cells are heterogenous with respect to constitutive expression of receptors for IL-3, IL-6, G-CSF and GM-CSF and to a lesser extent SCF.

The fate of candidate haemopoietic stem cells during *ex vivo* culture was examined in studies designed to determine which HGF are required to maintain survival and stimulate the division of single HPC. As a single cytokine, thrombopoietin (TPO) was shown to be the most potent survival factor for $CD34^+CD38^-$ cells. These studies also suggested that the most primitive cells within the $CD34^+CD38^-$ fraction require simultaneous stimulation with

combinations of early acting HGF to induce cell division. Remarkably, the combination of IL-3, IL-6, G-CSF, SCF, FLT3L and TPO (36GSFT) was shown induce division in greater than 90% of single $CD34^{+}CD38^{-}$ cells. Subsequent studies demonstrated that this response was attributed to the combination of SCF, FLT3L and TPO (SFT). Finally, these studies suggested that primitive HPC cells within the $CD34^{+}CD38^{-}$ fraction could be hierarchically ordered according to their requirement for stimulation with combinations of HGF to induce cell division. It is proposed that the most primitive HPC within the $CD34^{+}CD38^{-}$ fraction are those that exhibit an obligate requirement for simultaneous stimulation with 36GSFT.

Collectively, the studies contained within this thesis lay a solid foundation for *ex vivo* manipulation of human haemopoietic progenitor cells under stroma-free cytokine dependent culture conditions.

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