

# Development of Selective Amplifier Genes for Hematopoietic Stem Cell Gene Therapy

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## Abstract

To overcome the low efficiency of gene transfer into hematopoietic stem cells, we developed a novel system for selective expansion of transduced cells. The system utilizes “SAGs (selective amplifier genes)” which encode fusion proteins consisting of a growth-signal generator and its molecular switch. First, a fusion gene encoding a chimeric receptor between G-CSF receptor (GCR) and hormone-binding domain of estrogen receptor (ER) was constructed. We deleted the G-CSF-binding domain from the chimeric gene and introduced a mutation (Y703F) to block the differentiation signal. In addition, a mutant ER (TmR), which binds to 4-hydroxytamoxifen (Tm), was also used in place of a wild type ER. When the murine bone marrow cells transduced with  $\Delta$ Y703F-GCRTmR gene were transplanted into lethally irradiated mice, the proportion of transgene-positive leukocytes in peripheral blood increased in response to Tm administration. In a cynomolgus macaque model, half of six monkeys that received the SAG showed in vivo increase of transduced hematopoietic cells following E<sub>2</sub> or tamoxifen administration. Recently, we are developing the second-generation SAGs, which encode chimeric receptors between extracellular and transmembrane portion of erythropoietin receptor (EPOR) and intracellular portion of GCR or thrombopoietin receptor (Mpl). We transduced human cord blood CD34<sup>+</sup> cells with EPO-driven SAG (EPOR-GCR or EPOR-Mpl). The cells transduced with EPOR-Mpl proliferated most efficiently. The EPOR-Mpl also expanded and preserved c-Kit<sup>+</sup> cells most efficiently. Preliminary experiments suggest that the EPOR-Mpl conferred potent EPO-dependent growth advantage on the transduced hematopoietic cells in vivo in a murine system. The SAG system may circumvent the low efficiency of gene transfer.

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