Lentiviral Gene Therapy for p47^{phox} Deficient Chronic Granulomatous Disease

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Abstract
Chronic Granulomatous Disease (CGD) is an inherited primary immunodeficiency disorder with an incidence of ~ 1:200,000 live births. This disease is caused by mutations in the NAPDH oxidase, the phagocytic enzyme responsible for pathogen killing. As a result, CGD patients suffer from recurrent bacterial and fungal infections and often life threatening inflammatory complications. Allogeneic Haematopoietic Stem Cell Transplantation (HSCT) remains the only proven curative treatment for patients with CGD. The use of reduced intensity conditioning regimens (to limit toxicity) and the extended use of HLA-matched or single antigen mismatched unrelated grafts can now achieve excellent donor myeloid chimerism and >90% overall survival rate. However, it is not always possible to find a suitable donor and autologous gene therapy has become an attractive alternative option. A phase I/II clinical trial of lentiviral gene therapy is currently underway for X-linked CGD, the most common form of the disease. We propose to use a similar strategy to tackle p47^{phox} deficient CGD, caused by mutations in the *NCF1* gene encoding the cytosolic p47^{phox} subunit of the NADPH oxidase. p47^{phox} deficient CGD is the most common form of autosomal recessive CGD and accounts for approximately 25-30% of patients in the Western world (this is likely to be higher in some consanguineous populations worldwide). We have developed and tested a self-inactivating lentiviral vector containing a codon-optimized p47^{phox} transgene under the transcriptional control of the chimeric cathepsin G/c-fes myeloid promoter (pCCLChim-p47). When used in a p47^{phox} deficient myeloid cell line and in monocyte-derived macrophages from p47^{phox} CGD patients, the lentiviral vector was able to restore p47^{phox} expression and oxidase activity to normal levels. In a murine model of stem cell gene therapy for p47^{phox} deficient CGD, the pCCLChim-p47 vector induced high expression of the p47^{phox} protein in granulocytes from blood and bone marrow of gene therapy treated mice and restored levels of NADPH-oxidase activity that were comparable to those found in wt animals (with an average of~ 1 vector copy per cell). As expected, the expression of the p47^{phox} protein was mainly confined to myeloid cells in blood, bone marrow and spleen. The percentage of functional neutrophils remained stable over time up to six months, suggesting that the vector is not prone to epigenetic inactivation. The presence of corrected granulocytes in secondary transplanted animals also indicates that we can successfully transduce haematopoietic stem cells. Overall this study shows that the pCCLChim-p47 vector is a promising tool for the clinical gene therapy of p47^{phox} deficient CGD.