

# High Efficiency Gene Transfer to Human CD34+ Cells

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## Abstract

We have previously reported the development of improved MLV-based retroviral vectors whose prototype is entitled MT (Kim et al, *J. Virol.* 72:994-1044; Yu et al, *Gene Therapy* 7:797-804). The MT vector does not contain any viral coding sequences, and thus the possibility of homologous recombination between the vector and the packaging genome is virtually nil. Indeed, in a shotgun RCR detection assay, an MT-based vector did not produce any RCR. On the contrary, the MFG vector, containing parts of all three viral coding sequences (gag, pol, and env), generated a significant number of RCR. In addition to being safe, MT-based vectors produce levels of gene expression and viral titer comparable to or higher than other vectors currently available within the community. Based on this vector, we have constructed a number of retroviral vectors that can be used for the treatment of a variety of human diseases. Our major target diseases are those that can be treated with or the status of which can be significantly improved with bone marrow transplantation. To obtain the most significant therapeutic effects, it is necessary to achieve the highest possible gene delivery efficiency, drive the highest level of gene expression, and prevent expression of the inserted therapeutic gene from being negatively influenced by the genome environment. To these ends, we compared various LTRs for their effects on the level of gene expression, tested the effect of cis-acting elements that may influence chromatin structure or position effect of the inserted gene, and studied different transduction conditions for their gene delivery efficiency. Data recently obtained from these experiments will be presented.

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There are a number of human diseases that are using BMT for therapeutic and supplementary purposes. They include a variety of blood cell origin cancer and solid tumors as well as other hematologic malignancies. In addition, a large number of hereditary diseases can be treated with BMT; for example, SCID, CGD, and Gaucher disease among others. However, many patients with these diseases cannot benefit from BMT due to a lack of immune matched donors.

To these patients, gene delivery technology may provide an excellent therapeutic opportunity because using this technology, autologous bone marrow or peripheral blood cells can be genetically engineered to contain the therapeutic gene, by relatively simple procedures. Indeed, the efficacy and safety of gene therapy technology has been tested for a number of diseases.

The most successful trials include gene therapy for X- or ADA-SCID. It is now expected that gene delivery to human CD34+ cells will be greatly expanded to other human diseases, including cancer.

There are three major factors that can highly influence the success of gene therapy trials involving CD34+ cells. First, the number of cells receiving cells should be high enough to give clear therapeutic effects.

The level and duration of gene expression are two other major issues. For reasons that are not yet clear, the level and duration of gene expression are low or short, especially over time after engineered cells have been reinfused into human subjects. For stem cell gene therapy to be successful in the real world, all of these problems must be addressed.

Despite the fact that a number of gene therapy trials

have now been done using CD34+ cells, much to our surprise, the very basics of stem cell gene therapy have not been studied as intensively as they should have been. For example, stem cells have to be cultured for a few days until genetically engineered cells are placed back into the patient. However, it is not yet clear what kind of medium formulation can produce the best results in terms of gene delivery efficiency and the status of CD34+ cells. Consequently, optimal conditions for gene transfer to CD34+ cells have not yet been clearly established.

During my presentation, I will show some of the recent data on these areas. Furthermore, as one approach to increasing the level of gene expression in CD34+ cells, we have been screening various LTRs and trying to select the best promoter that can provide the highest level of gene expression in these cells. Surprisingly, it was found that different LTRs behave very differently depending on the target cells as well as the nature of the genes that we are inserting to the retroviral vector. Implications for our findings will be discussed.