

***In vivo* Lentiviral Gene Delivery Prevents Bone Marrow Aplasia in FA Group C Knockout Mice**

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Objectives: The feasibility of gene therapy for Fanconi anemia (FA) has been well established with FA mouse models using *ex vivo* gene transfer approaches. However, the availability of hematopoietic stem cells (HSC) in FA mice, even with a 33% reduction of HSC in adult mice, is not a major issue. Since FA patients were found to have reduced numbers of stem/progenitor cells early in the disease even before severe pancytopenia, this may represent a significant obstacle to *ex vivo* gene transfer approaches. In addition, *ex vivo* gene transfer protocols were found to be hazardous for FA cells inducing apoptosis, loss of stem cell function and leading to clonal hematopoietic diseases.

Methods: To circumvent negative effects of *ex vivo* culture in FA stem cells, we tested the corrective ability of direct *in vivo* gene delivery into FancC^{-/-} bone marrow (BM) using intrafemoral (IF) injection of lentiviral particles coding for the FancC gene.

Results: Using this approach, we show that FancC^{-/-} hematopoietic stem cells are efficiently transduced while retaining their reconstitution ability and giving rise to cells from all blood lineages. *In situ* transduction of FancC^{-/-} HSC with the FancC gene corrected their self-renewal defect as shown by secondary transplants. Also, FancC intra-femorally injected FancC^{-/-} mice were resistant to MMC-induced BM failure. Moreover, efficient transduction of HSC was achieved in BM-depleted FancC^{-/-} mice meaning that the remaining HSC can be efficiently transduced. These IF-injected mice were also resistant to MMC-induced BM failure.

Conclusion: We show that direct *in vivo* lentiviral gene transfer into HSC by intrafemoral injections can be achieved without adverse effect in FA mouse models. Functional correction of HSC was achieved in unconditioned and BM-depleted FA mice suggesting that *in situ* gene therapy would be feasible in the context of FA.

Translational Applicability: Direct intrafemoral injection of viral particles coding for a FA gene may be an alternative gene therapy approach for FA. This approach would circumvent the need for *ex vivo* culture and manipulation of HSC that compromise their function.