

## Genetically Corrected Stem Cells Induce a Spontaneous Proliferation Advantage in a FA-D1 Mouse Model

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**Objective:** We have recently shown that mice harboring a deletion in the last exon of the Brca2 gene (FA-D1 mice) constitute the FA mouse model that more closely reflects the disease observed in FA patients[1]. Using this mouse model we have previously demonstrated the ability of bone marrow (BM) cells from wild type animals to progressively engraft FA mice in the absence of any pharmacological treatment [1]. However, a similar *in vivo* proliferation advantage has never been reported for FA hematopoietic stem cells (HSCs) subjected to gene therapy. Therefore, the aim of our study was to investigate whether genetically corrected FA-D1 HSCs develop a proliferation advantage in FA-D1 mice, thus allowing the progressive hematopoietic repopulation of the animals with genetically corrected cells.

**Methods:** A lentiviral vector carrying the human BRCA2 gene (BRCA2-LV) was first constructed. Thereafter, the efficacy of this vector to correct the deficiency in FA-D1 HSCs was investigated.

**Results:** *In vitro* results first showed that BRCA2-LVs were capable of normalizing the deficient expansion ability of BM progenitors. In the next set of experiments, the capacity of BRCA2-transduced FA-D1 HSCs to engraft FA-D1 mice was investigated. To this aim, Lin-BM cells from FA-D1 male mice were transduced either with BRCA2-LVs or with control EGFP-LVs, and then transplanted into FA-D1 females previously conditioned with mild irradiation (3Gy). The analysis of PB and BM samples from recipient mice showed in all tested animals, a progressive increase in the engraftment of FA-D1 mice with donor hematopoietic cells, up to an almost complete reconstitution, when FA-D1 BM cells were transduced with BRCA2-LVs. Colony forming cell (CFC) assays conducted with BM samples from FA-D1 recipients showed a marked increase in the proportion of donor-derived progenitors harbouring the BRCA2-LV provirus, from day 60 to day 180 post-transplantation, thus confirming the *in vivo* proliferation advantage of BRCA2-transduced FA-D1 progenitors. Consistent with this data, a progressive increase in the resistance of BM CFCs to MMC was observed along the post-transplantation period. Additionally, RT/Q-PCR analyses demonstrated an increase of BRCA2 expression over the post-transplantation period. Finally, LAM-PCR studies showed an oligoclonal hematopoiesis in these mice confirming the efficacy of gene therapy to restore the proliferative potential of FA HSCs.

**Translational Applicability:** Our data constitute the first formal demonstration showing that FA-HSCs treated by gene therapy develop a natural *in vivo* proliferation advantage in the absence of any selection pressure. These results strongly suggest that gene therapy will facilitate the progressive hematopoietic repopulation of FA patients with genetically corrected HSCs, without any pharmacological treatment, and will improve their hematological status mimicking the phenomena observed in a number of mosaic patients.

Refs: 1: Navarro, *et al.*, Mol Ther. 14, 525-35: 2006