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TALEN mediated gene editing in a mouse model of Fanconi anemia

Maria José Pino-Barrio[1,2,3], Yari Giménez[1,2,3], Mariela Villanueva[1,2,3], Marcus Hildenbeutel[6,7], Rebeca Sánchez-Dominguez[1,2,3], Sandra Rodríguez-Perales[4], Roser Pujol[3,5], Jordi Surrallés[3,5], Paula Río[1,2,3], Toni Cathomen[6,7,8], Claudio Mussolino[6,7], Juan Antonio Bueren[1,2,3] ✉ & Susana Navarro[1,2,3] ✉

The promising ability to genetically modify hematopoietic stem and progenitor cells by precise gene editing remains challenging due to their sensitivity to *in vitro* manipulations and poor efficiencies of homologous recombination. This study represents the first evidence of implementing a gene editing strategy in a murine *safe harbor* locus site that phenotypically corrects primary cells from a mouse model of Fanconi anemia A. By means of the co-delivery of transcription activator-like effector nucleases and a donor therapeutic FANCA template to the *Mbs85* locus, we achieved efficient gene targeting (23%) in mFA-A fibroblasts. This resulted in the phenotypic correction of these cells, as revealed by the reduced sensitivity of these cells to mitomycin C. Moreover, robust evidence of targeted integration was observed in murine wild type and FA-A hematopoietic progenitor cells, reaching mean targeted integration values of 21% and 16% respectively, that were associated with the phenotypic correction of these cells. Overall, our results demonstrate the feasibility of implementing a therapeutic targeted integration strategy into the m*Mbs85* locus, ortholog to the well-validated *hAAVS1*, constituting the first study of gene editing in mHSC with TALEN, that sets the basis for the use of a new *safe harbor* locus in mice.

Fanconi anemia (FA) is a rare genetic disorder associated with mutations in any of the twenty-two FA genes, known as FANC genes[1,2]. The genetic products of these genes belong to a DNA repair pathway known as the FA/BRCA pathway, which is involved in the repair of interstrand cross-link (ICL) lesions. FA patient cells are characterized by the accumulation of DNA damage at an increased rate as compared to healthy cells, due to an ineffective FA/BRCA DNA repair pathway. Furthermore, most patients show congenital abnormalities at birth, cancer predisposition[3–5], and bone marrow failure[6,7]. Due to the risks associated to allogeneic hematopoietic stem cell transplantation, alternative curative treatments have been proposed. This is the case of gene therapy approaches which aim at the correction of autologous hematopoietic stem and progenitor cells (HSPC) with therapeutic lentiviral vectors. The efficiency and safety of these strategies in preclinical stages have previously been demonstrated[8–13] and are nowadays tested in clinical trials[14–19]. Targeted gene therapy approaches are evolving as a promising new alternative approaches to avoid random integration issues arising from the use of integrative vectors.

Due to the fact that the most frequent complementation group in FA patients is FA-A (60–70%), and given that mutations in FANCA are highly heterogeneous[20–22], a therapeutic strategy to precisely insert a therapeutic *FANCA* expression cassette into a *safe harbor* locus[23,24] would be applicable to all *FANCA* mutations[25,26] and could be in principle extended to other FA subtypes.

The human *AAVS1* locus located on the first intron of the *MBS85* (PPP1R12C) gene on chromosome 19[27] meets the requirements of a *safe harbor* locus in a wide range of cell types[28–34]. It has an open chromatin status and also contains a putative insulator element[35]. Thus, based on the favorable results observed in human cells *in*

[1]Division of Hematopoietic Innovative Therapies, CIEMAT/CIBERER, 28040, Madrid, Spain. [2]Advanced Therapies Unit, IIS-Fundación Jimenez Diaz (IIS-FJD, UAM), 28040, Madrid, Spain. [3]Center for Biomedical Network Research on Rare Diseases, Instituto de Salud Carlos III, Madrid, Spain. [4]Molecular Cytogenetics Group, Human Cancer Genetics Program, Centro Nacional de Investigaciones Oncológicas (CNIO), Melchor Fernandez Almagro, 3, 28029, Madrid, Spain. [5]Genome Instability and DNA Repair Group, Department of Genetics and Microbiology, Universitat Autònoma de Barcelona, 08193, Barcelona, Spain. [6]Institute for Transfusion Medicine and Gene Therapy, Medical Center - University of Freiburg, 79106, Freiburg, Germany. [7]Center for Chronic Immunodeficiency, Medical Center - University of Freiburg, 79106, Freiburg, Germany. [8]Faculty of Medicine, University of Freiburg, Freiburg, Germany. ✉e-mail: juan.bueren@ciemat.es; s.navarro@ciemat.es