EP731 THE CRISPR/CAS9 SYSTEM EFFICIENTLY REVERTS THE TUMORIGENIC ABILITY OF BCR/ABL AND RESTORING THE NORMAL HAEMATOPOIESIS IN A MOUSE MODEL OF CHRONIC MYELOGENOUS LEUKAEMIA E. Vuelta1*, C. Aguilera-Manzano1 , J. L. Ordoñez2 , P. Hernández-Carabias1 , L. Méndez1 , R. Saldaña3 , J. Sevilla4 , *F. Sánchez-Guijo2* , J. M. Hernández-Rivas2 , I. García-Tuñón2 , M. Sánchez-Martín1 1 Servicio de Trasgénesis (Nucleus), Universidad de Salamanca, 2 Instituto de Investigación Biomédica de Salamanca (IBSAL), Salamanca, 3 Servicio de Hematología, Hospital de especialidades de Jerez, Jerez, 4 Hospital Infantil Universitario Niño Jesús, Madrid, Spain

Background: Chronic myelogenous leukaemia(CML) is a haematological neoplasia driven by translocation t(9;22) in the haemopoietic stem cell(HSC) compartment, generating the fusion oncoprotein BCR-ABL with a constitutive tyrosine kinase(TK) activity. The specific TK inhibition(TKI) is the base for current treatments. However, TKI resistances are observed in up to 25% of patients so, new therapeutic approaches for these patients are needed. The recently developed CRISPR system could be a definitive therapeutic option for these patients. The generation of human-mice model of bone marrow transplantation would allow to explore the potential of this technology to correct genetic disorders in hematological diseases Aims: To study the ability of CRISPR technology for disrupting the BCR/ABL oncogene sequence in CML stem cells and for restoring the normal haematopoiesis.

Methods: For the mouse stem cell transplantation a CML mouse model expressing the human BCR/ABL was used as a donor of CML leukemic stem cells(LSCs). In parallel, human CD34+ cells(hLSCs) from new diagnosed CML patients were also used to perform a bone marrow transplantation improving a xenograft mouse model. Human and mouse LSCs were electroporated with the CRISPR components, including two sgRNAs targeting the TK domain of BCR/ABL and were transplanted into the immunodeficient NSG mice. CRISPR genome edition was determined by PCR and Sanger sequencing. BCR/ABL oncogene expression was evaluated by qPCR to ensure total mRNA abolition. Finally, protein expression was measure by immunofluorescence of LSC. To study the ability of edited LSCs for restoring the normal hematopoiesis, peripheral blood cell populations were studied 2 and 4 months after transplantation, by blood smears and flow cytometry. Human-mouse transplant host were euthanized 6 months after transplantation, and BCR/ABL expression of hCD45+ cell population from bone marrow was analyzed by qPCR. Myeloid and lymphoid populations were measure by flow cytometry to analyze the hematopoiesis derived from the graft of human LSCs

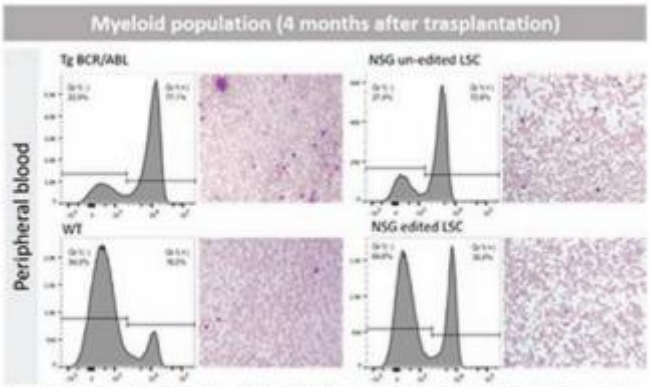

Figure 1. Analysis of peripheral blood 4 months after transplantation into NSG mice. The myeloid cells Gr1+ were quantified by Flow cytometry, and visualized by blood smears.

Results: In CML-LSCs from human and mice the CRISPR system induced a deletion in the BCR/ABL sequence triggering lower expression of both in RNA and protein level. Of note, 6 months after transplantation, the presence of the deletion in mature lymphoid and myeloid cells, exhibiting the totipotent ability of edited LSC, to contribute to all hematopoietic lineages in the host mice. Peripheral blood of mice transplanted with mouse un-edited LSCs(controls), showed a high percentage of myeloid cells, like those found in leukemic donor mice. However, in transplanted mice with edited cells, myeloid population was significantly reduced, reaching the levels observed in wild type mice. Moreover, the lymphoid population in transplanted mice with CML-edited cells were higher than in controls, reaching the level observed in wild type mice. Accordingly, in the bone marrow of mice transplanted with human un-edited LSC an inverse correlation between high CD117+ and low CD19+ cell populations was observed. In addition, in human edited-LSC transplants we found low CD117+ cell population accompanied with high CD19+ cell population, in agreement with observed in normal hematopoiesis derived from healthy human stem cells transplanted into NSG mouse, suggesting the recovery of normal hematopoiesis Summary/Conclusion: CRISPR technology allows to induce BCR/ABL null mutations in the CML-LSCs, disrupting the myeloid bias and restoring the normal hematopoiesis. These results are a proof-of-principle of the therapeutic potential of CRSPR genome edition tool in haematological diseases