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# Molecular Evidence of *Ex Vivo* Gene Editing in a Mouse Model of Immunodeficiency

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**Abstract**

Gene editing is the introduction of directed modifications in the genome, a process boosted to therapeutic levels by the use of designer nucleases. Building on the experience of *ex vivo* gene therapy for severe combined immunodeficiencies, it is likely that gene editing of haematopoietic stem/progenitor cells (HSPC) for correction of inherited blood diseases will be an early clinical application. Here we show molecular evidence of gene correction in a mouse model of primary immunodeficiency. *In vitro* experiments in *Prkdc scid* fibroblasts using designed zinc finger nucleases (ZFN) and a repair template demonstrated molecular and functional correction of the defect. Following transplantation of *ex vivo* gene-edited *Prkdc scid* HSPC, definite proof of the ability of these cells to mediate some level of reconstitution has been obtained by deep sequencing of tissues from some of the recipients, which carried the expected genomic signature of ZFN-driven gene correction (correction of the *scid* mutation associated to the introduction of a *Bsa*WI diagnostic site). This signature has been observed in peripheral blood mononuclear cells (PBMC), thymus, spleen and purified spleen CD3 and CD8 T-cells. Levels are variable in the positive primary transplanted animals, higher with integration-proficient lentiviral vector (IPLV)-ZFN than integration-deficient lentiviral vector (IDLV)-ZFN, and were also observed in secondary recipients. In some primary and secondary transplant recipients we also detected double-positive CD4/CD8 T-cells in thymus and single-positive T-cells in blood, but no other evidence of immune reconstitution. We consider the presence of the genome editing signature proof that reconstitution can be achieved, albeit inefficiently both in terms of the number of positive animals and the levels in different tissues. This *Prkdc* model is deficient in non-homologous end-joining, and this deficiency may be responsible for the frequency of a recurrent 44-bp deletion including the ZFN target site that we have observed in multiple samples *in vitro* and *ex vivo*. This highlights the importance of considering the possible effect of DNA damage repair defects on gene editing strategies.

Acknowledgements: the authors received financial support from the 7th EU Framework Programme (PERSIST project, grant agreement no. 222878) and the Primary

Immunodeficiency Association.
H.H.A.-R. and C.R. contributed equally to this work