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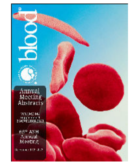

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POSTER ABSTRACTS

509.BONE MARROW FAILURE AND CANCER PREDISPOSITION SYNDROMES: CONGENITAL

CRISPR/Cas9 Strategy for Correcting *ELANE* Mutations and Restoring Neutrophil Differentiation in Severe Congenital Neutropenia: Insights from Patient-Derived iPSCs

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Introduction

The *ELANE* gene, encoding neutrophil elastase (NE), has been identified as the primary causative gene for more than half of patients with severe congenital neutropenia (SCN). For patients refractory to standard therapy (G-CSF), the only available option is an allogeneic hematopoietic stem cell transplant. Therefore, there is a great clinical interest in the development of novel gene therapies, including CRISPR/Cas9, which has shown promise for the correction of *ELANE* mutations and the restoration of neutrophil function. Here, we introduce a gene editing strategy using CRISPR/Cas9 to repair *ELANE* gene mutations in patient-derived induced pluripotent stem cells (iPSCs), aiming to restore proper neutrophil development and function.

Methods

We utilized a targeted NGS panel composed of 725 genes related to hematological disorders to sequence 282 pediatric patients suffering from chronic neutropenia. Patient-derived epithelial cells collected from urine were reprogrammed into iPSCs using the EBV-derived oriP/EBNA-1 system carrying transcription factors. CRISPR-Cas9 technology was employed to repair mutations in patient-derived iPSCs. The efficiency of transfection and gene editing was subsequently assessed by analyzing the targeted region by Sanger sequencing.

Control iPSCs (healthy donors) and iPSCs from patients were differentiated into neutrophils with specific cytokine cocktail (BMP4, CHIR99021, VEGF, SCF, bFGF, SB431542, SCF, IL-3, TPO, Flt-3, G-CSF). Cells were harvested at day 25 and analyzed for surface markers resembling the distinct myeloid progenitors. Flow cytometry antibody panel (CD14, CD45, CD49d, Siglec-8, CD101, CD11b, CD35, CD16) and gating strategy allowed to identify myeloid populations such as proNeu1, preNeu, immature-Neu and mature Neu representing myeloblasts, promyelocytes, myelocytes/metamyelocytes and band/segmented neutrophils, respectively. The morphology of myeloid precursors and mature neutrophils as well as the localization of neutrophil elastase in control iPSCs were evaluated by confocal microscopy.

Results

From a sequenced cohort of 282 patients, causative mutations were identified in 49 individuals, including 17 with *ELANE* mutations, and 9 novel variants were discovered. For this study, we selected two patients exhibiting different phenotypes for further investigation. One patient carries the NM_001972.3:c.[163T>C];[=], NP_001963:p.[(Cys55Arg)];[(=)] *ELANE* mutation and presents severe neutropenia (ANC=100/µL), currently undergoing G-CSF treatment. The second patient carries the NM_001972.3:c.[597+5G>A];[=], NP_001963:p.[(Val190_Phe199del)];[(=)] *ELANE* mutation which demonstrates in the fluctuating blood neutrophil counts characteristic for cyclic neutropenia. iPSCs were generated from both patients. Alkaline phosphatase and immunocytochemical staining confirmed that the reprogrammed cells express pluripotent stem cell markers and can differentiate into the three germ layers. Genetic defect repair was observed in 13.3% of tested cell colonies from the patient with the c.163T>C Cys55Arg *ELANE* mutation and in 30% of sequenced colonies from the patient with the c.597+5G>A V190_F199del *ELANE* mutation. Molecular cytogenetics (SNP-array) showed no significant genomic rearrangements or off-targets in iPSCs selected for differentiation. iPSC cells from healthy donors, derived from c.597+5G>A V190_F199del *ELANE* patient and the repaired clone were successfully differentiated into neutrophils (CD16+ CD35+) and their myeloid precur-

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sors (CD49d+ CD11b+). The percentage of immature and mature neutrophils (CD16+ and CD35+) derived from control or corrected iPSC was significantly higher compared to patient-derived neutrophils (Figure 1).

Conclusions

Our study demonstrates the successful use of CRISPR/Cas9 to repair *ELANE* gene mutations in patient-derived iPSCs, aiming to restore proper neutrophil development. Patient-derived iPSCs exhibit impaired differentiation at the myelocytes/metamyelocytes stage, as evidenced by a notably lower population of immature and mature neutrophils when compared to CRISPR/Cas9 repaired and healthy donor iPSCs. This observation aligns with the bone marrow phenotype observed in patients with SCN. Our findings indicate that iPSCs generated from patients' epithelial cells serve as a valuable cellular model for further research.

Disclosures Mlynarski: *Novartis:* Membership on an entity's Board of Directors or advisory committees.

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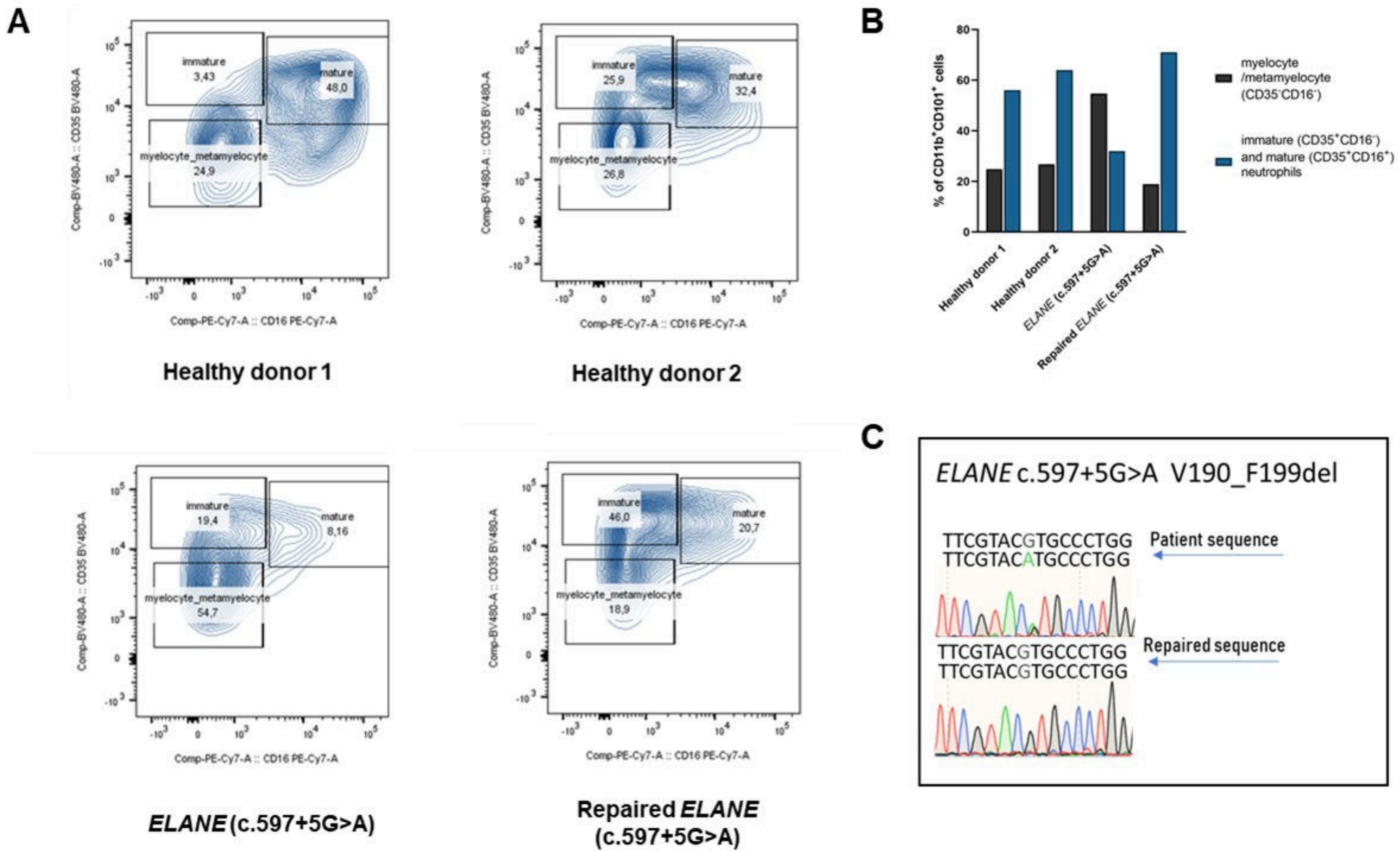

Figure 1. A) Distribution of myelocytes/metamyelocytes, immature, and mature neutrophils in CD11b+ CD101+ subpopulation differentiated from iPSCs generated from healthy donors, a patient with the c.597+5G>A V190_F199del *ELANE* mutation, and CRISPR/Cas9-corrected clone. B) The percentage of myelocytes/metamyelocytes and combined percentage of immature and mature neutrophils, in CD11b+ CD101+ cells differentiated from iPSCs generated from healthy donors, a patient with the c.597+5G>A V190_F199del *ELANE* mutation, and CRISPR/Cas9-corrected cells. C) Sanger seqencing results of the c.597+5G>A V190_F199del *ELANE* patient-derived iPSCs repaired using CRISPR/Cas9.

Figure 1

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