YOUNG EuNet-INNOCHRON GROUP

INITIATIVE 2

Screening of acquired AML/MDS associated genetic lesions in patients with severe neutropenia

Introduction

Severe congenital neutropenia (CN) is a pre-leukemia bone marrow failure syndrome presented with profound neutropenia early after birth due to markedly diminished granulocytic differentiation of bone marrow hematopoietic stem and progenitor cells (HSPCs). Patient with severe congenital neutropenia has high risk of leukemia transformation which was estimated to be 22% after 10 years. The risk of leukemia is even more increased in the CN patients with poor G-CSF response. (Skokowa et al. 2017). The identification of acquired nonsense CSF3R mutations is usually the first evidence of leukemogenic transformation. Screening for CSF3R, RUNX1 and other AML/MDS associated mutations (ASXL1, SUZ12, EP300, NPM1) can be helpful for early detection of malignant transformation (Skokowa et al. 2014).

Project aims:

- development of strategy for early detection of pre-leukemia clones in samples of CN patients with high sensitivity and accuracy;
- characterization of somatic mutation landscape which precedes leukemogenic transformation in CN;
- identification of the most optimal bone marrow and peripheral blood cell type for sensitive identification of somatic mutations in CN;
- retrospective analysis of somatic mutations detected at CN-, early and late stages of leukemogenic transformation;

Funded by the Horizon 2020 Framework Programme of the European Union

Participation in the initiative is open and free for all members of the EuNet-INNOCHRON

For the enrollment of patients in the study please contact Maksim Klimiankou, PhD Maksim.Klimiankou@med.uni-tuebingen.de.
For question regarding the clinical diagnosis AML/MDS or neutropenia please contact Prof. Dr. Julia Skokowa, Julia.Skokowa@med.uni-tuebingen.de

Inclusion and exclusion criteria for patients in the study

- severe chronic neutropenia (neutrophils <0.5 × 109/l);
- long-term treatment with G-CSF or defined molecular genetic diagnosis of CN;
- patients with drug-induced agranulocytosis or the chemotherapy-induced agranulocytosis are excluded

Important!

Signed “Informed consent form for adult patients or minor patients' parents (legal Guardian)” is required (the form can be found at https://severe-chronic-neutropenia.org/en/forms).
It is recommended that patient(-s) are registered in the Severe Chronic Neutropenia International Registry using “Registration Form” (the form can be found at https://severe-chronic-neutropenia.org/en/forms).

Sample requirements:

We accept:

1. Extracted DNA – minimum 2 µg in TE buffer with concentration > 20 ng/µl in DNase-free 1.5 ml snap-cap microcentrifuge tube;
2. Blood or bone marrow samples (minimum 2ml) in an EDTA tube;

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Samples should be sent to:

Maksim Klimiankou, PhD
Division of Translational Oncology
Department of Oncology, Hematology, Clinical Immunology and Rheumatology
University Hospital Tübingen
Otfried-Müller Str. 10
72076 Tübingen
tel: +49-7071/2980258

Project workflow

DNA of CN patients will be subjected to panel sequencing using unique molecular identifiers (UMIs). Integration of UMIs in sequencing reads enables strand specific molecular indexing and thus sequencing error correction which allows to catch up to 0.25% variant allele frequency of acquired mutations. Capture probes of the panel cover entire coding sequence of ASXL1, BCOR, BCORL1, CBL, CEBPA, DNMT3A, EP300, EZH2, FLT3, IDH1, IDH2, JAK2, KDM6A, KRAS, NPM1, NRAS, PHF6, PTPN11, RAD21, RUNX1, SETBP1, SF1, SF3B1, SRSF2, STAG2, SUZ12, TET2, TP53, U2AF1, ZRSR2, CSF3R and "hot-spot" positions in the other 209 genes mutated in AML, MDS and ALL. Sequencing will be performed with target raw reads depth 40000x in PE150 mode. The results will be provided as a panel sequencing report with estimation of clinical significance of identified mutations (Figure 1).

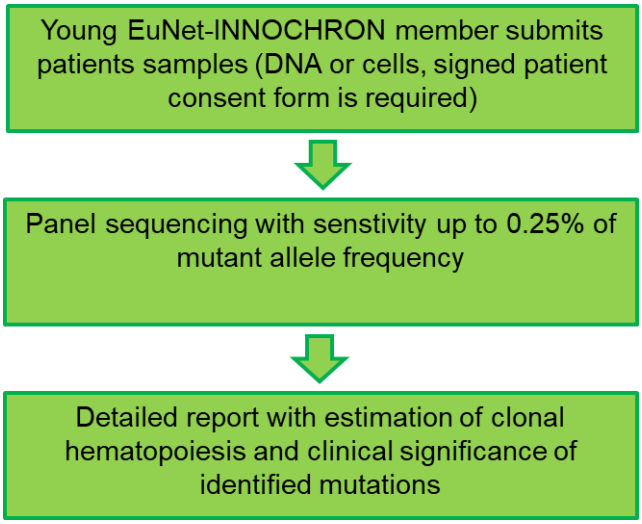

Figure1. *Project workflow.*

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