C7. NEXT GENERATION SEQUENCING FOR THE STUDY OF ACUTE MYELOID LEUKEMIA. A PILOT STUDY FOR THE TARGETED SEQUENCING OF LEUKEMIA-ASSOCIATED GENES USING THE GS JUNIOR PLATFORM

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Introduction.

Acute myeloid leukemia is a hematological disease associated with cytogenetic abnormalities at the hematopoietic stem cell level. Leukemogenesis involves several "hits" targeting genes that that regulate cell growth and differentiation, leading to maturation arrest and aberrant proliferation. The genes that are most requently mutated are NPM1, RAR, CBF, as well as FLT3, c-kit and MLL. Recent studies have shown that some other genes may take an active part in the development of the AML phenotype, such as CBL, KRAS and TET2. The purpose of the present pilot study was to observe the mutation status of these genes with the help of next generation sequencing technology in a number of three AML patients at the time of diagnosis.

Materials and Methods.

The aim of this pilot study was the Targeted Sequencing of AML-associated genes using the GS JUNIOR platform from Roche, and the genes studies were TET2, CBL and KRAS. For this, the peripheral blood collected from the patients at the time of diagnosis was separated using standard density gradient centrifugation, and DNA was extracted from the white blood cells. The amplicons used for sequencing were obtained using the GS GType TET2/CBL/KRAS Primer Set which consists of oligonucleotide PCR primers for the amplification of exons 3-11 of TET2, exons 8-9 of CBL, and exons 2-3 of the KRAS genes. This process also included the attachment of molecular identification labels (MID) to each patient sample. The amplicon libraries that were obtained were quantified, quality checked and amplified by means of emulsion PCR, then the samples from all three patients were sequenced in a single run on the GS Junior machine.

Results and Discussions.

Upon completion of the sequencing run and data analysis, we have observed a total number of eight mutations, of which seven were in the TET2 gene, one in the CBL gene, and none in KRAS. Three of the mutations in TET2 were deletions at intron levels, the other four were substitutions, while the mutation observed in the CBL gene – homozygous in all three patients – was an exonic deletion which is most likely to cause a frame shift. Mutations of these three genes alter cellular biology at multiple levels and require not only the activation of receptor proximal signaling events but also an increase in cellular glucose metabolism. Pathways that are activated by CBL gain-of-function mutations can be efficiently targeted by small molecule drugs. Since the results of this pilot study may bring knowledge with the potential of influencing therapeutic approaches for AML, further research will be performed in this direction.