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POSTER ABSTRACTS

803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

Comprehensive Characterisation of the Copy Number Landscape in Acute Lymphoblastic Leukemia Using DigitalMlpa

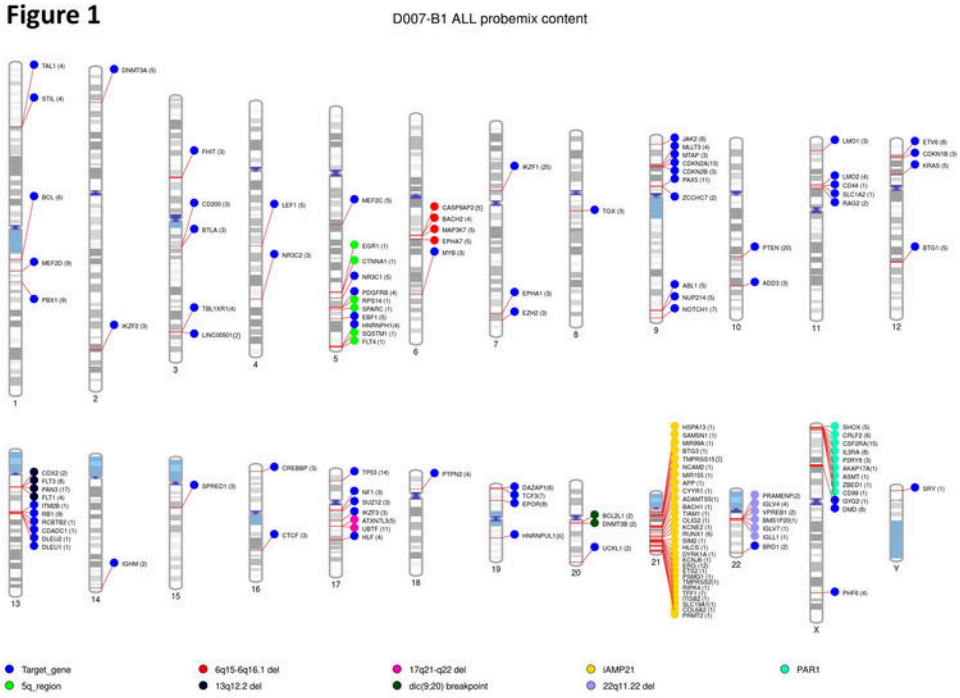
Anne Benard-Slagter, PhD[1], *Sander Palit, PhD*[1], *Charlotte Enright*[1], *Borbála Péterffy*[2], *Donát Alpár, PhD*[3], *Ivo Clemens*[4], *Suvi Savola, PhD*[1]

[1] Oncogenetics, MRC Holland, Amsterdam, Netherlands
[2] 1st Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
[3] HCEMM-SU Molecular Oncohematology Research Group, Department of Pathology and Experimental Cancer Research, Semmelweis University, Budapest, Hungary
[4] Software department, MRC Holland, Amsterdam, Netherlands

Introduction: Acute lymphoblastic leukemia (ALL) is a heterogeneous disease affecting various signaling pathways including cell differentiation and cell cycle control. ALL subtypes are defined by their genetic abnormalities, encompassing gross chromosomal aberrations, ploidy changes, translocations or rearrangements, regional or focal (intragenic) copy number alterations (CNAs), gene fusions and point mutations. Novel biomarkers for risk stratification are still being discovered, further mapping the diverse genetic ALL landscape. Testing for these newly identified genetic biomarkers and characterising the copy number (CN) landscape in ALL samples can provide useful new insights for risk stratification and patient-tailored treatment regimens.
Methods: For multiplex detection of CNAs in ALL samples, a new test version of SALSA® digitalMLPA™ Probemix D007 Acute Lymphoblastic Leukemia was developed. Hereafter, this new test version will be referred to as D007 Acute Lymphoblastic Leukemia. This assay contains probes for CNA detection of 70 key genes and regions frequently altered in B-cell ALL and T-cell ALL (*Figure 1*). In addition, this probemix contains digital karyotyping probes covering each chromosomal arm for the detection of gross chromosomal aberrations (except the p-arm of acrocentric chromosomes). A selection of these digital karyotyping probes also serve as reference probes. Finally, a set of single nucleotide polymorphism (SNP) probe pairs, targeting both alleles of each of the SNPs, was included for sample identification and guiding the correct CN baseline determination, facilitating optimal data normalisation in samples with complex karyotypes. To test the performance of this new test version of the probemix, DNA samples from 29 ALL cell lines were analysed. digitalMLPA-derived PCR amplicons were sequenced on a NextSeq 1000 Sequencing System (Illumina). FastQ files were analysed using Coffalyser digitalMLPA™ (MRC Holland) and a custom-built algorithm was used for copy number determination using SNP probe pairs.
Results: digitalMLPA data of all 29 cell lines was analysed and compared to Cancer Cell Line Encyclopedia (CCLE) copy number data (Depmap, Copy Number Public 23Q2 download). CNAs were detected with D007 Acute Lymphoblastic Leukemia, including clinically relevant biomarkers such as the IKZF1 ^{plus} profile, *PAX5*alt, *TCF3* fusions, focal 22q11.22 deletion (incl. *VPREB1*), intrachromosomal amplification of chromosome 21 (iAMP21), hypo- and hyperdiploidy. Other CNAs of emerging significance in ALL risk stratification included 6q15-q16 deletions, *DNMT3B*, *CDKN1B* and *KRAS* alterations. SNP probe pairs were effectively used to facilitate correct CN baseline determination, and results were confirmed using CCLE data (Depmap, CCLE_ABSOLUTE_combined 2019 download). Correct CN baseline detection is particularly important for samples with complex karyotypes, such as the P12-ICHIKAWA cell line. In this cell line, our data revealed that most chromosomal regions targeted by the SNP probe pairs had a copy number $\geq$3 (*Figure 2*), consistent with the hypotetraploid karyotype previously described for this cell line.
Conclusions: Using this new test version of SALSA® digitalMLPA™ D007 Acute Lymphoblastic Leukemia, numerous CNAs can be detected in a single reaction. Results obtained with this probemix showed a high correlation with CN data from the CCLE database in a panel of 29 ALL cell lines. digitalMLPA provides valuable insights into the CN of key target sequences relative to reference sequences in the genome. Moreover, our novel approach using pairs of probes that target both SNP alleles provides an additional layer of information, guiding CN and ploidy status determination in highly complex karyotypes. The SNP probe pair based approach for detection of CN levels in complex samples shows promising results and will be further validated in an independent cohort of 140 ALL patient samples. These results will be presented at the ASH meeting.

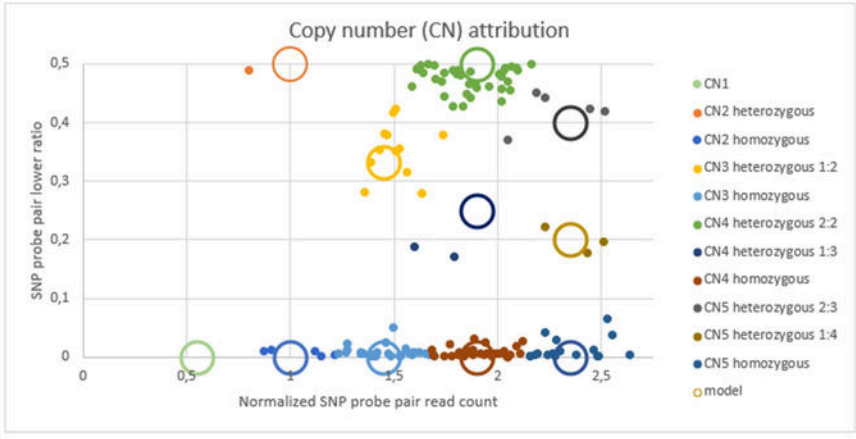
Disclosures Benard-Slagter: *MRC Holland:* Current Employment. **Palit:** *MRC Holland:* Current Employment. **Enright:** *MRC Holland:* Current Employment. **Clemens:** *MRC Holland:* Current Employment. **Savola:** *MRC Holland:* Current Employment.

Figure 1



Target genes and regions targeted in the new test version of D007 Acute Lymphoblastic Leukemia. Locations of SNP probe pairs and digital karyotyping probes are not included in this figure. Numbers between brackets indicate the number of probes per gene included in the probemix.

Figure 2



Copy number baseline determination using SNP probes in the P12-ICHIKAWA cell line with hypotetraploid karyotype. Each data point represents a SNP probe pair. The X-axis shows the normalized read count for the SNP probe pairs. The Y-axis shows the ratio between the lower of the 2 read counts of a SNP probe pair, divided by their sum. The circles represent the positions where datapoints are expected by the model depending on copy numbers and zygosity. Datapoints are attributed to the best matching copy number and zygosity.

Figure 1

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