Background: Amplon-based next-generation sequencing (NGS) is increasingly used for identification of clonal immunoglobulin (IG) and T-cell receptor (TR) gene rearrangements as markers for minimal residual disease (MRD) monitoring in acute lymphoblastic leukaemia (ALL). A standardized assay integrating amplicon library preparation as well as bioinformatic data analysis for marker identification has been established and validated in a multicentre study by the EuroClonality-NGS working group, including laboratories from the EuroMRD consortium (Brüggemann et al., Leukemia, in press). The use of NGS for MRD detection represents a universal assay which offers better specificity compared to the currently used real-time quantitative (q)PCR as well as information about background repertoire, however a reliable quantitation of MRD levels by this approach still represents a challenge.

Aims: 14 laboratories from the EuroMRD consortium and EuroClonal-ity-NGS working group performed IG / TR marker identification and MRD quantitation in 3 follow-up samples to assess the reproducibility of MRD detection and quantitation.

Methods: All participating laboratories followed the standardized library preparation protocols developed by the EuroClonality-NGS working group and performed 2 sequencing runs. The 1st run aimed at marker identification using potential target IGH, IGK, TRB, TRG and TRD rearrangements in the diagnostic sample. Based on the screening results, at least one gene locus was selected by each laboratory for a 2nd run of all 3 follow-up samples diluted into a central polyclonal buffy coat control with 50 input DNA used (equivalent to 75,000 cells) for MRD detection and assessment of false positivity. MRD levels in these samples were assessed by qPCR independently. Libraries were prepared by 2 rounds of PCR in 12 laboratories, whereas 2 laboratories used a modified 1-round approach. The EuroClonality-NGS-developed In-Tube Quality Control (cIT-QC) - spike-ins of known DNA quantity, was used by 2 labs. Sequencing was performed on Illumina MiSeq (2x250 v2 kit) or IonTorrent S3 XL instruments. Results were analysed with ARRRes/Interrogate.

Results: The 6 rearrangements (3 IGH-VJ, 1 IGH-DJ, 1 TRD, 1 TRG) present in the diagnostic sample above the 5% threshold were identified as markers in 71/76 cases (93%). MRD detection was performed in the IGH-VJ system by all 14 laboratories; IGH-DJ by 4, TRD by 4 and, TRG by 2, with mean coverage of 992,129 reads per sample. In one case IGH-VJ libraries did not meet the quality control standards for analysis and were therefore excluded. In 12 of the remaining 13 laboratories, all 3 IGH-VJ targets were detected in all 3 follow-up samples, as well as in all IGH-DJ, TRD and TRG libraries. MRD levels (4.35E-3; 1.8E-3 and 7.2E-4) correlated well with the expected dilution (8E-3; 4E-3 and 2E-4) and qPCR results in TRG systems, but appeared over-estimated and 1.7E-4) correlated well with the expected dilution (8E-3; 4E-3 and 2E-4) and qPCR results in TRG systems, but appeared over-estimated compared to the currently used real-time quantitative (q)PCR as well as information about background repertoire, however a reliable quantitation of MRD levels by this approach still represents a challenge.

Summary/Conclusion: We have shown that the EuroClonality-NGS-developed approach can be successfully used for MRD-qPCR marker identification. In addition, MRD detection using this assay showed very good inter-laboratory concordance. For accurate quantitation of MRD the use of the cIT-QC is essential and should be further validated in a large-scale multicentre testing before the method can be used in a clinical setting.

Background: In 2014, we used flow-cytometry (FC) to describe a sub-type of pediatric B cell precursor acute lymphoblastic leukaemia (BCP ALL) with a lineage switch of leukemic blasts into monocytoid cells (swALL) during early treatment (Slamova et al., 2014). Although part of the swALL cases harbored deletion of ERG (ERGdel) and/or IKZF1 alterations, the genetic background remained unknown in the majority of cases. Recently, DUX4-rearranged (DUX4+) ALL was described as a new subset of BCP ALL. Based on its association with ERGdel, we hypothesized that DUX4+ ALL may represent a BCP ALL subtype highly prone to swALL. After the lineage switch, the monocytoid cells lose BC markers (typically CD19) while they retain Immunoglobulin and T-cell receptor gene rearrangements (IG/ TR), which results in a discrepancy between BCP-immunophenotype oriented FC-based and IG/TR-oriented quantitative (q) PCR-based minimal residual disease (MRD) detection.

Aims: In the current study we aimed to describe the genotypic composition of swALL. Next, we wanted to evaluate in detail the impact of swALL phenomenon on discrepancies between FC-based and PCR-based MRD assessment during the induction and consolidation phases of treatment and its homogeneity across genetic/biological ALL subtypes.

Methods: Occurrence of swALL phenomenon was assessed in 738 patients consecutively diagnosed between 09/2007-02/2019 via 8-color FC using a panel of monoclonal antibodies against BCP and myelomonocytic markers as described previously (Slamova et al., 2014). RNA sequencing (RNAtarget IG, IGK, TRK, TRG, TRD, TRG, TRG, TRG, TRG) showed over-estimated (cito/genetic markers in order to classify them into additional genetic/biological subtypes. MRD was assessed via FC and qPCR in peripheral blood at day (d) 8 and in bone marrow at d15, d33 and week (w) 12.
between MRD results at d15 at the level of 1e-3 was 97% in DUX4 swALL, but only 17% in PAX5-P80R swALL. The main cause of discordance was the underestimation of MRD by FC compared to qPCR. The poor correlation of both methods at d15 in PAX5-P80R indicates rapid loss of B cell phenotype. Correlation of both methods was uneven across ALL subtypes. At d33, 41% and 33% of patients with DUX4 and PAX5-P80R swALL, respectively, were discordantly called to have MRD ≥ 10-3 by PCR but not by BCP FC (which could theoretically lead to discordant risk stratification). On the contrary, the MRD categorization using the same cut-off level at d15 was fully concordant in high-hyperdiploid and ZNF384 swALLs.

Summary/Conclusion: We identified DUX4 and PAX5-P80R ALL as the most prevalent subtypes among swALL. We showed that the correlation between FC- and qPCR-based MRD is influenced by the treatment time point and genetic background of ALL. Blasts in PAX5-P80R mutated swALL cases lose B cell antigens early and already at day 15 often majority of blasts is of monocytic phenotype. Loss of B cell phenotype in DUX4 cases is more gradual and at day 15 we observe blasts with decreased expression of CD19 but still clearly positive. Supported by NV18-03-00343, NV18-07-00430, 16-32568A, UNCE/MED/015

PF177 NATION-WIDE PROSPECTIVE, REAL-TIME MONITORING OF PEGYLATED E.COLI AND ERWINIA ASPARAGINASE THERAPY IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AND NON-HODGKIN LYMPHOMA IN BELGIUM

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Background: Asparaginase (ASNase) is an important anti-leukemic drug in the treatment of childhood acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). Depletion of asparagine by ASNase results in selective apoptosis of lymphoblasts which depend on an external source of asparagine for their cell growth. A substantial proportion of patients develops anti-ASNase neutralising antibodies, resulting in allergic reactions or silent inactivation (SI), characterized by rapid clearance and inactivation of ASNase.

Aims: Prospective, real-time therapeutic drug monitoring of peg-ASNase (Oncaspar®) and Erwinia ASNase (Erwinase®) in children treated for ALL and NHL in Belgium.

Methods: Patients (1-18y) with newly diagnosed ALL or precursor B- or T-lymphoblastic NHL from 8 Belgian pediatric hematopoietic- oncology centres were enrolled between 01/2013 and 11/2017. All patients were treated according to the treatment guidelines of the FORTC-CLG 58081 study. ASNase activity was quantified using the AHA test (described by Lanvers et al., 2002) using a SpectraMax M3 spectrophotometer (Molecular Devices). ASNase activity ≥1000U/L was considered to be sufficient for complete depletion of asparagine. Erwinia ASNase was given as second line after allergic reaction or silent inactivation to Peg-ASNase. One dose of Peg-ASNase 2,500IU/m² was replaced by 6 doses of 20,000IU/m² Erwinia ASNase in 2 weeks.

Results: In total, 286 children (118 girls and 168 boys) with newly diagnosed ALL (n = 260) and NHL (n = 26) were enrolled in the prospective real-time ASNase monitoring programme. Clinical allergic reactions were seen in 33 (11.5%), and silent inactivation in 14 (4.9%) patients treated with peg-ASNase. Most allergies were CTCAE 4.03 grade 2-3 and occurred after the second or third administration. SI was mainly seen after the second administration and in many cases the initiation of BM representation. Patients were more at risk for hypersensitivity reactions after an ASNase-free period. Forty-two of them were switched to Erwinia ASNase. Three patients (7.1%) experienced a clinical allergic reaction, and 1 (2.4%) a silent inactivation on Erwinia ASNase.

Median ASNase activity after the first peg-ASNase was 1254U/L (range:704-2027U/L) one hour after administration (peak), 921U/L (147–1727U/L) at day 7 (D7) and 574U/L (<5-1807U/L) at day 14 (D14). After the second administration in induction, patients reached higher median activity levels 2091U/L (c≤5-208U/L) (peak), 1181U/L (c≤5-2107U/L) at D7 and 666U/L (c≤5-1111U/L) at D14. After peg-ASNase in re-induction, median ASNase activity was 1325U/L (peak), 1338U/L (c≤5-5621U/L) at D7 and 802U/L (c≤5-346U/L) at D14. Median Erwinia ASNase activity 2 days after administration (D2) was 321U/L (<14-1193U/L) and 76U/L (c≤5-292U/L) at day 3 (D3), with significantly more D3-samples ≤100U/L (62.5% vs 10%, P = ≤0.001). According to the route of administration, median activity at D2 was significantly higher for intramuscularly (IM) Erwinia ASNase administrations (385U/L, [19–1195U/L] than for intravenous (IV) administrations (159U/L, [14–1097U/L], 61.5% IV-treated patients and 90.5% IM-treated patients achieved an activity above 100U/L in ≥75% of the D2 samples.

Summary/Conclusion: This prospective nation-wide, multi-center study shows that monitoring of ASNase activity during treatment of children with ALL and NHL is feasible and informative. Allergy and SI occurred after both peg-ASNase and Erwinia ASNase administration. Treatment with Erwinia ASNase warrants close monitoring of activity levels and optimally, adherence to a two-day interval of IM administrations.

PF178 DEFINITION OF REPRESENTATIVE BONE MARROW SAMPLE BASED ON PARALLEL EVALUATION OF PERIPHERAL BLOOD DURING THE THERAPY OF ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: Acute lymphoblastic leukemia (ALL) treatment leads to elimination of the blasts and subsequent regeneration of non-malignant populations. Amount of residual disease (MRD) is the strongest prognostic marker and its value differs between bone marrow (BM) and peripheral blood (PB). Therefore for patients stratification is necessary to evaluate non-hemodiluted BM sample. Our AIEOP BFM 2009 protocol considers a BM sample non-hemodiluted if ≥2% of its cells are erythroid precursors (EP). EPs are defined as CD19neg/orCD7neg/CD45neg cells.

Aims: To evaluate utility of the currently used marker of BM representation.

2. To define new, more exact approach for definition of representative BM.

Methods: Patients (n = 528) diagnosed with ALL between 2007 and 2015 were included into the study. We analyzed proportion of EPs defined as CD19neg/orCD7neg/CD45neg in BM and PB for the new definition of representative BM (lymphocytes, granulocytes, monocytes, erythroid precursors, mature B cells, T cells and NK cells in both BM cell precursor and T ALL; CD4+, CD8+ and CD45neg/CD15neg lymphocytes in T-ALL only).

Results: We identified high proportion of apoptotic cells in EPs defined as CD19neg/orCD7neg/CD45neg (6.5–96%, median 55%) The population defined as CD19neg/orCD7neg/CD45neg/CD37pos was more viable (apoptotic cells 0–66%, median 9%) p < 0.0001. Then we evaluated proportion of CD19neg/orCD7neg/CD45neg in PB. We found that as many as 29% of PB samples have >2% of EP (the value that is currently used as a marker of BM without hemodilution).

2. We defined a new assessment of BM representativeness based on comparison of non-malignant populations between BM and PB with increasing difference between the proportion of any population between BM and PB. With this approach we can calculate maximal possible hemodilution and its influence on stratification of the patient. We evaluated 342 patients with available paired BM-PB samples and identified 6 patients with highly hemodiluted BM samples who might have been misstratified.

Summary/Conclusion: We defined a new, more accurate approach for assessment of representative BM. Patients would be more at risk for non-malignant populations between PB and BM. By this approach we can exclude hemodilution in majority of the samples.

The currently used marker of representativeness – proportion of EPs, defined as CD19neg/orCD7neg/CD45neg – is not sufficient for assessment of BM representativeness. If proportion of EPs is required, CD71 should be added for the definition.

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