between MRD results at d15 at the level of 1e-3 was 97% in DUX4 swALL, but only 17% in PAX5-P80 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 ZNF384r 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, UNEC/MED/015

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

V. Mondelaers 1,*, T. Lammens 1, L. Dedeken 2, A. Uyttebroeck 3, B. Brichard 4, J. van der Werff ten Bosch 5, K. Norga 5, N. Francotte 5, C. Piette 5, Y. Benoit 5, B. De Moorloose 5

1Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, 2Pediatric Hematology-Oncology, Hôpital Universitaire des Enfants Reine Fabiola (IUH-RFRF-UFRF), Brussels, 3Pediatric Hematology-Oncology, University Hospitals Leuven, Leuven, 4Pediatric Hematology-Oncology, Cliniques Universitaires Saint-Luc (UCU), 5Pediatric Hematology-Oncology, University Hospital Brussels, Brussels, 6Pediatric Hematology-Oncology, University Hospital Antwerp, Antwerp, 7Department of Pediatric Oncology, CHR La Citadelle, Liège, Belgium

**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:**

1. **Patients:** 1-19y with newly diagnosed ALL and 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 1Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, 2Pediatric Hematology-Oncology, Hôpital Universitaire des Enfants Reine Fabiola (IUH-RFRF-UFRF), Brussels, 3Pediatric Hematology-Oncology, University Hospitals Leuven, Leuven, 4Pediatric Hematology-Oncology, Cliniques Universitaires Saint-Luc (UCU), 5Pediatric Hematology-Oncology, University Hospital Brussels, Brussels, 6Pediatric Hematology-Oncology, University Hospital Antwerp, Antwerp, 7Department of Pediatric Oncology, CHR La Citadelle, Liège, Belgium

2. ** ASNase activity levels 2091U/L (<5–5208U/L) (peak), 1181U/L (<5–2107U/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 (<5–5208U/L) (peak), 1181U/L (<5–2107U/L) at D7 and 666U/L (<5–1151U/L) at D14. After peg-ASNase in re-induction, median ASNase activity was 1254U/L (<5–4112U/L) at D7 and 3359U/L (<5–5621U/L) at D7 and 802U/L (<5–3464U/L) at D14. Median Erwinia ASNase activity 2 days after administration (D2) was 321U/L (14–1193U/L) and 76U/L (<5–529U/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 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**

B. Vakrmanova 1,*, M. Novakova 1, J. Starý 2, O. Hrusak 1,2, E. Mejdrikova 1,2

1Childhood Leukemia Investigation Prague, Department of Paediatric Haematology and Oncology, Second Faculty of Medicine, Charles university, 2Motol University Hospital, Prague, Czech Republic

**Background:** Acute lymphoblastic leukemia (ALL) treatment leads to elimination of the blasts and subsequent regeneration of non-malignant populations. Amount of minimal 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:** 1. To evaluate utility of the currently used marker of BM representation.

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

**Methods:**

1. 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 in parallel also in PB at day 15 together with viability dye DAPI. We tested whether a new definition of EPs including an added CD71 marker (transferrin receptor expressed since early phases of erythropoiesis) improves its specificity.

2. Then we evaluated other non-malignant populations (n = 342) 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 CD4negCD8neg-T lymphocytes in T ALL only).

**Results:**

1. 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)CD45negCD71pos was more viable (apoptotic cells 0–66%, median 9%) p <0.0001. Then we evaluated population 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, the possibility of hemodilution lowers. According to 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 BM representativeness. If proportion of EPs is required, CD71 should be added for the definition.

Supported by grants NV18-03-00343, NV18-07-00430 and LO1604.