regimen, as well as higher probability of relapse, progression or death. Therefore, the absolute quantification of these LS in PB may be considered potential biomarkers capable of predicting response and prognosis in DLBCL. Also, we believe it may mirror the cellular composition of the immune TME.

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ANALYTICAL VALIDATION OF A 10-COLOR FLOW CYTOMETRY PANEL FOR ASSESSMENT OF MEASURABLE RESIDUAL DISEASE OF ACUTE MYELOID LEUKEMIA

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The assessment of measurable residual disease (MRD) in acute myeloid leukemia (AML) by flow cytometry (FC) is challenging and requires full standardization to provide reliable results for patient management. Here is shown the analytical validation of a 10-color panel for AML MRD detection. **Material and methods:** 6 normal, 50 regenerative and 68MRD positive bone marrow (BM) samples were prepared using bulk lysis for acquisition of at least 1 million cells per tube. The panel composition was addressed to detect leukemia-associated immunophenotypes (LAIPs) and different-than-normal (DfN) immunophenotypes (clonal evolution), as well as leukemia stem cell (LSC) identification, using 5 tubes with 6 backbone markers (CD33APC/CD34PECy5.5/CD38APC-H7, CD45V500/CD117PE-Cy7,HLA-DRV450), combined with CD15/CD13/CD11b/CD7, CD2/CD56/CD123, CD97/CD99/CD54/CD244/CD18, CLL1+TIM3 /CD123/CD96/CD19, CD36/CD300e/CD45RA/CD14/CD64 in FITC/PE/AF700/BV421/BV605, respectively. Fluorescent markers were selected according to minimum spillovers between fluorescence channels and their stability after treatment. This panel was validated by comparing with our established 8-color panel. Samples were acquired in BD FACSCanto II and BD FACSLyric 10, adjusted according to EuroFlow's standard operating procedures. An unstained blank tube was run prior to sample acquisition to assess compensation and carry-over contamination was avoided by cleaning with distilled water between tubes. Infinicyt (Cytognos™) software was used for data analysis. Statistical analysis: R software (v 4.1.2) and Excel were used for statistical analysis, considering a p value $\leq$ 0.05 as significant. Bland-Altman plots and Pearson's correlation coefficient were used to assess agreement values between 10- and 8-color panels. Levey-Jennings plots, Ranksum tests, and chi-square tests were used to assess variances on the median fluorescence intensity (MFI) of the markers in CD34+ progenitor cells in normal and regenerated BM and in blast cells. The chi-square test was also used to compare the expressions of the monoclonal markers at diagnosis and during treatment. The linearity of the tests to determine the lower limit of quantification (LLOQ) was assessed using Pearson's correlation coefficient. **Results:** There was high accuracy and reproducibility comparing the performance of both panels and both flow cytometers (R2 > 0.96). The analytical sensitivity was determined by the limit of blanc (LOB) calculated using normal BM samples not stained with CD34 and CD117, limit of detection (LOD) with LOB+1.645SD; and lower limit of quantification LLOQ, using sequential dilutions of AML samples. Linearity was assessed using the results of blast cell percentages obtained from three samples diluted in duplicate, with high correlation between the measurements (R2 = 0.99; p-value < 0.001). The expression patterns of markers were evaluated according to their MFI in CD34+ progenitor cells from 6 normal and 50 regenerative BM samples compared with the MFI in blast cells from 68 MRD+ samples and there was significance difference of most values ($p < 0.05$). The hemodilution control was done by the percentages of mast cells (CD117+ bright/HLA-DR-) < 0.002% and of mature granulocytes (CD13 and CD11b bright) > 90%. **Conclusion:** The results showed a successful process of laboratory validation for AML MRD assessment, as recommended by the international consensuses (ICCS and ELN) and to authorize its use for clinical decision making.

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AVALIAÇÃO DA ATIVIDADE ANTITUMORAL DO LÁTEX DE EUPHORBIA UMBELLATA

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Objetivos: Determinar a concentração inibitória mínima (IC_{50}) de linhagens tumorais hematológicas e de linfócitos humanos saudáveis (PBMCs) a partir do látex liofilizado da *Euphorbia umbellata*, bem como seu índice de seletividade (SI), a fim de avaliar se a planta em questão possui atividade antitumoral. **Material e métodos:** Células Jurkat, Raji e K-562 foram plaqueadas (1×10^5 células/poço) em triplicatas biológicas e tratadas com o látex liofilizado da *E. umbellata* (800 μg/mL; 400 μg/mL; 200 μg/mL e 100 μg/mL) por 24h. Após esse período, elas foram marcadas com 7-AAD. O mesmo protocolo foi realizado com PBMCs, a partir da coleta do sangue periférico de 6 pacientes saudáveis em tubo de heparina. As amostras foram processadas com Ficoll-Paque® (1,078 g/mL) na razão 1:2, com separação por gradiente de densidade para obtenção de PBMCs. A quantificação de morte celular em ambos os experimentos foi realizada em citômetro de fluxo BD FACS Canto II™, com aquisição e análise de 10.000 eventos por meio do software Diva 6.0 (BD Biosciences). As curvas do IC_{50} para todas as linhagens celulares testadas foram realizadas por regressão não-linear, utilizando o software Graphpad Prism® 9 (versão 8.0.2). Por fim, o SI foi obtido a partir da razão entre o IC_{50} de PBMCs e o IC_{50} das células neoplásicas. Adicionalmente, foi realizado o screening fitoquímico do látex da planta, efetuando-se os