

manejo das coagulopatias. Recomenda-se uma análise aprofundada para compreender melhor as causas desse aumento e melhorar as estratégias de tratamento e diagnóstico.

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TIME-LAPSE MICROSCOPY DRUG SCREENING FOR FUNCTIONAL PRECISION MEDICINE IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA

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Relapse remains a leading cause of cancer-related deaths in children with acute lymphoblastic leukemia (ALL). Current treatments rely on conventional chemotherapy and/or bone marrow transplantation, yet ALL's genetic and pharmacological diversity suggests personalized therapies may be advantageous. Our goal was to establish a drug screening system against primary pediatric ALL cells for personalized predictions. Therefore, we standardize a method based on multi-image bright field microscopy, which we named ELDA (Ex vivo Leukemia Drug Advisor). In ELDA, primary ALL cells are cultured in 384-well plates with bone marrow stromal cells and a collagen matrix. In this microenvironment, we tested a panel of drugs at 5 concentrations. After drug addition, the plate is subjected to bright field microscopy, where each well is photographed every 30min for 60h. In the end, the images are analyzed by software that discriminating between living and dead cells according to the ALL movement. Living cells move their membrane, but when they die, the movement stops. After standardizing, we validated the results generated by ELDA through orthogonal methodologies, such as Calcein-AM and MTT assay. ELDA showed results comparable to those obtained with MTT and calcein, but uses only 4,000 ALL cells per well, needs no cellular dye, and viability is measured on real-time, providing more information about pharmacodynamics. After this validation, we tested 50 drugs against 88 newly diagnosed pediatric B-ALL. Drug sensitivity differs across B-ALL molecular subtypes. The subtype ETV6-RUNX1 were more sensitive to the tested drugs than other subtypes, while TCF3-PBX1 and High-Hyperdiploid showed intermediate response, and B-other, iAMP21 and KMT2A-r were more resistant. Furthermore, we confirmed the prognostic value of a drug resistant profile combining dexamethasone, vincristine, and asparaginase (XVA score) in terms of presence of minimal residual disease (MRD) at day 33. 45% of the samples belonging to resistance group to XVA, according to ELDA, had positive MRD, while only 16% of samples belonging to the sensitivity group to XVA had positive MRD. Finally, we chose two

cases of ALL with the same genetic mark (iAMP21), but with MRD negative and positive, respectively. The first case (MRD negative) showed high sensitivity to dexamethasone and vincristine, while the second case (MRD positive) was resistant to this drugs according to ELDA. We transplanted these samples into immunosuppressed mice in the PDX (Patient-derived xenograft) model. When these mice acquired the leukemia (confirmed by flow cytometry), we started treatment with dexamethasone and vincristine for 4 weeks. The mice transplanted with cells from case number 1 responded very well to the treatment, having their lifespan extended by 6 weeks (2.5 more than the control group that was not treated). On the other hand, the mice transplanted with cells from case number 2 did not respond to the treatment, having no increase in survival in relation to the control group, confirming the resistance/sensitivity profile previously found by ELDA. These results show that ELDA is a feasible method for ALL pharmacotyping and highthroughput drug screening, ideal to identify actionable targets not yet explored in the conventional means of treating ALL and functional precision medicine.

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ANTIOXIDANTES PROMOVE EFEITO PROTETOR DURANTE A FORMAÇÃO DE METEMOGLOBINA E NOS DANOS AO MATERIAL GENÉTICO INDUZIDOS PELA DAPSONA/HIDROXILAMINA IN VITRO

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A dapsona (DDS) é um fármaco utilizado na poliquimioterapia da hanseníase, que é responsável por reações adversas como a metemoglobinemia, relacionada ao metabólito da DDS, a dapsona-hidroxicilamina (DDS-NOH). Na tentativa de redução de efeitos hematotóxicos, são estudadas alternativas terapêuticas com os antioxidantes. O presente trabalho objetivou avaliar o efeito da Nacetilcisteína (NAC) e *Agaricus brasiliensis* na metemoglobina (MetHb) e no dano em DNA induzidos pela DDS-NOH in vitro. A dapsona (DDS) é um fármaco utilizado na poliquimioterapia da hanseníase, que é responsável por reações adversas como a metemoglobinemia, relacionada ao metabólito da DDS, a dapsona-hidroxicilamina (DDS-NOH). Na tentativa de redução de efeitos hematotóxicos, são estudadas alternativas terapêuticas com os antioxidantes. O presente trabalho objetivou avaliar o efeito da Nacetilcisteína (NAC) e *Agaricus brasiliensis* na metemoglobina (MetHb) e no dano em DNA induzidos pela DDS-NOH in vitro. A DDS-NOH foi capaz de induzir 25% de MetHb in vitro. Em relação ao pré-tratamento, os antioxidantes preveniram em mais de 20% a formação de MetHb induzida pela DDSNOH, enquanto que no pós-tratamento houve reversão de até 10% de MetHb para ambos antioxidantes. Apenas a NAC mostrou ser eficiente na