3D co-culture system for the development of a chemoresistance prediction platform in B-ALL.

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ABSTRACT

The expansion of malignant lymphoid precursors within the bone marrow results in B-cell acute lymphoblastic leukemia (B-ALL), the main population affected are children. The conventional 2D culture is unable to emulate the *in vivo* conditions so it was necessary to use a model that allows to replicate them. A more advanced three-dimensional *in vitro* model are organoids made from resected tumor tissue of patients or cell lines. They are capable of mirroring the specific characteristics of their native tumor entity. Such organoids possess the ability to represent the variety of cell types constituting the original tumor as well as enabling the complex cellular cross-talk occurring in the native tumor environment. Specifically, leukemic organoids makes possible to evaluate some characteristics of the leukemic niche, including the resistance of leukemia-initiating cells that could be responsible for relapse due to treatment ineffectiveness.Therefore, organoids have been exploited for testing of therapy sensitivity and personalized treatment approaches.

OBJECTIVE

To establish a 3D platform for the evaluation of the tumor microenvironment in the presence of different drugs in B-ALL.

METHODS

Generic organoids were formed from stromal mouse cells OP9 and lymphocyte-like Nalm-6 cells, they were treated with concentrations 50,25,12.2,6.25,3.12 ng/ml (Balandran *et al*., 2021). Samples were acquired in the cytometer to detect CD45⁺ and 7AAD to determine the treatment effectiveness on the cells

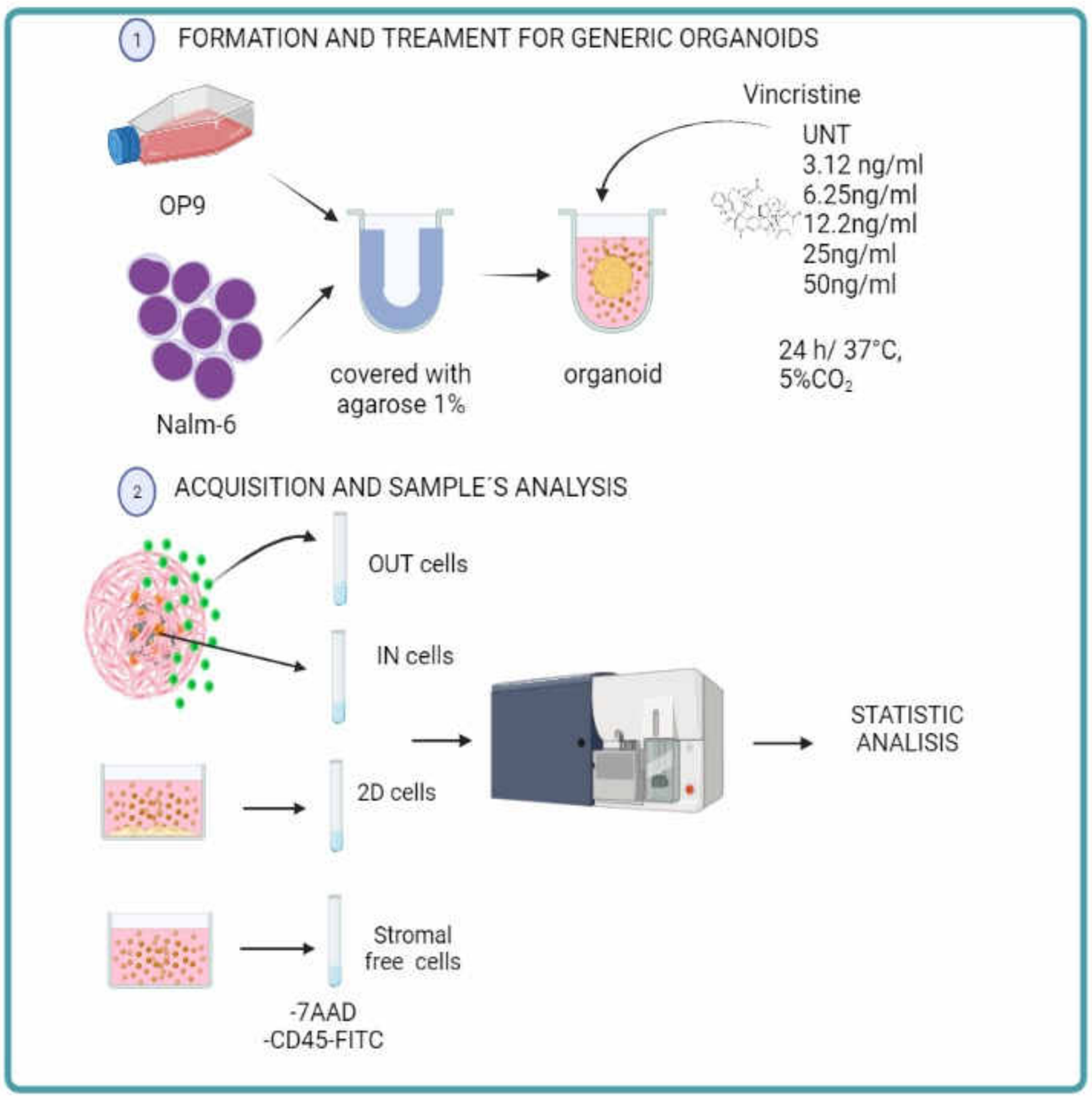

Figure 1. General methodology. 1) Formation and treatment for generic organoids. **2)** Acquisition and sample´s análisis.

RESULTS

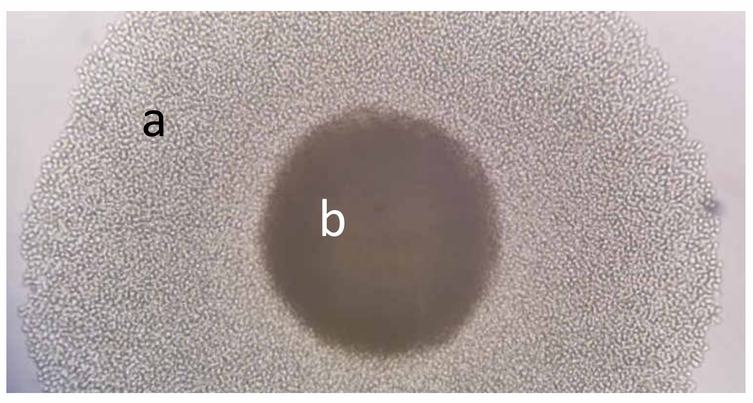

Figure 2. Generic organoids were formed from an OP9 mouse stromal cell line with leukemic cells of the Nalm-6 line. a) Leukemic cells (Nalm-6) outside the organoid. **b)** Organoid formed by stromal mouse cells OP9, inside them leukemic cells Nalm-6 **.**

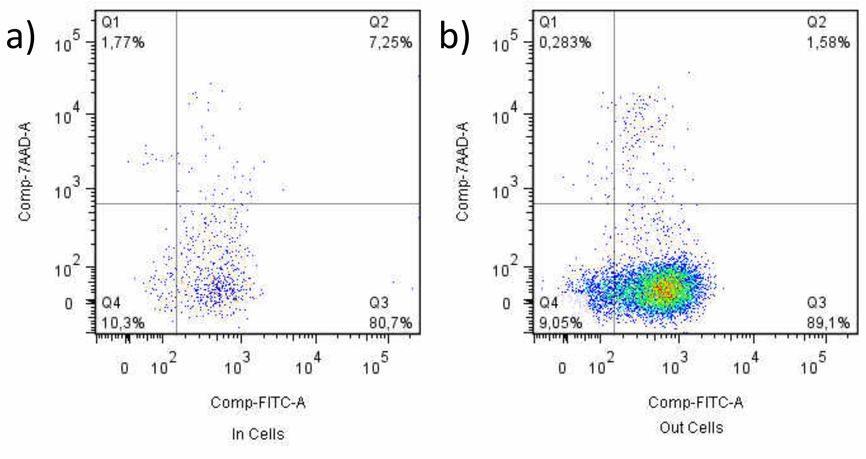

Figure 3. Leukemic organoid density plot. Read viability (7AAD) and CD45⁺ marker of b lymphocytes (FITC). a) Leukemic cells Nalm-6 inside the organoid (in) **b)** Leukemic cells Nalm-6 outside the organoid (out).

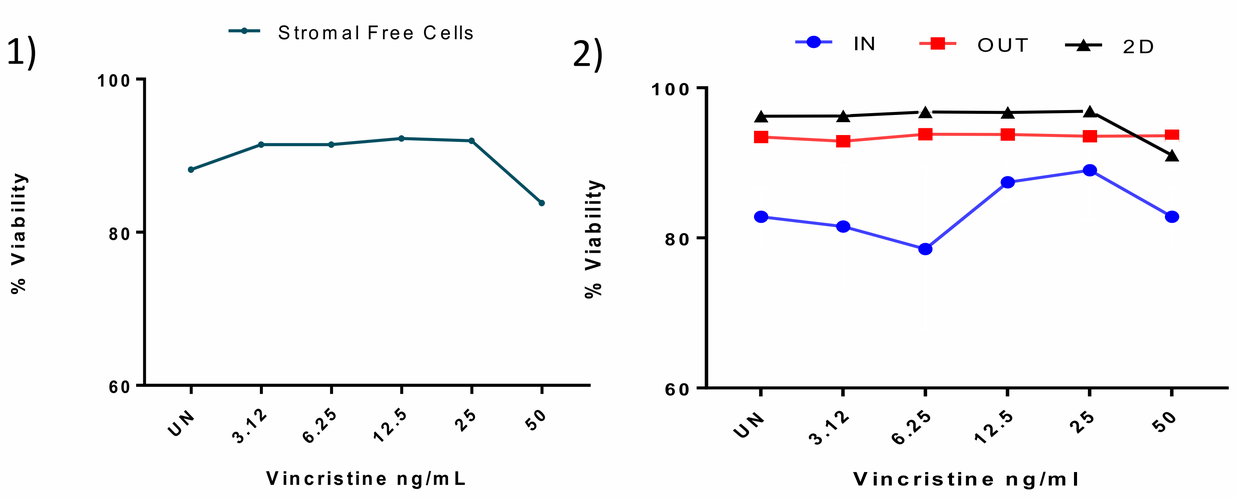

Figure 4. Effect of the vincristine on Nalm-6 cell line. 1) Stromal Free cell, One way-ANOVA and ns between the concentrations. **2)** . 2D culture, organoids in cells, organoids out cells with Kruskal-Wallis test using the Dunn post-hoc it is found that P>0.05

CONCLUSION

Generic organoids were correctly formed since leukemic cells were found inside the organoid. After that, the standardization with vincristine was done; the preliminary results showed Nalm-6 cell line have chemoresistance to vincristine, even at high concentrations at which cell samples isolated from patients showed sensitivity[1] ,these results let us establish this cell line as a chemoresistant control to this treatment. This is the initial phase of the investigation and it will let us evaluate different drugs and even new treatments, with patients´ leukemic cells.

REFERENCES

1. Balandrán, J. C., Purizaca, J., Enciso, J., Dozal, D., Sandoval, A., Jiménez-Hernández, E., Alemán-Lazarini, L., Perez-Koldenkova, V., Quintela-Núñez Del Prado, H., Rios de Los Ríos, J., Mayani, H., Ortiz-Navarrete, V., Guzman, M. L., & Pelayo, R. (2016). Pro-inflammatory-related loss of CXCL12 niche.
promotes acute lymphoblastic leukemic progression at the expense of normal lymphopoiesis. Frontiers in Immunology, 7, 666. https://doi.org/10.3389/fimmu.2016.00666
2. Inaba, H., & Mullighan, C. G. (2020). Pediatric acute lymphoblastic leukemia. Haematologica, 105(11), 2524–2539. https://doi.org/10.3324/haematol.2020.247031

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