Chronic lymphocytic leukemia patient-derived xenografts recapitulate clonal evolution to Richter transformation

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Introduction

Chronic lymphocytic leukemia (CLL) is a B-cell neoplasm with a very heterogeneous biological and clinical behavior. In 5-10% of patients, CLL transforms into diffuse large B-cell lymphoma (DLBCL) through known as Richter transformation (RT). Establishing patient-derived xenografts (PDXs) from primary CLL tumors can improve the understanding of the disease's pathogenesis and help to evaluate effective new therapies. Generation of adequate pre-clinical mouse models reflecting CLL/RT biology has not yet been successfully achieved and PDX models might be a poweful tool.

In this study we have generated two new RT-PDXs by co-xenotransplanting CLL or RT tumoral B cells with autologous activated T cells into immunocompromised NOD-*scid* IL2rγ^{null} (NSG) mice. For the first time a RT-PDX has been developed from a CLL sample that mimicked the evolution of CLL to RT uncovering intrinsic features of RT cells of therapeutical value.

Establishing two new PDXs, one coming from CLL

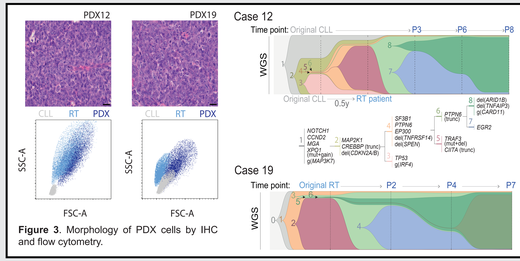

Figure 3. Morphology of PDX cells by IHC and flow cytometry.

Figure 4. Clonal evolution along the PDX generation inferred from WGS in cases 12 and 19. Each subclone is depicted by a different color and number. The height of each subclone in each time point (vertical dashed line) is proportional to their CCF. The phylogeny of the subclones with the main driver alterations is shown

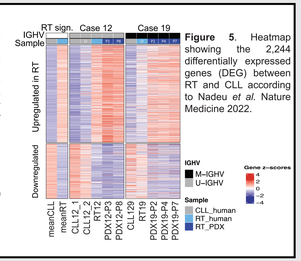

Figure 5. Heatmap showing the 2,244 differentially expressed genes (DEG) between RT and CLL according to Nadeu *et al.* Nature Medicine 2022.

IACS-010759 can circumvent Venetoclax resistance

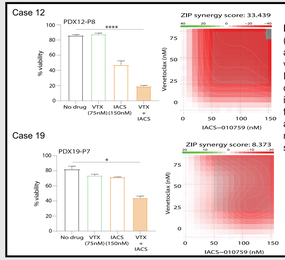

Figure 9. Percentage of alive cells (Annexin V-) from PDX12 and PDX19, after 48h of treatment with 75nM of venetoclax (VTX) alone, 150nM of IACS-010759 (IACS) alone and the combination of VTX + IACS. Drug interaction landscape and synergy score for the two drugs was calculated according to ZIP model. ZIP score 0-10 reflects an additive effect whereas ZIP score >10 reflects a synergistic effect.

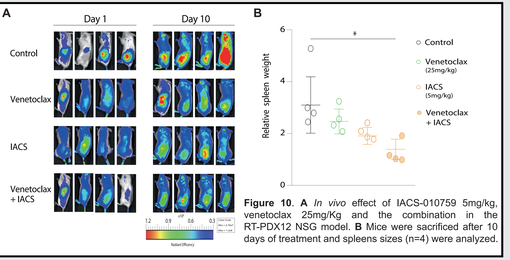

Figure 10. **A** *In vivo* effect of IACS-010759 5mg/kg, venetoclax 25mg/Kg and the combination in the RT-PDX12 NSG model. **B** Mice were sacrificed after 10 days of treatment and spleens sizes (n=4) were analyzed.

Materials & Methods

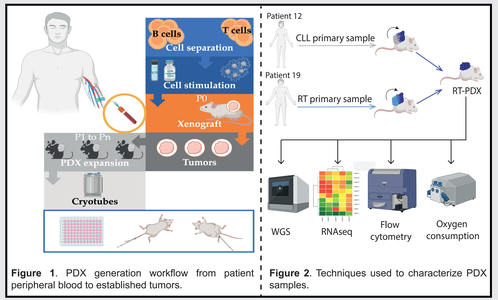

Figure 1. PDX generation workflow from patient peripheral blood to established tumors.

Figure 2. Techniques used to characterize PDX samples.

PDX cells are sensitive to OXPHOS and glycolisis inhibitors

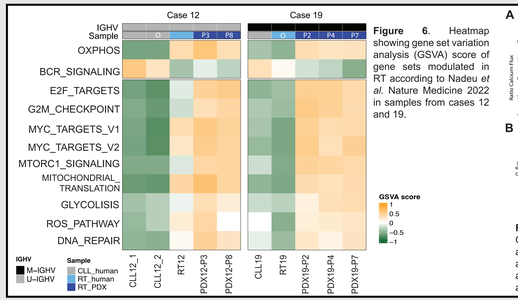

Figure 6. Heatmap showing gene set variation analysis (GSVA) score of gene sets modulated in RT according to Nadeu *et al.* Nature Medicine 2022 in samples from cases 12 and 19.

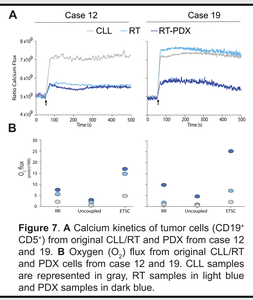

Figure 7. **A** Calcium kinetics of tumor cells (CD19$^+$ CD5$^+$) from original CLL/RT and PDX from case 12 and 19. **B** Oxygen (O_2) flux from original CLL/RT and PDX cells from case 12 and 19. CLL samples are represented in gray, RT samples in light blue and PDX samples in dark blue.

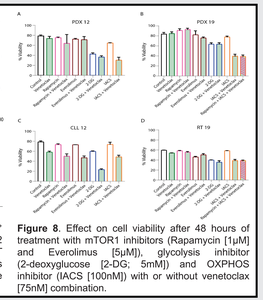

Figure 8. Effect on cell viability after 48 hours of treatment with mTOR1 inhibitors (Rapamycin [1μM] and Everolimus [5μM]), glycolysis inhibitor (2-deoxyglucose [2-DG; 5mM]) and OXPHOS inhibitor (IACS [100nM]) with or without venetoclax [75nM] combination.

Conclusions

1. Two **new RT-PDX models** were generated, **one from a CLL sample**, confirming the presence of small RT subclones in the early stages of the disease.

2. Both **RT-PDXs showed** a similar **genomic and transcriptomic profile** compared to their RT counterparts.

3. The **OXPHOS^{high}-BCR^{low} profile** may be responsible for resistance to targeted therapies.

4. Cells are **sensitive to OXPHOS inhibitor** (IACS-010759) and glycolysis inhibitor (2-DG).

5. Only **IACS-010759 is able to overcome venetoclax resistance** *in vitro*.

6. ***In vivo* results** are in line with *in vitro* results with **a modest effect when combining** IACS-010759 + venetoclax.

7. These PDX models are relevant to study the biology of the disease.

References

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