LYMPHOMA TISSUE EXPLANTS TO ANTICIPATE RESPONSE TO TARGETED THERAPIES

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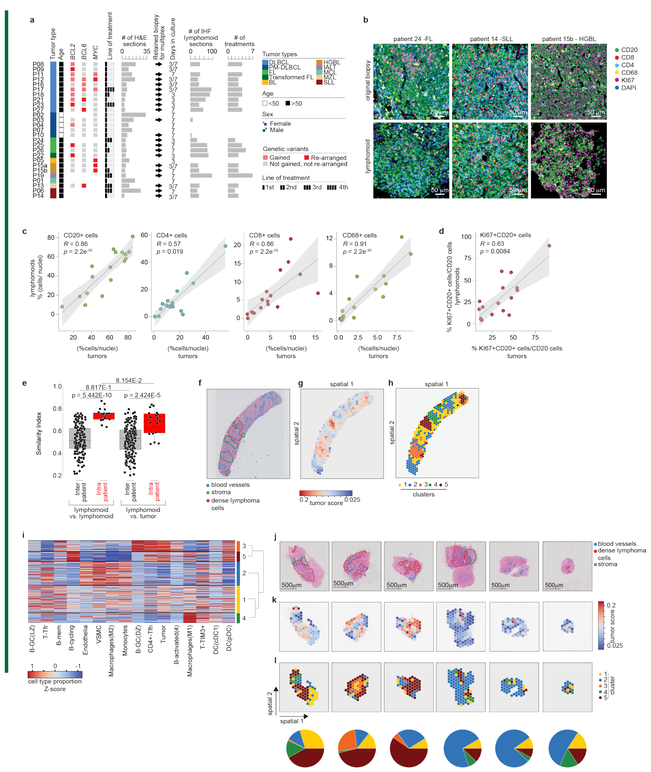
Experimental Hematology / Oncology

Albert Santamaria-Martínez[1,2], Justine Epiney[1,2,3], Divyanshu Srivastava[1,2,4], Daniele Tavernari[1,2,4], Dina Milowich[5], Thorsten Krueger[6], Rafael Duran[7], Igor Letovanec[5], Giovanni Ciriello[2,4,1], Anne Cairoli[3], Elisa Oricchio[1,2]

Swiss Institute for Experimental Cancer Research, EPFL; [2]Swiss Cancer Center Léman; [3]Hemato-oncology department, CHUV; [4]University of Lausanne; [5]ICH, Hôpital du Valais; [6]Thoracic surgery department, CHUV; [7]Interventional radiology department, CHUV. SWITZERLAND

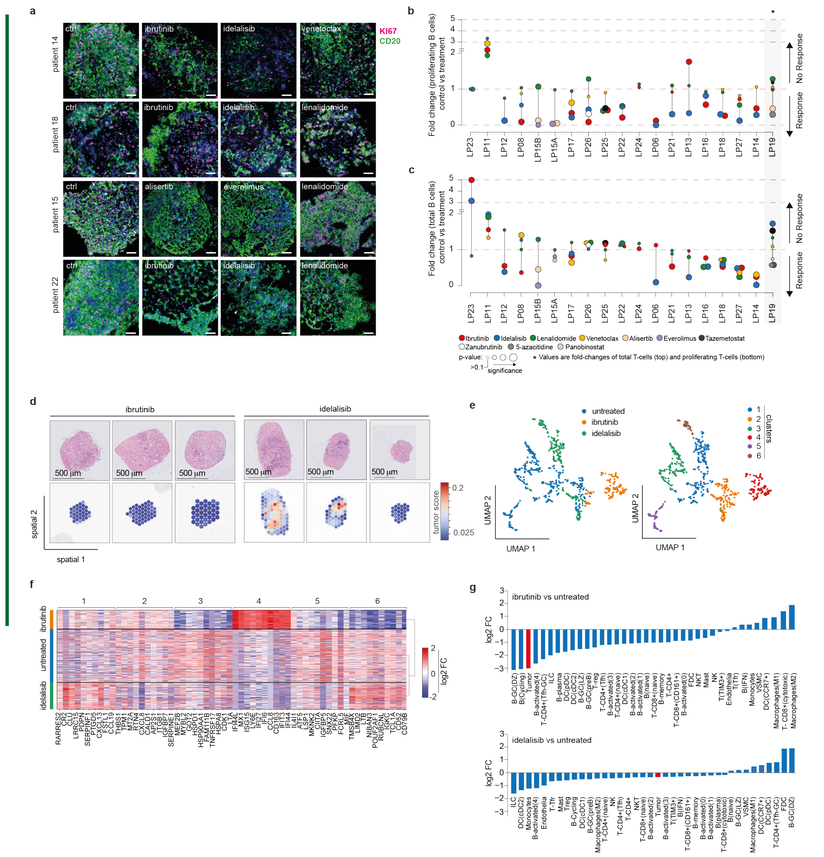
B-cell malignancies are a heterogeneous group of tumors originating from B cells blocked at various stages of differentiation. Lymphomas acquire multiple genomic alterations over time, but particularly during early stages of tumor development and in indolent tumors, lymphoma cells depend strongly on the tumor microenvironment to grow. Thus, in vitro models (i.e. patient-derived cell lines) can be used to study advanced and aggressive phases of the disease, but they poorly recapitulate early stages of tumor development and progression. Modeling lymphoma ex vivo has been hampered by the lack of suitable 3D models and the complexity of translating organoid technology from other cancer types into lymphoma. Therefore, research mainly relies on the use of transgenic and xenograft animal models. To overcome these challenges, we have developed a method to maintain lymphoma tissues in culture ex vivo -that we call lymphomoids-, which can be used to test response to anti-cancer therapies directly on patient tumor tissues. With this approach, we are able to preserve the tumor tissue composition and the interactions between tumor cells and their microenvironment.

1 LYMPHOMOIDS RETAIN TUMOR HETEROGENEITY



Characterization of human lymphomoids obtained from fresh tumor biopsies. (a) Graphical summary of all the cases collected in this study. (b) Representative images of multicolor immunofluorescence staining of indicated markers of three human lymphoma biopsies (upper panel) and their respective lymphomoids (lower panel).(c) Spearman's correlation coefficient (two-sided) analyses of untreated lymphomoids vs original tumor biopsies on the different markers analysed by multiplex IHF.. (d) Spearman's correlation coefficient analyses of untreated lymphomoids vs original tumor biopsies on proliferating CD20+ cells analysed by multiplex IHF. (e) Similarity index comparisons in the indicated conditions. (f) H&E staining of patient 8 DLBCL biopsy. Tissue features are highlighted with lines of different colors. (g-h) Representation of 10X Genomics Visium spots color-coded by the tumor score (g) and based on unbiased clustering (h). (i) Heatmap of cell type enrichment in each spot of the biopsy determined using Bayesprism deconvolution. Only top 4 differentially enriched cell types in each cluster are shown. The cell state proportions are z-scale normalized for each cell type. The dendrogram shows the distances between the different clusters. (j) H&E staining of six lymphomoids maintained in culture for 3 days. Tissue features are highlighted with lines of different colors.(k-l) Representation of 10X Genomics Visium spots color-coded by the tumor score (k), based on cluster scores obtained in the original tissue (l-top), and pie-charts summarizing the cluster composition of each lymphomoid (l-bottom).

2 LYMPHOMOIDS TO TEST DRUG SENSITIVITY



Response to targeted therapies using patient-derived lymphomoids. a) Representative images of immunofluorescence staining for KI67 and CD20 in lymphomoids derived from 4 different biopsies. Scale bar 50 µm. b-c) Proportion of proliferating CD20+ cells (b) and total CD20+ cells (c) compared to untreated controls. Each treatment is color-coded and the p-values (dot size) were calculated using non-parametric ANOVA followed by Dunn's test. e) UMAP projection of 10X Genomics Visium spots colored by sample (left) and by cluster (right). f) Heatmap of differentially expressed genes in each cluster and sample obtained in the 10X Genomics Visium experiment. Clustering of spots based on gene expression in untreated and treated lymphomoids showed that ibrutinib-treated lymphomoids formed a distinct cluster (cluster 4) while clusters 1 and 2 were composed of both untreated and idelalisib-treated samples (e). Importantly, in ibrutinib-treated lymphomoids, we noticed upregulation of several inflammatory genes (e.g., IFI27, IFI6, IFIT3) and loss of expression of B-cell markers (f), indicating that ibrutinib affected viability and proliferation of B-cells and triggered the activation of inflammatory anti-tumoral responses. g) Comparison of cell type enrichment in ibrutinib (top) and idelalisib (bottom) treated lymphomoids versus control.

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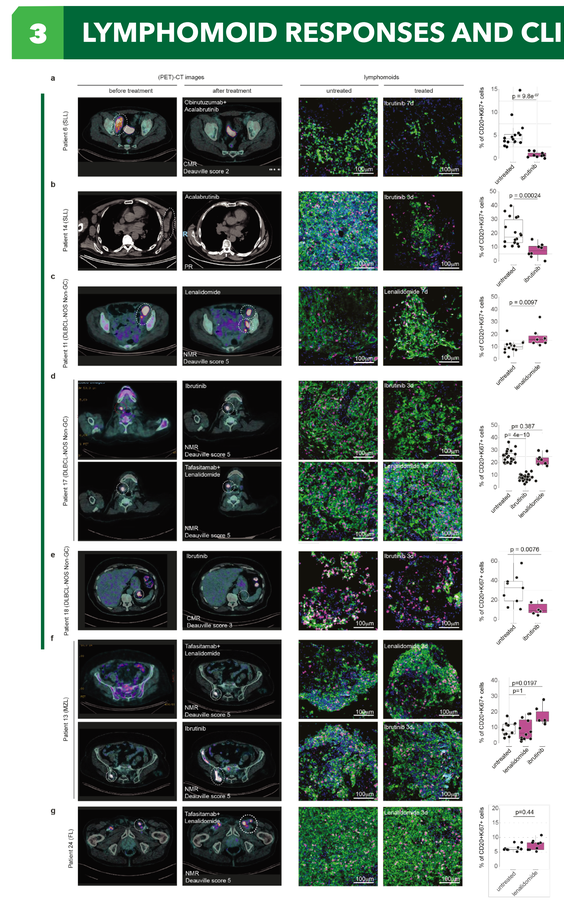
3 LYMPHOMOID RESPONSES AND CLINICAL OUTCOMES



Matched results obtained in the clinic and on lymphomoids in seven patients. (a-f) PET-CT before and after treatment, representative image and quantification (of the CD20 and KI67 immunofluorescence signal of the lymphomoids derived from patient 6 (a, ctrl n=16; ibrutinib n=9), patient 14 (b, ctrl n=18; ibrutinib n=8), patient 11 (c, ctrl n=14; lenalidomide n=7), patient 17 (d, ctrl n=21; ibrutinib n=33, lenalidomide n=10), patient 18 (e, ctrl n=44, ibrutinib n=12), patient 13 (f, ctrl n=14; ibrutinib n=6, lenalidomide n=12), and patient 24 (g, ctrl n=9; lenalidomide n=9). The data are shown as boxplots where the upper and lower hinges represent the 25th and 75th percentiles and the center is the median, and were analyzed by either two-sided Wilcoxon rank-sum test or Kruskal-Wallis followed by Dunn's posthoc test. CMR: complete metabolic response; NMR: no metabolic response; PR: partial response.

4 CONCLUSIONS

We have developed a novel ex vivo culture system that allows to rapidly test the efficacy of different therapies in B-cell lymphoma patients. We are currently working on increasing the sample size to further establish the robustness and reliability of our approach. In addition, in parallel to their preclinical application, the lymphomoids could serve as a system to understand tissue remodeling after treatment using spatially resolved molecular assays, which may help uncovering new clinically relevant aspects of the biology of lymphoid malignancies.

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