

Patient-Derived 3D Ex Vivo Lymphoma Spheroids Platform Enables High-Throughput Immunotherapy Screening and Prediction of Glofitamab Resistance in Relapsed/Refractory B-NHL



Translational



Hematology

Fabien Gava, 1-4,*,#,§,¶ Paul Marcoux, 1-4,*,#,§,¶ Neus Serrat, 6,7 Sonia Quertinmont, 1-4,#,§,¶ Marie Tosolini, 1-4,#,§,¶ Pauline Gravelle, 1-5,#,§,¶ Cally Quelen, 1-4,#,§,¶ Juan G Valero, 6,8 Fanny Bouquet, 9,#,§,¶ Catia Fonseca, 9,#,§,¶ Sylvia Hefer, 10,#,§,¶ Laura Codari Deak, 10,#,§,¶ Ron McCord, 11,#,§,¶ Eugene Chiang, 11,#,§,¶ Karin Tarle, 12,13,#,§,¶ Mickael Roussel, 12,13,#,§,¶ Pierre Sesques, 14 Caroline B et, 15 Cédric Rossi, 16 Sylvain Carros, 17 Franck Marschhauser, 18,#,§,¶ Guillaume Cartron, 19 Christina Schmie der jo hann, 20-23,§,¶ Sascha Diehrich, 20-23,§,¶ Peter-Martin Bruch, 20-23,§,¶ Wolfgang Huber, 24,25,§,¶ Pierre Brousset, 1-5,§,¶ Patricia Perez-Galan, 6,7 Loic Tseboert, 1-4,26,#,§,¶ Christine Bezombes, 1-4,#,§,¶ Camille Laurent, 1-5,#,§,¶

1Univ Toulouse, INSERM, CRCT, Toulouse, France. 2Institut Claudius Regaud-UCT-Oncopole, France. 3Laboratoire d'Excellence TOUCAN-2, Toulouse, France. 4Institut Carnot Lymphome CALYM. 5Department of Pathology, IUCT-Oncopole, Toulouse, France. 6Fundació Clinic per a la Recerca Biomèdica - Institut d'Investigacions Biomèdiques August Pi i Sunyer, Barcelona, Spain. 7Centro de Investigación Biomédica en Red-Oncología (CIBERONC), Madrid, Spain. 8Dpt of Hematology, Hospital Clinic, Barcelona, Spain 9F. Hoffmann-La Roche, Basel, Switzerland. 10Roche Pharma Research and Early Development, Roche Innovation Center Zurich, Schlieren, Switzerland. 11Genentech, San Francisco, CA USA 12Institut National de la Santé et de la Recherche Médicale (INSERM), Unité Mixte de Recherche U1236, Université Rennes, Etablissement Français du Sang Bretagne, LabEX IGO, Rennes, France. 13Pôle Biologie-Centre Hospitalier Universitaire (CHU) Rennes, Rennes, France. 14Department of Hematology, University Hospital of Lyon, Lyon, France 15Institut de Génétique Humaine, Centre Hospitalier Universitaire, Montpellier, France. 16Clinical Haematology Dijon University Hospital Dijon France. 17Department of Hematology CHU Grenoble Alpes La Tronche France. 18Department of Hematology, CHU Lille, Lille, France. 19Department of Clinic of Hematology, CHU Montpellier, Montpellier, France 20Department of Hematology, Oncology and Clinical Immunology, Medical Faculty and University Hospital Düsseldorf, Heinrich Heine University Düsseldorf, Düsseldorf, Germany. 21Center for Integrated Oncology Aachen-Bonn-Cologne-Düsseldorf (CIO ABCD), Düsseldorf, Germany. 22Molecular Medicine Partnership Unit (MMPU), Heidelberg, Germany. 23Department of Medicine V, Heidelberg University Hospital, Heidelberg, Germany. 23Spatial and Functional Screening Core Facility, Medical Faculty, University of Düsseldorf, Düsseldorf, Germany 24Europe Molecular Biology Laboratory (EMBL) Heidelberg, Heidelberg, Germany 25Molecular Medicine Partnership Unit (MMPU), Heidelberg, Germany 26Department of Hematology, IUCT-Oncopole, Toulouse, France. *co-authors, #imCORE network, §TRANSCAN network

ABSTRACT

Relapsed/refractory (R/R) B-cell non-Hodgkin lymphomas (B-NHL) remain largely incurable, and predictive biomarkers for guiding immunotherapy are scarce. The TOU-H1 study—TAiloring LYmphoma therapy with Immune Escape Signatures from 3D avatars of B-cell NHL (TALYIES) aimed to develop a 3D ex vivo platform for immunotherapy screening and to identify signatures of response and resistance to T-cell-based therapies.

We generated Patient-Derived Lymphoma Spheroids (PDLs), a scaffold- and matrix-free 3D model derived from peripheral blood or lymph node biopsies of patients with R/R follicular lymphoma, transformed follicular lymphoma, or diffuse large B-cell lymphoma. PDLs preserve the cellular composition, T-cell activation, and immune escape profiles of the original tumors, enabling multiplexed screening of single-agent and combination therapies within clinically relevant timeframes.

Using PDLs from 39 R/R B-NHL samples, we assessed responses to immune checkpoint inhibitors (aPD-L1/atezolizumab; aTIGIT/tiragolumab; aPD-1-LAG-3/tobemstomig; aPD-1-TIM-3/lomvastomig), bispecific T-cell engagers (aCD20-TCB/glofitamab), co-stimulatory BsAbs (aCD19-4-1BBL/englumafusup alpha; aCD19-CD28), and ADCs (a-CD79b-MMAE/polatuzumab vedotin), both alone and in combination. B-cell depletion following ex vivo treatment revealed heterogeneous patient responses. A key strength of this platform is its ability to simultaneously monitor tumor B-cell depletion and T-cell activation, integrating 3D imaging, immune profiling, viability assessment, and drug response analysis in the same sample. Notably, ex vivo responses to bsAbs correlated with clinical outcomes in 8 of 9 patients, highlighting PDLs as a predictive tool for personalized treatment decisions.

Screening with PDLs identified candidate resistance markers and response signatures. High responders exhibited CD8⁺ T-cells with strong cytotoxic and activation profiles, whereas low responders showed exhausted CD8⁺ T-cells and enriched functionally active CD4⁺ T-follicular helper (Tfh) cells in close proximity to malignant B-cells, supporting tumor survival via IL21 and CXCL13 signaling. Anti-TIGIT co-treatment enhanced glofitamab efficacy in low responders, and Tfh depletion further increased B-cell elimination. Analysis of pretreatment RNA-seq data from 48 R/R B-NHL patients confirmed that high Tfh abundance correlates with poor glofitamab response. These findings identify predictive biomarkers that could be used as tools for selecting patients with high probability of glofitamab response.

This TOU-H1 study enables the preparation of two manuscripts (Marcoux et al, Blood in revision, and Gava et al, submitted to Hemasphere) and presentations at ICML 2025 and 2023. This study has opened up very large perspectives, notably including the submission of a new project to the imCORE call "Translational Research imCORE project to Uncover Mechanisms of response and resistance to immunotherapy in Lymphoma (2L Relapsed/Refractory DLBCL)".

SCIENTIFIC IMPACT

This scalable PDLs platform recapitulates immune-tumor interactions, enabling rapid assessment of immunotherapy efficacy and identification of bsAb resistance mechanisms, including CD8⁺ T-cell exhaustion and Tfh-mediated tumor support, in aggressive lymphomas (R/R FL, tFL, DLBCL). Easily implemented in any cytometry-equipped lab, it allows patient-specific evaluation of immune evasion. PDLs supports high-throughput drug screening, 3D tumor avatar generation, and integration into clinical trials. Combined with spatial profiling (CODEX, scRNA-seq), it can uncover predictive response signatures and rapidly guide non-responding patients toward effective therapies, advancing personalized treatment strategies.