ELSEVIER

Blood 142 (2023) 852–854

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

Single-Cell Longitudinal Characterization of FL Heterogeneity and Residual Disease in the Bone Marrow from Low-Tumor Burden FL Enrolled in the Flirt Clinical Trial

Alicia Beyou[1], *Simon Leonard*[2], *Ilenia Papa*[2], *Noushin Mossadegh-Keller, PhD*[1], *Sabrina Baaklini, PhD*[1], *Lionel Spinelli, PhD*[1], *Owen Hoare*[1], *Bertrand Nadel, PhD*[3], *Pierre Milpied, PhD*[1], *Joelle Dulong*[2], *Céline Monvoisin*[2], *Gabriel Brisou*[1], *Guillaume Cartron*[4], *Karin Tarte, PhDPharmD*[2], *Sandrine Roulland, PhD*[5]

[1] Aix Marseille Université, CNRS, INSERM, Centre d'Immunologie de Marseille-Luminy, Marseille, France
[2] Department of Immunology, Rennes University, CHU Rennes, UMR1236, Rennes, France
[3] Centre D'Immunologie De Marseille-Luminy, Marseilles, France
[4] IGMM UMR 5535 CNRS UM, University of Montpellier, Montpellier, France
[5] Centre d' Immunologie de Marseille - Luminy, Marseille, France

Follicular Lymphoma (FL) remains a clinically challenging disease as most patients undergo a succession of clinical response/relapse cycles suggesting that current therapies do not eradicate residual tumor cells. Genetic studies suggest that FL relapses seed from a reservoir of rare Cancer Precursor Cells (CPCs), which likely remain resistant to treatment due to their homing to propitious niches such as the bone marrow (BM) and/or to the acquisition of a specific functional B-cell state. Yet, the rarity of such CPCs and the use of bulk sequencing technologies have so far hampered their direct characterization. Profiling CPC remains thus a major challenge in the FL field. Here, we identify and characterize FL cells before/after rituximab monotherapy within the BM of low tumor burden FL, looking for CPC-like cells supporting minimal residual disease.

We harnessed a unique collection of longitudinal BM aspirates from 15 newly diagnosed low-tumor burden FL patients enrolled in the phase III clinical trial FLIRT (NCT02303119) evaluating Rituximab monotherapy and sampled at diagnosis (M0) and at one-year post-treatment start (M12). We generated a multi-modal single-cell dataset combining gene expression, B-cell receptor (BCR) and T-cell receptor (TCR) repertoire (Fig1A). We used BCR sequence analysis as a pivotal method to assign the clonal malignant identity within variably infiltrated BM at diagnosis and track the clonally related residual disease FL cells at M12. We compared FL B cell transcriptional heterogeneity after rituximab therapy. We also integrated BM T cell transcriptomes from both timepoints to study compositional changes of the BM microenvironment linked to a B-cell depletion treatment.

We profiled 9,222 BM infiltrating FL cells. Single cell clustering showed a unique transcriptional signature for malignant cells of each FL patient, indicating a major inter-patient heterogeneity. By contrast, the non-malignant B cell clusters were mixed across patients and organized into transcriptional signatures encompassing normal B-cell differentiation steps (Fig1B, left). We then used unsupervised metaclustering of malignant B cells to uncover shared transcriptional states across patients, yielding 6 metaclusters (Mcl) expressing distinct gene expression programs. Mcl gene expression signatures observed in FL BM shared some similarities with the transcriptional programs observed in FL lymph nodes such as non-cycling Germinal Center-like (Mcl.1) or Mem-like (Mcl.3 and Mcl.4) states. This suggests that FL transcriptional heterogeneity is conserved between distinct niches. BM FL cells also upregulated several pathways, including glycolytic metabolism (*GAPDH*), NF-κB signaling, negative regulators of cell cycle, or entry into quiescence programs.

By analyzing BM samples obtained at M12, we were able to track and profile very rare clonally-related FL cells in 80% of FL patients with a range of 2 to 65 residual FL cells per patient. Those residual FL cells present a heterogeneous cellular phenotype partly redundant with the transcriptional heterogeneity found at diagnosis indicating that residual cells cover a plurality of cell states whose links with clinical outcome is underway. Differential expression analysis in one case also revealed a transcriptional shift in residual FL cells (including overexpression of *BCL2*) compared to BM tumor cells at diagnosis (Fig1B, right), providing novel insights into putative CPC identity with possible implications for personalized therapies. Finally, exploration of the FL T-cell microenvironment revealed abundant TFH-like cells at diagnosis that were barely detectable in healthy donors. Interestingly, high frequency of the TFH-like subset was maintained at remission suggesting a potential role as a niche for therapy-resistant B cells and later relapses.

In summary, our single cell RNAseq analysis in the frame of a clinical trial provide the first longitudinal map of FL heterogeneity in the BM and characterize putative CPCs resistant to Rituximab-therapy. Ongoing efforts are now focusing on the microenvironment drivers of this intra-patient BM diversity and on specific molecular features of BM residual cells that could represent therapeutic vulnerabilities in this particular low-tumor burden FL population.

Disclosures Nadel: *BMS:* Research Funding; *diatech pharmacogenetics:* Consultancy, Honoraria. **Milpied:** *BMS:* Research Funding. **Brisou:** *Novartis:* Consultancy. **Cartron:** *MabQi, Ownards Therapeutics, Abbvie, Roche, Bristol Myers Squibb:* Membership on an entity's Board of Directors or advisory committees; *MedxCell, Ownards Therapeutics, MabQi, Emercell, F. Hoffmann-La Roche Ltd, BMS, Abbvie:* Consultancy; *Jansen, Gilead, Novartis, F. Hoffmann-La Roche Ltd, BMS, Abbvie:* Honoraria; *MedxCell:* Consultancy; *Novartis:* Honoraria; *Roche:* Consultancy, Honoraria; *Ownards Therapeutics:* Consultancy; *Janssen:* Honoraria; *MabQi:* Consultancy; *AbbVie:* Consultancy, Honoraria; *Gilead:* Honoraria; *Emercell:* Consultancy; *BMS:* Consultancy, Honoraria. **Roulland:** *BMS:* Consultancy, Other: Travel Support, Research Funding.

https://doi.org/10.1182/blood-2023-186070

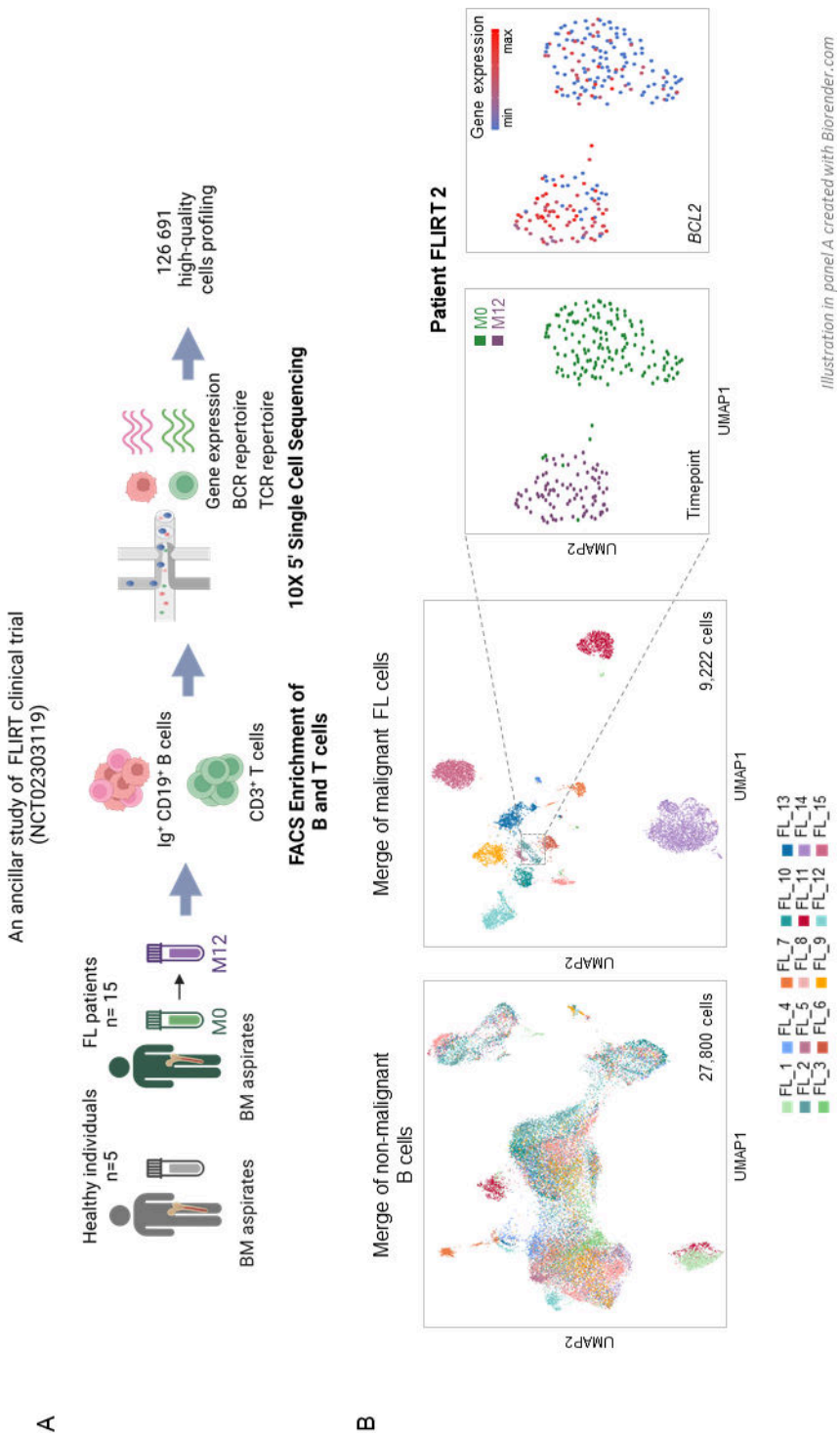

Figure 1