ELSEVIER

Blood 142 (2023) 716–717

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

603.LYMPHOID ONCOGENESIS: BASIC

Lymphoma-Derived IL-10 Is a Key Immunomodulatory Factor at the Tumor Microenvironment of Activated B-Cell Diffuse Large B-Cell Lymphoma and Influences In Vivo Responses to Immunotherapy

Marcos Garcia-Lacarte, PhD[1,2], *Sara C Grijalba*[1,2], *Francisco J Novo, MDPhD*[1,2], *Adrián Arnaiz-Leché*[1,2], *Javier Melchor, PhD*[1,3], *Marién Pascual, PhD*[3], *Enrique Goñi*[4,2], *Sandra Morales-Sánchez*[5], *María A Burrell, PhD*[5], *Oscar Blanco, MDPhD*[6], *Iñigo Clemente-Larramendi*[1], *Jose A Martinez-Climent, MDPhD*[3,2,7], *Noelia Casares, PhD*[2,8], *Juan J Lasarte, PhD*[8,2], *Pablo Sarobe, PhD*[8,2,9], *Miguel Canales*[10,2], *Sergio Roa, PhD*[1,2,7]

[1] Department of Biochemistry and Genetics, University of Navarra, Pamplona, Spain
[2] Navarra Institute for Health Research (IdiSNA), Pamplona, Spain
[3] Hemato-Oncology Program, Cima Universidad de Navarra, Pamplona, Spain
[4] Computational Biology Program, Cima Universidad de Navarra, Pamplona, Spain
[5] Department of Pathology, Anatomy and Physiology, University of Navarra, Pamplona, Spain
[6] Institute of Biomedical Research of Salamanca (IBSAL), Department of Pathology, University of Salamanca, Salamanca, Spain
[7] Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Cáncer (CIBERONC), Madrid, Spain
[8] Immunology and Immunotherapy Program, Cima Universidad de Navarra, Pamplona, Spain
[9] Instituto de Salud Carlos III, Centro de Investigación Biomédica en Red de Enfermedades hepáticas y digestivas (CIBERehd), Madrid, Spain
[10] Clínica Universidad de Navarra Cancer Center (CCUN), Pamplona, Spain

Diffuse large B-cell lymphoma (DLBCL) is the most common subtype of aggressive non-Hodgkin's lymphoma with ~40% of patients experiencing refractory or relapsed disease to R-CHOP immunochemotherapy. These higher risk patients often exhibit gene expression profiles that resemble a late germinal center-derived activated B-cell (ABC) phenotype, where diverse specific mutations in genes along BCR and TLR signaling pathways converge into constitutive activation of the NF-κB pathway. Identification of factors that contribute to the survival of malignant ABC-DLBCL cells is crucial for designing specific interventions. In this setting, previous *in vitro* studies in DLBCL cell lines have evidenced that IL-10 is a direct target of NF-κB signaling that sustains lymphoma cell survival by an auto-stimulatory loop via STAT3 signaling. However, the action of IL-10 could be more complex within the lymphoma microenvironment, as IL-10 can have both stimulatory and inhibitory effects depending on the cellular source of IL-10, the timing of its secretion, and the type of immune cells that receives signals via IL-10 receptor (IL-10R). To explore *in vivo* the relevance of lymphoma-derived IL-10, we have generated a novel quintuple transgenic mouse model that we call pBIC10, after crossing our previous ABC-DLBCL-like pBIC mice carrying constitutive NF-κB signaling (Pascual *et al.*, Blood 2019) to mice with a floxed *Il10* gene for conditional knock-out of IL-10 specifically in malignant ABC-DLBCL cells. As expected, increased intracellular IL-10 signaling was detected in primary murine DLBCL cells from pBIC mice but not in IL-10 deficient pBIC10 mice, which promoted lymphoma cell survival *ex vivo* as revealed by perturbation studies of the IL-10/JAK1/STAT3 autocrine loop with blocking monoclonal antibodies or selected inhibitors. However, *in vivo* genetic deletion of lymphoma-derived IL-10 did not offer improved overall survival but, on the contrary, notably accelerated DLBCL progression in pBIC10 mice (Figure 1A, left). A comprehensive integration of multiparametric flow cytometry and transcriptomic analyses using bulk and single-cell RNA-seq of lymphoma cells and the tumor microenvironment coupled with BCR- and TCR-seq, identified lymphoma-derived IL-10 as a key immunomodulator of the DLBCL microenvironment, including unexpected protective paracrine functions in the progression of DLBCL. By comparing IL-10-proficient (pBIC) and -deficient (pBIC10) murine DLBCL models, we observed a multifaceted role of IL-10 produced by DLBCL cells, enabling immune-effector processes (facilitating immune chemotaxis, antigen presentation, and IFNγ responses) that yielded higher percentages of long-lived stem-like CD8 $^{+}$TCF-1 $^{+}$PD-1 $^{lo/int}$ T cells and dendritic cells, while restraining PD-1/LAG3-driven T-cell exhaustion and reducing immunosuppression by CD4 $^{+}$CD25 $^{+}$FOXP3 $^{+}$ regulatory T (Treg) cells. Thus, depletion of lymphoma-derived IL-10 decreased T-cell cytotoxicity and increased Treg-driven immunosuppression, which collectively accelerated lymphoma development. Consequently, T-cell targeted immune-checkpoint therapy (with anti-PD-1 antibodies) was effective only in IL-

10-proficient DLBCL but not in IL-10-deficient pBIC10 mice (Figure 1A, right). Further comparative studies between pBIC and pBIC10 mice showed that tumor vascularization and expression of B-cell calcium channels, which have been previously associated with sensitivity to rituximab, were counteracted by lymphoma-derived IL-10. Accordingly, anti-CD20-based targeting of lymphoma cells (with rituximab surrogate antibodies) showed best *in vivo* responses in IL-10-deficient DLBCL mice with respect to pBIC mice (Figure 1A, right). Consistent with this preclinical data, analyses of transcriptional IL-10-associated hallmarks to stratify DLBCL patients (Schmitz *et al.*, NEJM 2018) identified better clinical responses to R-CHOP in patients with pBIC10-like DLBCL phenotype (Figure 1B). Collectively, our study provides new functional and mechanistic insights into the role of lymphoma-derived IL-10 in the pathogenesis of DLBCL and the restraint of an immunosuppressed tumor microenvironment, which holds predictive potential as a biomarker for immunotherapy responses in DLBCL.

Disclosures Martinez-Climent: *Priothera:* Research Funding; *Roche-Genentech:* Research Funding; *Janssen:* Research Funding; *BMS-Celgene:* Research Funding; *Palleon:* Research Funding; *AstraZeneca:* Research Funding. **Canales:** *Takeda:* Consultancy; *Roche:* Consultancy; *BMS:* Consultancy; *Incyte:* Speakers Bureau; *Janssen:* Consultancy; *Karyopharm:* Consultancy; *Kite:* Consultancy; *Kyowa:* Consultancy; *Lilly:* Consultancy; *Kite:* Speakers Bureau; *Beigene:* Consultancy; *Janssen:* Speakers Bureau; *Incyte:* Consultancy; *Kyowa:* Speakers Bureau; *Roche:* Speakers Bureau; *Takeda:* Speakers Bureau. **Roa:** *Roche-Genentech:* Research Funding.

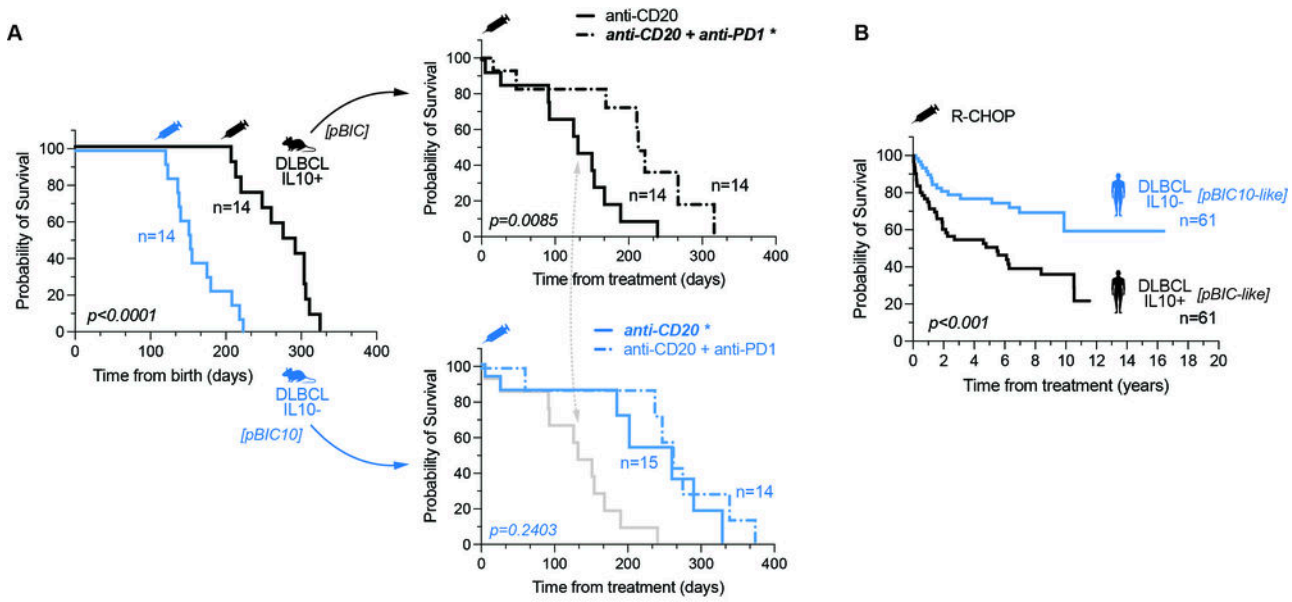

Figure 1: *Overall survival analyses and preclinical immunotherapy trials in the IL-10-proficient (pBIC) and IL-10-deficient (pBIC10) mouse models for ABC-DLBCL. Patients in the Schmitz et al. NEJM 2018 cohort had received CHOP or CHOP-like chemotherapy plus rituximab.*

Figure 1

https://doi.org/10.1182/blood-2023-177612