



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

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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- κ B 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- κ B 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- κ B 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 IFN γ 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-

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.

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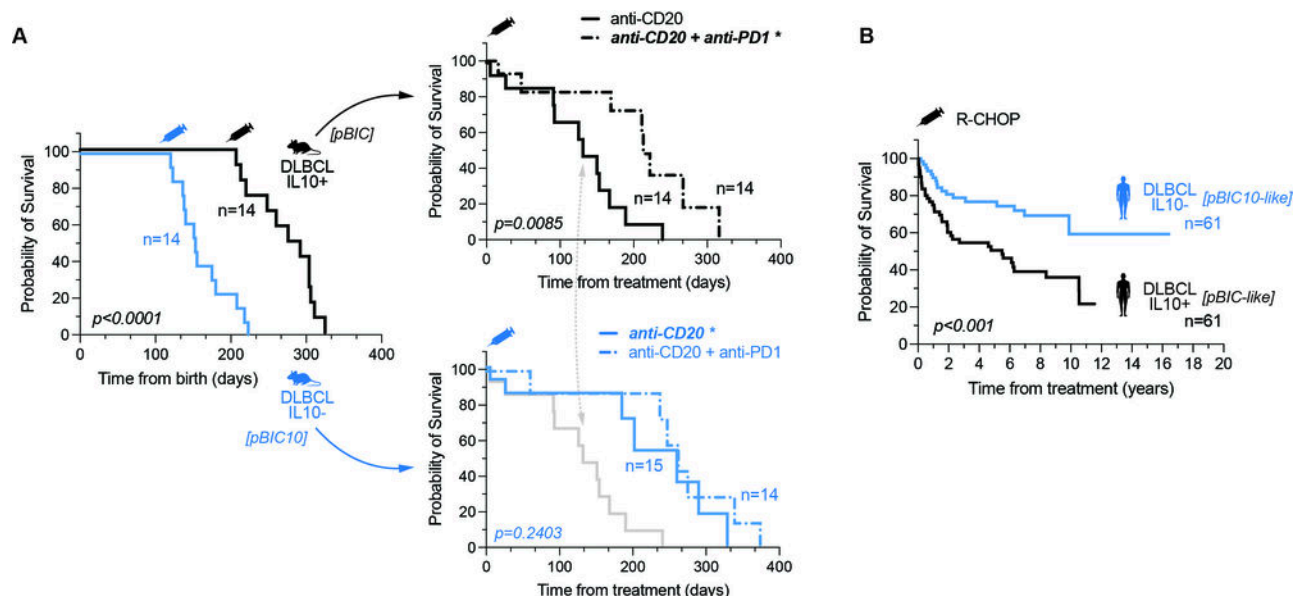


Figure 1: Overall survival analyses and preclinical immunotherapy trials in the IL-10-proficient (pBIC) and IL-10-deficient (pBIC10) mouse models for ABC-DLBCL. Patients in the Schmitz *et al.* NEJM 2018 cohort had received CHOP or CHOP-like chemotherapy plus rituximab.

Figure 1

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