

Immunomodulatory drugs may overcome the negative prognostic role of active Th17 axis in follicular lymphoma: evidence from the SAKK35/10 trial

Follicular lymphoma (FL) is a germinal center-derived B-cell neoplasm.¹ Similar to their non-malignant counterparts, FL cells are dependent on their tumour microenvironment (TME), consisting of T cells, macrophages, follicular dendritic cells and fibroblasts. Recently, we were able to show that the prognostic impact of different T cell subsets in FL depends on the treatment given.² In the present study, we performed gene expression profiling (GEP) to further explore the prognostic impact of the TME in FL, highlighting a new and important role for the subset of T-helper (Th) 17 T cells.

T cells constitute a major part of the FL microenvironment and can basically be subdivided into cytotoxic, Th- and regulatory T cells (Treg). The latter two subsets are important players in the functional maintenance of germinal centers and, thus, also vital components of the TME of FL. Special roles have already been attributed to subsets of Th cells, the T follicular helper cells (TFH)³ and to Treg.⁴

Recently, in the controlled randomised clinical trial SAKK 35/10 investigating whether addition of lenalidomide (R²) to a rituximab (R)-only regimen in treatment-naïve FL is beneficial,² we showed that the TME and especially the T cells yield prognostic impact for FL. High ratios of CD4 to CD8-positive T cells and PD1-positive T cells were linked to poorer progression-free survival (PFS), while higher amounts of GATA3-positive Th2 equivalents was linked to better PFS.⁵ We could also demonstrate that this impact is dependent on the therapeutic regimen given, with the immunomodulatory drug (IMiD) lenalidomide overcoming the negative impact of PD1 positive T cells while leaving the impact of GATA3 positive T cells unaffected.

In order to investigate the role of the TME in R- and R²-treated FL more profoundly, we performed GEP. 71 trial cases with available tissue were included in the present study. Details of GEP analysis and immunohistochemical staining for retinoic-acid-receptor-related orphan nuclear receptor gamma (ROR γ t) to detect Th17 cells are shown in the attached supplementary file. Cell content of TME was at least 50% in all cases, investigated to allow robustness of GEP analysis. All clusters detected in GEP were analysed in regard to their statistically-significant relevance for PFS.

A cluster group with low mRNA expression-levels of Th17 axis-related genes such as *IL17A*, *B* & *C* and *IL22* (Fig 1A) was associated with better PFS in the R-treatment arm

(median PFS 16.6 months, mean 21.2 months, 95% CI 13.5–28.8 in the Th17 high signature group *versus* median PFS 24.6 months, mean 45.9 months, 95% CI 31.9–61.0 in the Th17 low signature group; $P = 0.039$) (Fig 1B). Addition of lenalidomide seemed to improve the prognosis, particularly of this Th17 high signature group, since in the R² cohort this effect was not observable any more (Fig 1C). Concordantly, respecting quantification of Th17 cell-equivalents by immunohistochemistry (ROR γ t) (Fig 2A), PFS improvement was perceptible, though not reaching statistical significance, for patients with higher amounts of ROR γ t-positive cells only in the R² group (median PFS 30.7 *versus* 59.9 months; $P = 0.115$) (Fig 2B). For other T cell- and macrophage subset clusters, no statistically-significant differences regarding PFS were seen.

Th17 cells have a well-established role in autoimmunity and inflammatory diseases. Physiologically, these cells and their main product, IL17A, are necessary for protection against bacterial and fungal infections, as IL17A attracts neutrophils.⁶ IL17A can also promote the formation of germinal centers in autoimmune disease.⁷ By contrast, the role of Th17 cells in malignancies is less clear, with several studies showing both beneficiary and adverse effects in solid tumours. In lymphomas, the role of Th17 cells is yet not well investigated. Duffield *et al.* demonstrated that Epstein-Barr virus- (EBV) negative classic Hodgkin lymphoma (cHL) have a distinctive Th17-accentuated TME, while EBV-positive cHL have a Th1-primed TME.⁸ Another recent study in cHL could show that Th17 cells are increased in a subset of cases, and that soluble CD30 (CD30s) can shift the polarisation of T cells in favor of Th17.⁹ Ferretti *et al.* showed, both *in vivo* and *in vitro*, that IL17A promotes the growth of germinal center derived B-cell lymphomas, including diffuse large B-cell lymphomas (DLBCL) and FL.¹⁰ Another study in DLBCL showed, similar to our current observations, that high levels of IL17A promote resistance to R by suppressing p53-induced apoptosis and go along with an inferior prognosis.¹¹ In chronic lymphocytic leukaemia (CLL), higher levels of Th17 cells were correlated with better prognosis.¹²

Our study sheds some new light on the role of Th17 cells in FL. High IL17A levels seem to be related to an inferior prognosis of FL, especially in instances treated by R alone, and this effect seems to be overcome by IMiD, as it was not seen in the group treated with lenalidomide. On the

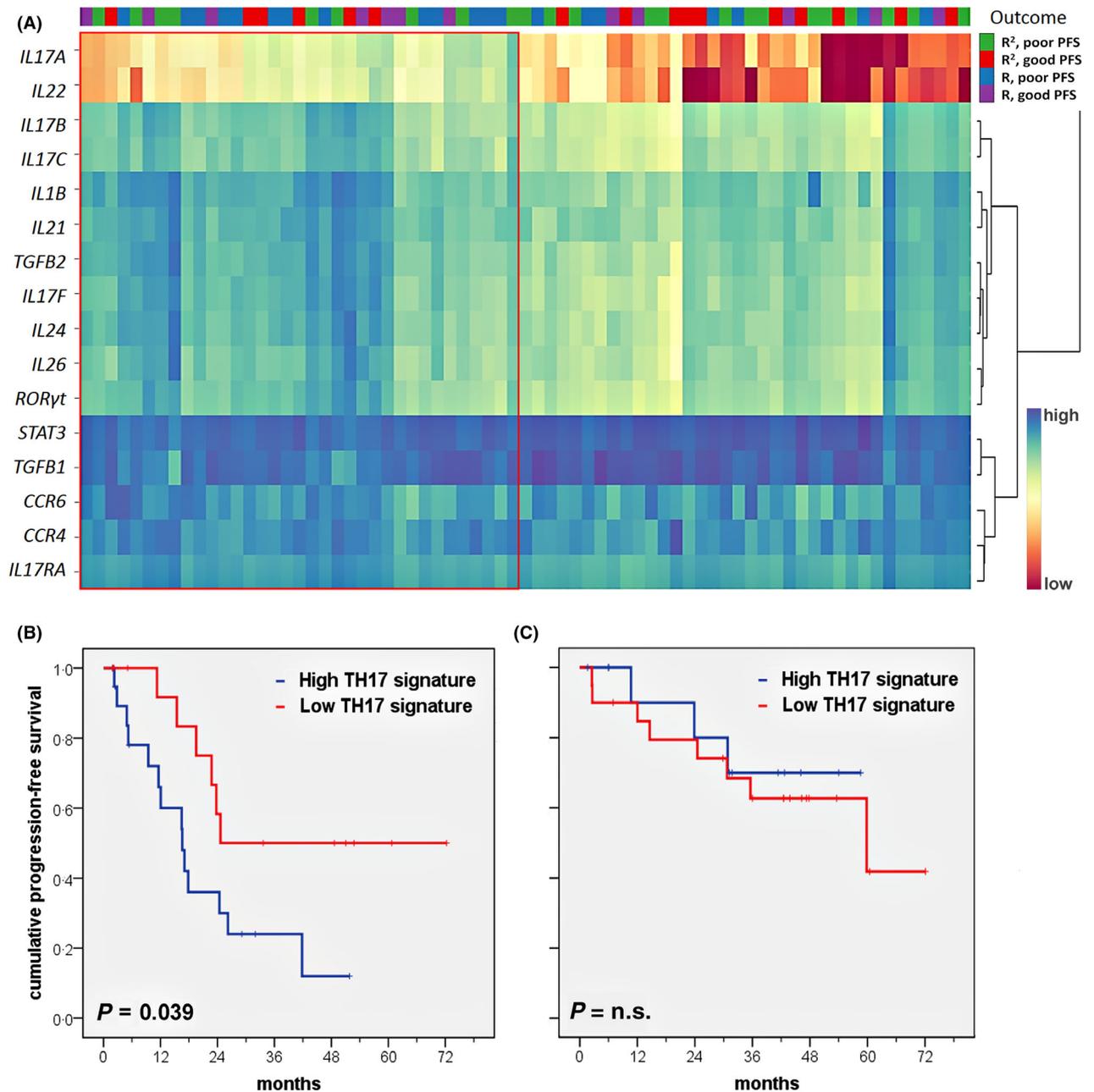


Fig 1. (A) Cluster analysis showing two distinct subgroups in regard to differential mRNA-levels of IL17A and related molecules (left: high Th17 signature; right: low Th17 signature). The plot in the lower right shows the relative amount of mRNA of the respective gene expressed, ranging from dark red (very low) to dark blue (very high); (B/C) Progression-free survival analysis for the impact of the two Th17 subgroups in regard to the two treatment groups (B: R-group; C: R²-group).

contrary, in cases treated with lenalidomide, higher amounts of Th17 cells even correlated with a better prognosis. These findings raise the question of selective influence of IMiD on different T cell subsets. In studies investigating the effect of lenalidomide in CLL, it was found that it induces Th17 cells while decreasing Treg. This is thought to be the background of the antitumour- and immunostimulating function of lenalidomide.¹³

Taken together, our findings show that the prognostic implication of Th17 cells in FL depends on the type of

treatment given. In the setting of lenalidomide, a Th17 TME signature does not seem to negatively affect prognosis; higher amounts of Th17 cells might even be beneficiary.¹³ Further studies should take into account our immunohistochemical- and GEP-based analysis results and investigate *in vivo* both serum levels of the cytokines released by the various Th subpopulations (and particularly by Th17 cells) and the dynamic amounts of these populations under treatment in order to better define the specific prognostic and predictive role of the TME in FL.

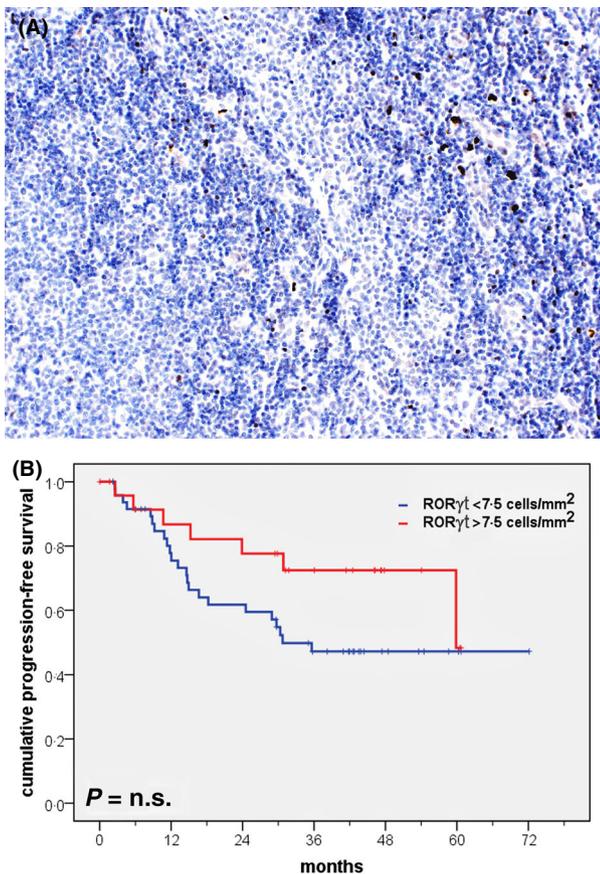


Fig 2. ROR γ t-positive T cells in follicular lymphoma. (A) Scattered ROR γ t-positive small reactive cells are mainly found in the interfollicular area (immunohistochemistry and hematoxylin counterstain, 200x); (B) Progression-free survival analysis for the impact of ROR γ t positive T cells in the R² group showing a trend towards improved survival for cases with higher amounts of Th17 cells.

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Conflict of interest

The authors declare no competing interests.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Material and Methods for gene expression profiling and immunohistochemistry

References

- Roulland S, Faroudi M, Mamessier E, Sungalee S, Salles G, Nadel B. Early steps of follicular lymphoma pathogenesis. *Adv Immunol.* 2011;**111**:1–46.
- Zucca E, Rondeau S, Vanazzi A, Østenstad B, Mey UJM, Rauch D, et al. Swiss Group for Clinical Cancer, R. & the Nordic Lymphoma, G. Short regimen of rituximab plus lenalidomide in follicular lymphoma patients in need of first-line therapy. *Blood.* 2019;**134**:353–62.
- Ochando J, Braza MS. T follicular helper cells: a potential therapeutic target in follicular lymphoma. *Oncotarget.* 2017;**8**:112116–31.
- Tzankov A, Meier C, Hirschmann P, Went P, Pileri SA, Dirnhofer S. Correlation of high numbers of intratumoral FOXP3+ regulatory T cells with improved survival in germinal center-like diffuse large B-cell lymphoma, follicular lymphoma and classical Hodgkin's lymphoma. *Haematologica.* 2008;**93**:193–200.
- Menter T, Tzankov A, Zucca E, Kimby E, Hultdin M, Sundstrom C, et al. Prognostic implications of the microenvironment for follicular lymphoma under immunomodulation therapy. *Br J Haematol.* 2020;**189**:707–17.
- Ishigame H, Kakuta S, Nagai T, Kadoki M, Nambu A, Komiyama Y, et al. Differential roles of interleukin-17A and -17F in host defense against mucocutaneous bacterial infection and allergic responses. *Immunity.* 2009;**30**:108–19.
- Xie S, Li J, Wang JH, Wu Q, Yang P, Hsu HC, et al. IL-17 activates the canonical NF- κ B signaling pathway in autoimmune B cells of BXD2 mice to upregulate the expression of regulators of G-protein signaling 16. *J Immunol.* 2010;**184**:2289–96.
- Duffield AS, Ascierto ML, Anders RA, Taube JM, Meeker AK, Chen S, et al. Th17 immune microenvironment in Epstein-Barr virus-negative Hodgkin lymphoma: implications for immunotherapy. *Blood Adv.* 2017;**1**:1324–34.
- Ferrarini I, Rigo A, Zamo A, Vinante F. Classical Hodgkin lymphoma cells may promote an IL-17-enriched microenvironment. *Leuk Lymphoma.* 2019;**60**:3395–405.
- Ferretti E, Di Carlo E, Ognio E, Guarnotta C, Bertoni F, Corcione A, et al. Interleukin-17A promotes the growth of human germinal center derived non-Hodgkin B cell lymphoma. *Oncoimmunology.* 2015;**4**:e1030560.
- Zhong W, Xu X, Zhu Z, Yang L, Du H, Xia Z, et al. Increased interleukin-17A levels promote rituximab resistance by suppressing p53 expression and predict an unfavorable prognosis in patients with diffuse large B cell lymphoma. *Int J Oncol.* 2018;**52**:1528–38.
- Jain P, Javdan M, Feger FK, Chiu PY, Sison C, Damle RN, et al. Th17 and non-Th17 interleukin-17-expressing cells in chronic lymphocytic leukemia: delineation, distribution, and clinical relevance. *Haematologica.* 2012;**97**:599–607.
- Idler I, Giannopoulos K, Zenz T, Bhattacharya N, Nothing M, Dohner H, et al. Lenalidomide treatment of chronic lymphocytic leukaemia patients reduces regulatory T cells and induces Th17 T helper cells. *Br J Haematol.* 2010;**148**:948–50.