

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

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Supplementary Design and Methods

Design and Methods

Patients, treatment regimens, definition of response, relapse and treatment failures

HIV infection (n=5) and post-transplant settings (n=6) were not considered exclusion criteria. Treatment was either standard or consistent with the investigational protocols active during the time the patients were diagnosed; only 5 diffuse large B-cell lymphomas (DLBCL) patients were given rituximab. Complete disease remission was defined as absence of disease on laboratory, imaging and physical examinations for at least 3 months after cessation of the last treatment regimen. Disease relapses were defined as recurrences after remission. Minor tumor shrinkage, immediate progressive disease, and early relapses (within 3 months after cessation of the last treatment regimen) were considered failure to achieve complete remission. Death with/of disease and failure to achieve complete remission defined treatment failures.

Tissue microarray construction

One [marginal zone- (MZL), peripheral T-cell- (PTCL) and angioimmunoblastic T-cell lymphomas (AITL)], two [DLBCL from Innsbruck and Basel, follicular lymphomas (FL), small lymphocytic lymphomas/chronic lymphocytic leukemias (SLL/CLL) from Innsbruck, Burkitt lymphomas (BL), anaplastic large cell lymphomas (ALCL) and classical Hodgkin lymphomas (cHL) from Innsbruck and Basel] or three [primary mediastinal B-cell lymphomas (PMBCL), DLBCL from Bologna, SLL/CLL from Bologna and cHL from Bologna] cores from every sample were arrayed.

Immunohistochemical double stains

For double-stains on conventional slides from tonsils, heat-induced antigen retrieval in Tec buffer (pH8.0, from Biologo, Kronshagen, Germany) was performed for 20 minutes at

100°C and the slides incubated for 32 minutes at 37°C with the primary anti-FOXP3 antibody on an automated immunostainer using the streptavidin-biotin-peroxidase detection system with diaminobenzidine as chromogen (brown). A second incubation for 120 minutes at 37°C with the primary anti-CD4 (1:80, from Neomarkers, Fremont, CA, USA) or anti-CD25 antibody (1:100, from Neomarkers) was subsequently carried out using the streptavidin-biotin-peroxidase detection system with 3-amino-9-ethyl-carbazole as chromogen (red).

Results

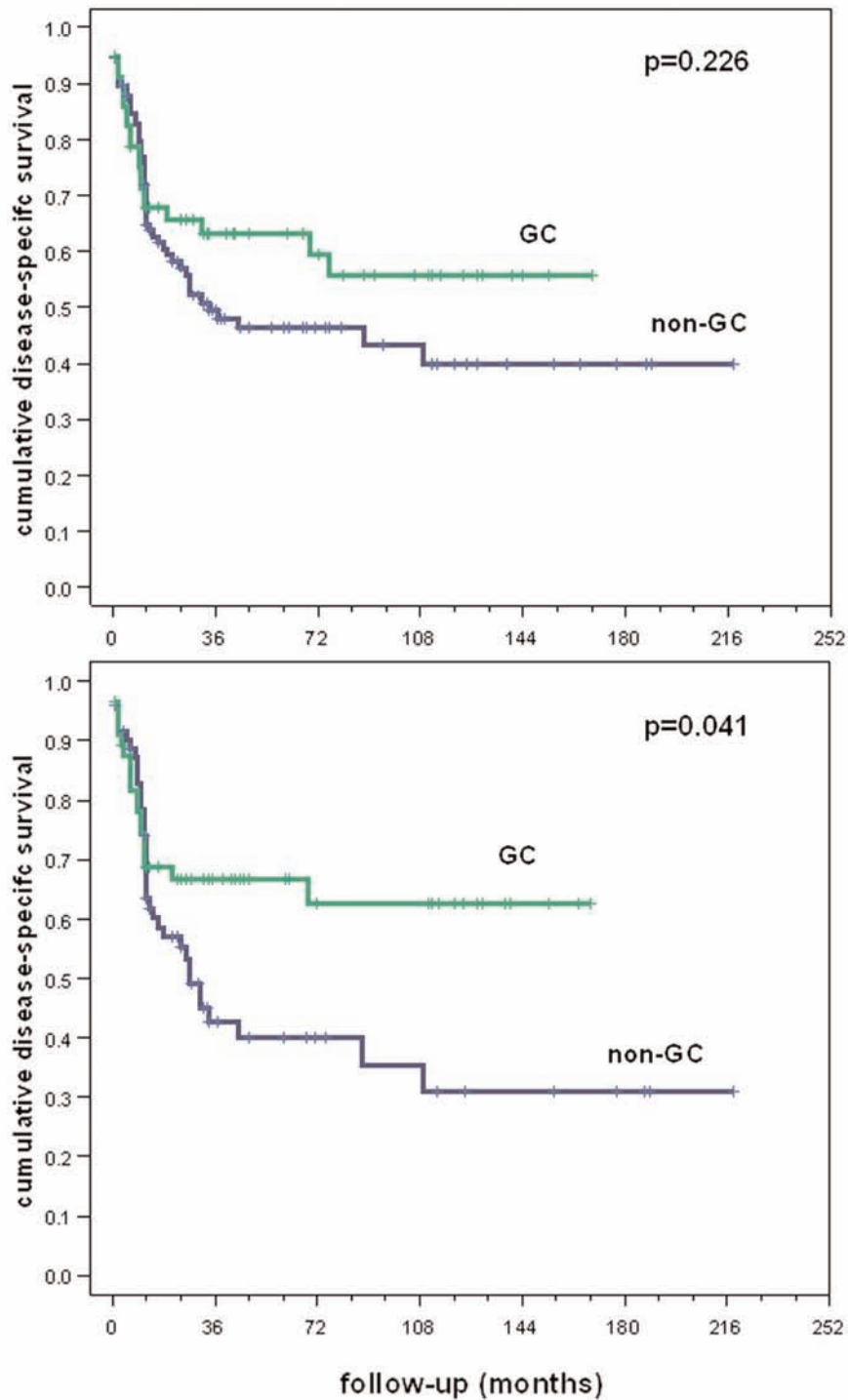
Life status data

The mean observation period for the 515 B- and T-cell lymphoma patients with known follow-up data was 51 months (range 0.5-219, median 33 months). Within that period, 179 (35%) patients died with/of disease, 27 (5%) due to second malignancies, 26 (5%) of cardio-vascular events and 13 (2.5%) due to other causes (infections, trauma). The mean observation period for the 152 cHL patients with known follow-up data was 115 months (range 0.5-219, median 105 months). Within that period, 17 (11%) patients died with/of disease, 18 (12%) due to secondary malignancies, 8 (5%) of cardio-vascular events and 9 (6%) due to other causes (infections, trauma).

Correlations of FOXP3⁺ cell quantity with lymphoma phenotype and expression of cell cycle-regulators

There were no additional correlations of the amount of FOXP3⁺ cells with other known phenotypic characteristics in the studied entity collectives such as deregulated expression of cell cycle regulating proteins (p21, p27, p53, bcl-2, cyclin A, D1, D3, E, Mib-1), angiogenesis (CD34, COX2, VEGF-A, Flk-1, Flt-1) and expression of other lineage-, differentiation-associated- and viral antigens (bcl-6, CD2, CD3, CD4, CD5, CD8, CD10, CD15, CD20, CD30, CD44s, CD79a, CD117, CD138, FOXP1, MUM1, LMP-1, ZAP-70).

Supplementary Figure



Comparison of the results for the prognostic molecular classification of our diffuse large B-cell lymphoma series according to the Hans' algorithm using cut-off values of the variables as suggested in the original publication (upper) and those suggested by the ROC curves (lower).