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LETTER TO THE EDITOR

The Role of Immunohistochemical Overexpression of p53 as Adverse Prognostic Factor in Primary Testicular Diffuse Large B Cell Lymphoma

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To the editor:

Primary testicular diffuse large B cell lymphoma (PT-DLBCL) is a rare and aggressive extranodal presentation of non-Hodgkin's lymphoma (NHL) accounting for less than 5% of testicular malignancies and less than 2% of NHL cases. PT-DLBCL displays a unique subtype within the highly heterogeneous group of DLBCL both in molecular-pathologic pattern as well as in biologic behavior [1]. However, risk-stratification of PT-DLBCL relies on common prognostic scores developed for nodal DLBCL like the International Prognostic Index (IPI) or the National Comprehensive Cancer Network- International Prognostic Index (NCCN-IPI). Recent studies further indicate that cell of origin (COO) and double-expressor lymphoma (DEL) status influence survival in nodal and extranodal lymphoma [2]. Nevertheless, these traditional prognostic tools do not account for biology of PT-DLBCL in detail. The tumor suppressor gene *TP53* encodes p53, functioning as a fundamental responder to cellular and oncogenic stress. In contrast to other malignancies p53 overexpression is not exclusively a result of *TP53* mutations in DLBCL. Previous studies have shown p53 overexpression as surrogate parameter of cellular –preferably oncogenic - stress in nodal DLBCL [1–3]. Nevertheless, there is lack of data with regard to p53 expression as easy available biomarker in PT-DBCL.

In this single-center study, we retrospectively analyzed all patients who presented to our institution with PT-DLBCL after orchiectomy and had formalin-fixed paraffin-embedded (FFPE) tissue available for immunohistochemical (IHC) assessment of p53 expression. Clinical data at baseline and outcome status were ascertained by review of health records of our hospital as previously described [4]. For assessment of double expressor lymphoma (DEL) status, double hit status (DHS) as well as next-generation sequencing of *TP53* see the Supplementary Materials and Methods. In addition, the Austrian Social Security system was queried for mortality outcomes. The study was approved by the local institutional review board (EK-Nr.: No. 32-306 ex 19/20).

Overexpression of p53 was defined as at least 50% positive tumor cells in IHC stains of testicular specimen. Patients were dichotomized into a binary p53 IHC expression variable at a cut-off at 50% as previously described [5] (Supplementary Materials and Methods).

Twenty-four males with a median age of 67 years [25th–75th percentile: 53–74] were included in the study (Supplementary Table 1, Supplementary Fig. 1). Overexpression of p53 was observed in 10 (42%) patients, and was more common in patients with higher clinical stage,

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a more unfavorable risk profile according to established prognosis scores (R-IPI, NCCN-IPI), and/or a higher risk for CNS involvement as per CNS-IPI (Supplementary Table 1). This suggests that p53 overexpression associates with adverse disease phenotypes in PT-DLBCL. Older age and BCL-2 or c-MYC overexpression associated numerically but not statistically significant with p53 overexpression.

Patients were relatively homogenously treated within 1st-line therapy. In detail, all but one (4%) patient received systemic treatment with an R-CHOP-like regimen (Supplementary Table 2) for a median of 6 cycles [5]. Twenty (83%) patients also received primary CNS prophylaxis with intrathecal long-lasting cytarabine (Depocyte®, $n = 11$), high-dose methotrexate ($n = 5$), and intrathecal methotrexate ($n = 4$), respectively. Immediate responses to 1st-line therapy included 21 complete remissions (88%) and 3 primary progressive diseases (13%). At progression or relapse, 7 patients received salvage therapies (Supplementary Table 3). One patient each was treated with autologous hematopoietic stem cell transplantation (ASCT) during 1st-line and salvage therapy, respectively.

Median follow-up at the data cut-off was 6.9 years, and 75% and 25% of the study cohort had follow-up intervals of at least 2.0 and 12.5 years, respectively. During this interval, seven (29%) patients developed relapse after prior remission, and 3 (13%) had primary progressive disease during 1st-line treatment. Moreover, 11 (46%) deaths were observed, of which 8 were adjudicated to PT-DLBCL progression and 3 to other causes, respectively. These outcomes corresponded to 10-year estimates of PFS, OS, progression risk, and cancer-specific mortality of 36% (95%CI: 12–59), 38% (14–62), 38% (17–60) and 48% (21–71), respectively (Supplementary Fig. 2).

Overexpression of p53 strongly predicted for dismal outcomes in PT-DLBCL. In detail, patients exhibiting overexpression of p53 showed significantly shorter 10-year PFS of 10% compared with 66% in those without/weak p53 expression. (log-rank $p = 0.001$). These results prevailed in 10-year OS as strongest predictor of clinical outcomes which estimates 12% in the p53 overexpression-group compared with 70% in patients without/weak p53 expression. (log-rank $p = 0.002$) Of note, after an observational horizon of 15 years every patient in the p53 overexpression group succumbed to PT-DLBCL.(Fig. 1) In univariable Cox analysis, p53 overexpression was associated with a 6.6-fold higher risk of progression (Hazard ratio (HR) = 6.56, 95%CI: 1.78–24.26, $p = 0.005$) and an 8-fold higher risk of death-from-any-cause (HR = 7.90, 95%CI: 1.69–36.85, $p = 0.008$), respectively. Of note, all three patients who had primary progressive disease during 1st-line therapy, exhibited p53 overexpression (Supplementary Table 4).

The strongest other univariable predictor of worse PFS and OS was a higher NCCN-IPI score (Supplementary Table 5). After adjusting for the NCCN-IPI, the adverse prognostic association between p53 overexpression and PFS (adjusted HR for p53 overexpression = 4.66, 95%CI: 1.16–18.80, $p = 0.030$) as well as OS (adjusted HR = 5.45, 95%CI: 1.11–26.71, $p = 0.037$) prevailed. Addition of p53 overexpression status to the NCCN-IPI numerically but not statistically significantly increased the NCCN-IPI's already high prognostic potential towards PFS (Harell's C index without and with p53 status: 0.73 vs. 0.78, p for difference = 0.262) and OS (0.80 vs. 0.84, $p = 0.306$), respectively.

In this study, we firstly defined overexpression of p53 measured by IHC as a valuable biomarker for clinical outcomes in PT-DLBCL. We could show that overexpression of p53 is a frequent event in PT-DLBCL affecting approximately 40% of patients and predicting worse clinical outcome independent of the established NCCN-IPI risk score. Even after adjusting for NCCN-IPI in a multivariate model outcomes were dismal. Of

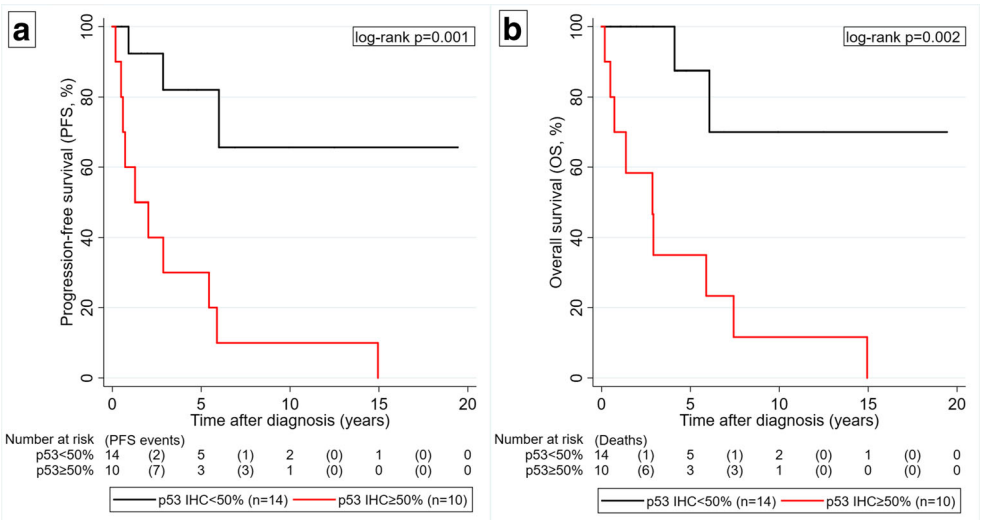

Fig. 1 Progression-free and overall survival experience of the study cohort – Kaplan-Meier curves by p53 expression status ($n = 24$). Panel **a** (left): Progression-free Survival, Panel **b** (right): Overall Survival. Red line: patients with p53 expression ≥50%, black line: patients with p53 expression <50%. Abbreviations: IHC – Immunohistochemistry

note, p53 overexpression is not due to somatic *TP53* mutations in most PT-DLBCL cases in our cohort hinting at a secondary response to different oncogenic hits. In our cohort only 2/14 sequenced patients showed somatic *TP53* mutations (Supplementary Table 6). Interestingly, *TP53* mutations were neither associated with IHC p53 expression status ($p = 0.312$) nor outcome variables. However, *TP53* mutation were significantly associated with c-MYC and BCL-2 translocation (double-hit lymphoma) which is in agreement with data generated in nodal DLBCL [3, 5].

In conclusion, our presented results suggest p53 overexpression as an independent prognostic factor in patients with untreated de novo PT-DLBCL which should be incorporated into future clinical studies.

Compliance with Ethical Standards

Conflict of Interest None of the contributing authors has any conflicts of interest, including specific financial interests and relationships and affiliations relevant to the subject matter or materials discussed in the manuscript.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Local institutional review board approval: *EK-Nr.: No. 32-306 ex 19/20*.

This article does not contain any studies with human participants or animals performed by any of the authors.

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