Circulating Levels of TNF Receptor II Are Prognostic for Patients with Peripheral T-cell Non–Hodgkin Lymphoma

Christina Heemann1,2, Markus Kreuz2,3, Irene Stoller1, Nils Schoof1,2,5, Frederike von Bonin1, Marita Ziepert3, Markus Löffer1, Wolfram Jung1, Michael Pfreamundschuh4, Lorenz Trümper1,2, and Dieter Kube1,2

Abstract

**Purpose:** Peripheral T-cell non–Hodgkin lymphomas (T-NHL) represent a small but heterogeneous and clinically aggressive subset of NHLs with a poor outcome. Cytokines or their receptors might be associated with the clinical outcome of these lymphomas. Therefore, we tested whether gene variations and serum levels of soluble TNF receptor (TNFR) I (sTNFRI), sTNFRII, interleukin (IL)-10, or sIL-4R are predictive for treatment response in T-NHLs.

**Experimental Design:** Peripheral blood DNA from 117 patients with T-NHL treated in prospective clinical trials was subjected to genotyping analysis. Whenever possible, pretreatment sera were obtained, and circulating levels of sTNFRI, sTNFRII, IL-10, and sIL-4R were determined with a specific capture enzyme-linked immunoassay.

**Results:** Patients characterized by TNFRI-609GG (rs4149570) showed a trend toward better event free survival [EFS; univariate: P = 0.041; multivariate: HR, 1.76; confidence interval (CI), 0.99–3.14 with P = 0.056]. A protective role of IL-10–1087A, −824T, and −597A reported in another study was not confirmed in our cohort. Patients with circulating levels of soluble TNFR II ≥2.16 ng/mL had a 2.07-fold increased relative risk for shorter overall survival (OS; univariate: P = 0.0034; multivariate: HR, 2.07; CI, 0.92–4.70 with P = 0.081) and a 2.49-fold higher risk for shorter EFS (univariate: P = 0.00068; multivariate: HR, 2.49; CI, 1.22–5.08 with P = 0.012). Elevations of circulating levels of sTNFRI, IL-10, and sIL-4R are frequent, but the clinical response in these patients is not significantly different.

**Conclusions:** Our findings suggest a critical role for TNF-TNFR signaling for the clinical outcome of patients with peripheral T-NHLs. *Clin Cancer Res; 18(13); 1–11. ©2012 AACR.*

Introduction

Mature peripheral T-cell non–Hodgkin lymphomas (T-NHL) represent a rare, morphologically, and clinically distinct group of aggressive NHL (aNHL) in Europe. A geographic variation in frequency of these lymphomas was observed ranging from 1.5% (Vancouver, BC, Canada) and 4.4% (Würzburg, Germany) to 18.3% (Hong Kong, China) of all NHLs diagnosed in these specific regions (1). Because of their relative rarity and histologic heterogeneity, T-NHLs have been the subject of only a limited number of studies investigating their biologic background; therefore, the pathobiology of T-NHLs is poorly understood (2). Compared with patients with B-cell NHL (B-NHL), a more aggressive clinical course and inferior treatment outcome for patients with T-NHLs has been reported in most studies (2–4). The only exception represents the anaplastic large-cell lymphoma (ALCL) that overexpresses the anaplastic lymphoma kinase (ALK; ref. 2). Promising treatment strategies for B-NHL do not generally improve T-NHL treatment (5). Nevertheless, in younger patients with T-NHLs, the addition of etoposide to standard CHOP therapy (cycloheximide, doxorubicin, vincristine, prednisolone) has been introduced in combination with CHOP therapy and/or stem cell transplantation, but most of them still need to be assessed in prospective clinical trials and a more effective standard therapy for T-NHLs has yet to be defined (6, 7). Reliable prognostic markers are urgently needed, as they would enable the definition of patient subpopulations based on biologic characteristics. The use of the International Prognostic Index (IPI) factors may provide to some extent a survival prediction for patients with T-NHLs, complementing histologic analyses (4, 8). Recent investigations of their relative rarity and histologic heterogeneity, T-NHLs have been the subject of only a limited number of studies investigating their biologic background; therefore, the pathobiology of T-NHLs is poorly understood (2). Compared with patients with B-cell NHL (B-NHL), a more aggressive clinical course and inferior treatment outcome for patients with T-NHLs has been reported in most studies (2–4). The only exception represents the anaplastic large-cell lymphoma (ALCL) that overexpresses the anaplastic lymphoma kinase (ALK; ref. 2). Promising treatment strategies for B-NHL do not generally improve T-NHL treatment (5). Nevertheless, in younger patients with T-NHLs, the addition of etoposide to standard CHOP therapy (cycloheximide, doxorubicin, vincristine, prednisolone) improved response rates (4). Several new agents have been introduced in combination with CHOP therapy and/or stem cell transplantation, but most of them still need to be assessed in prospective clinical trials and a more effective standard therapy for T-NHLs has yet to be defined (6, 7). Reliable prognostic markers are urgently needed, as they would enable the definition of patient subpopulations based on biologic characteristics. The use of the International Prognostic Index (IPI) factors may provide to some extent a survival prediction for patients with T-NHLs, complementing histologic analyses (4, 8). Recent investigations
in global gene expression of T-NHLs now provide a basis to improve their classification and the discovery of new therapeutic targets in the future (9, 10). Nevertheless, there is still a strong need to identify clinically applicable prognostic markers to improve treatment strategies.

There is evidence that the initiation or progression of lymphomas is supported by certain cytokines and chemokines, produced by lymphoma cells and/or cells in the tumor microenvironment, and their receptors (11, 12). Cytokines regulate the magnitude and profile of immune responses. In addition, they may act as paracrine or autocrine growth factors, thus supporting tumor growth and progression or can be involved in inflammatory processes (11–13). In this context, pro- and anti-inflammatory molecules such as TNF, interleukin (IL)-4, or IL-10 or their receptors have to be investigated.

TNF-α is a proinflammatory cytokine produced mainly by macrophages and T cells after respective stimulation. During cell transformation, TNF-α can be produced by neoplastic cells or cells in the tumor microenvironment and can act as an auto- or paracrine growth factor. TNF-α signals through binding to its receptors, TNF receptor I (TNFRI, p55) or TNFRII (p75), which can lead to proliferation, invasion, and metastasis of tumor cells leading to sustained tumor growth (14, 15) as well as to apoptosis (15).

TNF and the 2 receptors have been the focus of several studies. It was shown that single-nucleotide polymorphisms (SNP) or elevated plasma levels are associated to some degree with the risk and clinical outcome of some subtypes of lymphomas, solid tumors, and autoimmune diseases such as rheumatoid arthritis or allergy/asthma (16–23).

Circulating levels of soluble (s)TNFRI and sTNFRII were elevated in the majority of patients with NHLs and Hodgkin lymphomas with relatively high variability while remaining at low levels in healthy subjects (20, 24–26). Therefore, an increased production in patients with lymphoma was suggested. Moreover, risk groups were defined according to circulating levels of TNF and the 2 TNFRs, which significantly improved the predictive value of the IPI (20, 24, 26), whereby circulating serum levels of sTNFRII have been found to be more important (24).

However, the sample size of previous studies was limited, and T-NHLs were underrepresented. Recently, IL-10 has been the focus of a number of studies (13, 27, 28). A variety of cells including subsets of T cells, normal and neoplastic B cells, or stimulated monocytes and macrophages secrete IL-10, which is part of a balanced network of immunoregulatory cytokines (reviewed in refs. 29, 30). IL-10 is generally described to be an immunosuppressive and anti-inflammatory cytokine. Consequently, it has been found to exert antitumor immunity and to inhibit angiogenesis in experimental systems (31–33). However, IL-10 is also known to promote tumor cell proliferation and survival by acting as auto- or paracrine growth factor (13, 27, 28). Elevated IL-10 serum levels as well as defined IL-10 gene variations have been implicated in the pathogenesis and course of NHL and Hodgkin lymphomas (27, 34–39).

Furthermore, it has been revealed that IL-10 might play a role in the pathogenesis of T-NHLs. IL-10 mRNA has been detected frequently in different T-cell and natural killer (NK) cell lymphomas (40, 41). Because of its immunosuppressive function, IL-10 is thought to promote growth in a murine T-cell lymphoma cell line (42).

Lee and colleagues showed in an Asian cohort of 108 patients with T-NHLs a significant association between IL-10 gene polymorphisms and clinical outcome (43). Patients carrying at least one allele of the ATA haplotype (IL-10–1087A/G, −824C/T, −597A/C) showed a better overall survival (OS) and failure-free survival than in those without ATA haplotype. The authors stated that even though a definite association with the response to chemotherapy was not proven, their data imply that IL-10 may have some impact on the prognosis of T-NHLs.

We have recently observed an increased risk for patients suffering from aNHLs that carry the gene variation IL-10–7400Del/Del leading to a significantly shorter OS (44). However, the number of patients with peripheral T-NHLs in this cohort was too small (n = 45) to allow valid conclusions related to this lymphoma subgroup.

IL-4 is a key regulator of immunity. It is essential for the differentiation of naive helper T cells to T-helper (Th)2 cells. In a recent work, we found that the I75 variant of IL-4R I75V, a nonsynonymous SNP, combined with low levels of sIL-4R led to a favorable prognosis in patients with diffuse large B-cell lymphomas (DLBCL; ref. 45). In addition to this, an SNP in the promoter region of IL-4R gene (IL-4R–3223C/T) has been shown to be significantly associated with serum levels of sIL-4R (46).
To answer the question about the prognostic relevance of circulating levels of cytokines/cytokine receptors and their gene variations in T-NHLs we analyzed gene variations of the TNFRI, TNFRII, IL-4, and IL-10 genes and the respective circulating levels of IL-10 or soluble forms of the analyzed receptors in correlation with OS or event-free survival (EFS) of patients in a larger cohort treated within prospective clinical trials.

High circulating levels of sTNFRII are a negative predictor for treatment response in T-NHLs, suggesting a role for TNFRs for the clinical course of T-NHLs.

Patients and Methods

Patients and treatment

Between October 1993 and May 2007, 343 patients with mature nodal or extranodal biopsy-confirmed T-cell or NK cell lymphomas were included within prospective clinical trials of the German High-Grade Non-Hodgkin Lymphoma Study Group (DSS NHL) as described recently (4, 47–49). The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the ethics review committee of each participating center (4). Patients were eligible if they had previously untreated, biopsy-confirmed NHLs according to the Revised European-American Lymphoma Classification [translated into the World Health Organization (WHO) classification]. Clinical and histologic characteristics of the patients eligible for this study are shown in Table 1.

Genotyping analyses

DNA was isolated from whole blood samples of 117 T-NHLs, followed by fragment length analysis, TaqMan real-time PCR, and SNAPshot assay (38, 50, 51).

For the analysis of the −7400In/Del gene variation, a fragment length analysis was used (50). The IL-10 SNPs at −597A/C (rs1800872), −824A/T (rs1800871), −1087A/G (rs1800896), −3538A/T (rs1800890), −6208C/G (rs10494879), and −6752A/T (rs6676671); the TNFRI–609C/T (rs4149570), TNFRII R196M (rs1061622), as well as the IL-4R SNPs at −3226C/T (rs2057768), −1914C/T (rs2107356), I125V (rs1805015), and Q576R (rs1801275) were analyzed by TaqMan SNP genotyping assays. In addition, IL-10 SNPs at +4529 (rs3024498), −2726 (rs6693899), −2812 (rs6703630), −11668 (rs4072226), −11777 (rs4072227), and −12806 (rs17015865) were analyzed by using a SNAPshot Multiplex System according to the manufacturer’s instructions (Applied Biosystems). Briefly, DNA from patients and controls was used to amplify 3 different fragments containing the gene variations of interest (IL-10 −12 kb, −3 kb, or +4 kb). These PCR fragments were used in a primer extension reaction with fluorescent-labeled ddNTPs. SNP-specific primers of different length with the 3′-end directly adjacent to the respective gene variation were used. The analysis was then done using the AB3130 Genetic Analyzer from Applied Biosystems. The IL-10 gene variation at −3538 (rs1800890) was analyzed by both TaqMan and SNAPshot assays.

Table 1. Clinical and histologic characteristics of the patients with T-NHLs, analyzed for gene variations in IL-10 and cytokine receptors

<table>
<thead>
<tr>
<th>T-NHL patients included</th>
<th>Complete</th>
</tr>
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<tbody>
<tr>
<td>N = 117</td>
<td>N = 197</td>
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### Patients characteristics

<table>
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<th>Sex</th>
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<tr>
<td>Male</td>
<td>69 (59%)</td>
<td>120 (61%)</td>
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<tr>
<td>Female</td>
<td>48 (41%)</td>
<td>77 (39%)</td>
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<table>
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<th>Age, median (min.–max.)</th>
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<td>61 (18–78)</td>
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<table>
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<table>
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<td>99 (50.3%)</td>
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<table>
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<table>
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<td>47 (40.2%)</td>
<td>85 (43.2%)</td>
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### Histology

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<td>37 (18.78)</td>
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<table>
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<td>44 (22.34)</td>
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<table>
<thead>
<tr>
<th>T/NK</th>
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<tbody>
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<td>8 (4.06)</td>
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<table>
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<th>Hepatosplenic γδ</th>
<th>T-NHL patients included</th>
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<table>
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<th>T-cell NOS</th>
<th>T-NHL patients included</th>
<th>Complete</th>
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<tbody>
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<td>3 (2.56)</td>
<td>5 (2.54)</td>
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</table>

<table>
<thead>
<tr>
<th>T-cell, subtype</th>
<th>T-NHL patients included</th>
<th>Complete</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (1.71)</td>
<td>2 (1.02)</td>
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</table>

NOTE: Values in table are expressed as total number of patients and respective percentage are in parentheses (%), unless otherwise indicated. Abbreviations: AILT, angioimmunoblastic T-cell lymphoma; NOS, not otherwise specified for other than technical reasons; PTCL, peripheral T-cell lymphoma.

*LDH > N, age > 60 years, ECOG > 1, stage III/IV, and number of extranodal sites ≥ 2.
Further details and primer sequences are summarized within the Supplementary Tables S2 and S3. Several controls were included. Thus, each plate contained a well with DNA-free reaction mix to detect contamination with DNA. Five percent of the samples were also verified by direct sequencing.

ELISA
Sera were collected and stored frozen at the trial office biomaterials storage facility center at −70° C until measurement of serum factors. Sera were available for 80 patients with T-NHLs. Serum concentrations of soluble cytokine receptors and IL-10 were determined with ELISA kits (sTNFRI: Quantikine Human sTNFRI-Immunoassay, R&D Systems; sTNFRII: Quantikine Human sTNFRII-Immunoassay, R&D Systems; sIL-4R: Quantikine Human IL-4 sR ELISA, R&D Systems; IL-10: Eli-Pair, Diaclone), according to the manufacturer’s instruction. The median of serum level values of soluble receptors was used as the threshold to define high and low producers. For IL-10, the detection limit of the 5-pg/mL assay was set to discriminate high and low IL-10 levels. All samples and standards were run in duplicates.

Statistical analysis
For the analysis of polymorphisms in cytokine receptor genes, 117 patients with T-NHLs were selected from the NHL-B1/B2 and RICOVER60 studies considering the factors of the IPI [age > 60 years, lactate dehydrogenase (LDH) > normal (N), Eastern Cooperative Oncology Group (ECOG) > 1, stage III/IV, >1 extranodal involvement], bulky disease, and B-symptoms.

GENEPOP software was used for a first analysis of the data. These first analyses included tests for Hardy–Weinberg equilibrium, genotypic, and allelic differentiation.

WHO grades for leukocytopenia and infection and genotypic and allelic differentiation between groups were analyzed using the χ² test, and if required, Fisher exact test.

EFS was defined as time from first day of therapy (NHL-B1/B2) or random assignment (RICOVER60) to progressive disease under therapy, or failure to achieve complete response (CR) or CRu (i.e., no change or partial remission associated with additional therapy), additional therapy in excess of that prescribed in the protocol, relapse or death from any cause, whatever came first. OS was defined as time from first day of therapy (NHL-B1/B2) or random assignment (RICOVER60) to death from any cause.

Patients without an event in EFS or OS were censored at the last day with valid information for the respective endpoint. EFS and OS were estimated according to Kaplan–Meier and compared by log-rank trend test.

Multivariate analyses were conducted with the use of Cox proportional hazard models to estimate HRs for evolving an event. Nominal significance level was at 0.05 two-sided. We are aware of the problem of multiple comparisons and therefore have chosen to extract the most prominent aspect. Statistical analyses were conducted with R-2.6.2.

Results
Defined gene variations examined in T-NHL patients
One hundred and ninety-seven patients with mature nodal or extranodal biopsy confirmed T-cell or NK cell lymphoma were included into the study (Table 1). On the basis of the availability and quality of stored biomaterial, 117 patients with T-NHLs were eligible for respective sample preparation and genotyping analysis. Clinical and histologic characteristics of patients with T-NHLs are summarized in Table 1.

The effects of genetic variations were assessed in an exploratory fashion. DNA from patients with T-NHLs was genotyped for gene variations in the TNFRI (TNFRI-609G/T), TNFRII (TNFRII M196R), 12 gene variations in IL-10, and 5 in IL-4R SNPs.

Allele and genotype frequencies are shown in Table 2 and Supplementary Table S1. The Hardy–Weinberg test showed no significant differences between observed and suspected numbers of genotype frequencies.

Associations of the TNFRI-609G/T gene variations of the TNFRI gene with EFS of T-NHL patients
To estimate the impact of the analyzed gene variations on treatment outcome in patients with T-NHLs, univariate and multivariate analyses of the OS and EFS of all 117 patients suffering from T-NHLs were conducted with a median observation time of 50.5 months. The 3-year OS and EFS rates were 70% [95% confidence interval (CI), 62–79] and 56% (95% CI, 47–65), respectively.

Univariate analysis of polymorphisms in the TNFRI and TNFRII genes revealed differences in treatment outcome, associated with TNFRI-609G/T. As shown in Fig. 1A and B homozygous carriers of the TNFRI-609G major allele had a better EFS than in patients carrying TNFRI-609GT or TNFRI-609TT (P = 0.041). A consistent but not significant trend has been observed for OS (P = 0.099). The respective 3-year survival rates for patients homozygous for TNFRI-609G were longer than in carriers of TNFRI-609GT and TNFRI-609TT and are summarized within Supplementary Table S2. While no differences in OS rates were observed for the polymorphism TNFRII M196R-T/G (Fig. 1C), patients homozygous for the major allele TNFRII R196-T showed a trend toward shorter EFS rates (P = 0.066; Fig. 1D) than in patients carrying at least one minor allele TNFRII M196R-G (TNFRII M196R-GG and TNFRII M196R-GT). Accordingly, 3-year survival rates were shorter in patients homozygous for TNFRII R196-T than in carriers of TNFRII M196R-GG and TNFRII M196R-GT (Supplementary Table S2). When analyzing OS and EFS of patients with T-NHLs in correlation with IL-10 gene variations, shorter survival rates were observed for IL-10–7400Del/Del (Fig. 1E and F). The respective 3-year survival rates for patients carrying IL-10–7400Del/Del were shorter than in those of patients carrying IL-10–7400In/In and IL-10–7400In/Del.
Although the difference between patients showed a comparable tendency as described recently within a large cohort of 500 patients with aNHLs, the observed differences were not significant for patients with T-NHLs in the present study. Statistical power of these analyses was very low, due to the very low number of patients carrying IL-10–7400Del/Del (OS: \(P = 0.776\); EFS: \(P = 0.934\); ref. 44).

In a recent study, a protective effect of the ATA haplotype (IL-10–1087A, /C0 824T, /C0 597A) was shown (43). We tested for this effect in our T-NHL cohort focusing onto the SNP IL-10–824C/T representing the ATA haplotype best. Comparing patients characterized by the presence of at least one T-allele (IL-10–824TT, IL-10–824CT) with those patients homozygous for the C-allele (IL-10–824CC), a worse clinical outcome was observed for the patients group TT&TC. However, this was not significant (\(P = 0.178\)). Furthermore, no comparable trends for shorter cumulative OS or EFS were detected for the other IL-10 gene variations analyzed (data not shown).

Multivariate analysis adjusted for the IPI factors was conducted to identify gene variations with prognostic power independent of the IPI factors. In Table 3, the results of this multivariate analysis are shown for TNFRI–609G/T, TNFRII M196R-T/G, and IL-10–7400In/Del. An increased relative risk of 1.76 for shorter EFS rates was observed for carriers of at least one minor allele TNFRI–609T compared with homozygous carriers of TNFRI–609G (\(P = 0.056\)). This relative risk is comparable with the relative risk observed for elevated LDH, higher ECOG status, or age. The respective Cox model for TNFRII M196R revealed an increased relative risk of 1.54 (\(P = 0.153\)) for carriers of the major T-allele only for EFS but not for OS in line with the univariate analysis. This risk was also comparable with the risk estimated for the IPI factors elevated LDH, higher ECOG status, or age.

Multivariate analysis of IL-10–7400In/Del showed a 2.2 (OS) or 1.9 (EFS) times increased relative risk for carriers of IL-10–7400In/Del, which is close to that of having elevated LDH, higher ECOG status, or age but was not significant in our study. However, this result is also consistent with an enhanced relative risk for carriers of IL-10–7400Del/Del, which has been observed in a cohort of 500 patients with aNHLs (44).

For the IL-4R gene variations, no associations with EFS or OS of patients with T-NHLs were observed in univariate or multivariate analyses. Therefore, the protective effect of the IL-4R I75 variant we observed recently seems to be restricted to patients with DLBCLs or B-NHLs (45).

**Association of circulating sTNFRII levels with survival of T-NHL patients**

Cytokine serum levels are parameters that could to some extent reflect the actual host–lymphoma interaction and thereby influence the prognosis of patients with

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### Table 2. Genotype and allele frequency of the analyzed IL-10, TNFRI, and TNFRII gene variations in patients with T-NHLs (N = 117)

<table>
<thead>
<tr>
<th>Gene variation</th>
<th>Genotype</th>
<th>n (%)</th>
<th>Allele AA variant</th>
<th>n (%)</th>
<th>HWE exact</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-10–7400 (unknown)</td>
<td>Del/Del</td>
<td>5 (6)</td>
<td>Del</td>
<td>23 (54)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>In/In</td>
<td>59 (5)</td>
<td>In</td>
<td>77 (180)</td>
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</tr>
<tr>
<td>IL-10–1087 (rs1800896)</td>
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<td>18 (12)</td>
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<td>46 (108)</td>
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<td></td>
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<tr>
<td></td>
<td>AA</td>
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<td>A</td>
<td>44 (102)</td>
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<td></td>
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<td>TNFRI–609 (rs4149570)</td>
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<td>0.25</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>42 (35)</td>
<td>G</td>
<td>117 (263)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>39 (3.3)</td>
<td>G</td>
<td>117 (263)</td>
<td></td>
</tr>
<tr>
<td>TNFRII M196R (rs1061622)</td>
<td>GG</td>
<td>6 (5)</td>
<td>G</td>
<td>26 (57)</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>33 (28.3)</td>
<td>T</td>
<td>117 (263)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>54 (45.7)</td>
<td>T</td>
<td>117 (263)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** AA, amino acid; HWE, Hardy–Weinberg equilibrium.
lymphomas. Therefore, we measured circulating levels of sTNFRI, sTNFRII, IL-10, and sIL-4R in patients with T-NHLs by ELISA before treatment. The median of all measured serum levels of the analyzed soluble receptors was used as the respective threshold to define high and low producers. For sTNFRI, the median was 1.74 ng/mL; for sTNFRII, it was 2.16 ng/mL; and for sIL-4R, a median of 0.1035 ng/mL was calculated. In case of IL-10, the threshold was set on 5 pg/mL, according to the detection limit.

First, the analyzed gene variations were compared with high and low producers of sTNFRI, sTNFRII, IL-10, and sIL-4R. However, no significant associations were observed (data not shown).

Subsequently, OS and EFS rates of patients with T-NHLs were compared with high and low serum levels in univariate analyses.

A strong association of circulating levels of sTNFRII with OS and EFS was observed. Patients with T-NHLs with serum levels of TNFRII >2.16 ng/mL had significantly shorter OS (P = 0.003) and EFS (P = 0.0007; Fig. 2A and B).

The 3-year survival rates for patients with circulating serum levels <2.16 ng/mL of sTNFRII were 80% (95% CI, 68–95) for OS and 68% (95% CI, 55–85) for EFS, whereas for patients with circulating serum levels ≥2.16 ng/mL, the 3-year survival rates were 52% (95% CI, 38–71) and 38% (95% CI, 25–57) for OS and EFS, respectively.

Multivariate analyses adjusted for the IPI factors revealed enhanced relative risks for shorter survival (Table 4). The relative risk for shorter OS (HR, 2.1; P = 0.081) was comparable with the relative risk of having elevated LDH levels and even higher than the relative risk estimated for higher age, ECOG, and stage. The relative risk estimated for...
Table 3. Multivariate analysis of TNFRI–609G/T, TNFRII M196R–T/G, and distal IL–10–7400In/Del gene variations in relation to OS and EFS adjusted to the IPI factors (Cox model)

<table>
<thead>
<tr>
<th>Factor</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFRI–609G/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>2.42 (1.02–5.75)</td>
<td>0.046</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>2.12 (0.95–4.71)</td>
<td>0.067</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.62 (0.98–7.01)</td>
<td>0.056</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.70 (0.32–1.54)</td>
<td>0.372</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>5.25 (2.39–11.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TFNRI–609GTT vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFRI–609GG</td>
<td>1.61 (0.80–3.22)</td>
<td>0.181</td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>1.71 (0.78–3.74)</td>
<td>0.182</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>1.55 (0.82–2.93)</td>
<td>0.174</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.10 (0.86–5.10)</td>
<td>0.103</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.68 (0.36–1.31)</td>
<td>0.248</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>3.33 (1.74–6.38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFRI–609GTT vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFRI–609GG</td>
<td>1.76 (0.99–3.14)</td>
<td>0.056</td>
</tr>
<tr>
<td>TNFRII M196R–T/G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>2.45 (1.04–5.73)</td>
<td>0.040</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>2.25 (1.02–4.39)</td>
<td>0.045</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.46 (0.94–6.45)</td>
<td>0.068</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.73 (0.33–1.62)</td>
<td>0.445</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>5.01 (2.27–11.04)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFRII M196–TT vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFRII M196R–GT/GG</td>
<td>1.10 (0.55–2.21)</td>
<td>0.78</td>
</tr>
<tr>
<td>EFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>1.60 (0.75–3.43)</td>
<td>0.227</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>1.70 (0.90–3.20)</td>
<td>0.101</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>1.90 (0.80–4.52)</td>
<td>0.144</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.65 (0.34–1.25)</td>
<td>0.196</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>3.26 (1.70–6.25)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNFRII M196–TT vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNFRII M196R–GT/GG</td>
<td>1.54 (0.85–2.80)</td>
<td>0.153</td>
</tr>
<tr>
<td>IL–10–7400In/Del</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>2.25 (0.95–5.34)</td>
<td>0.065</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>2.41 (1.07–5.38)</td>
<td>0.033</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.73 (1.03–7.20)</td>
<td>0.043</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.75 (0.35–1.61)</td>
<td>0.467</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>4.64 (2.13–10.08)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Del/Del vs. In/Del and In/In</td>
<td>2.21 (0.74–6.65)</td>
<td>0.157</td>
</tr>
<tr>
<td>EFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>1.63 (0.75–3.51)</td>
<td>0.215</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>1.73 (0.92–3.28)</td>
<td>0.091</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.16 (0.46–5.15)</td>
<td>0.083</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.74 (0.40–1.38)</td>
<td>0.349</td>
</tr>
<tr>
<td>Extranodal involvement &gt; 1</td>
<td>2.89 (1.51–5.51)</td>
<td>0.001</td>
</tr>
<tr>
<td>Del/Del vs. In/Del and In/In</td>
<td>1.90 (0.72–4.97)</td>
<td>0.193</td>
</tr>
</tbody>
</table>

Abbreviation: RR, relative risk.
We show that patients characterized by TNFRI-609GG have a better EFS than other patients. The multivariate analysis of the TNFRI-609G/T variation revealed a 1.76-fold increased relative risk for carriers of the T-allele to have a poor prognosis for EFS (Table 3). The observed relative risk is comparable with that estimated for clinical parameters, elevated LDH, higher ECOG status, or age. Interestingly, an enhanced risk for shorter OS or disease-free survival for carriers of at least one T-allele at TNFRI-609G/T was observed in a study of 310 patients with non–small cell lung cancer (54). In addition, an association of the TNFRI-609T allele with susceptibility to hepatocellular carcinoma has been described (55). However, in contrast to these studies, in squamous cell carcinoma, the TNFRI-609TT genotype was protective (21). This study has to be defined as exploratory. Additional studies have to prove whether the TNFRI-609G/T gene variation will indeed become a prognostic factor for T-NHL outcome. Furthermore, we observed that patients suffering from T-NHLs carrying the IL-10 genotype IL-10–7400Del/Del tend to have a shorter cumulative OS, although our results are not significant (Fig. 1E and F and Table 3).

Up to now, only one study dealt with the implications of IL-10 gene variations for patients with T-NHLs, where the prognosis was significantly better for patients with T-NHLs carrying the ATA-haplotype (IL-10–1087A, −824T, −597A) with respect to OS and failure-free survival (43). This was not observed in our study. The rare number of patients homozygous for ATA (n = 4) showed an even worse prognosis. In addition, when analyzing patients carrying at least one allele of ATA in our study, a respective better prognosis was not observed. However, a comparison with the cohort from Lee and colleagues is difficult. In contrast to Asian cohorts, where the ATA haplotype is frequent; this haplotype is rather rare in Caucasian populations (51). The opposing results in both studies might be caused by the pronounced differences in elevated serum LDH levels (18.8% in our study, 53% in the study of Lee and colleagues) or in extranodal site involvement (19.7% in our study, 66% in the study of Lee and colleagues) that may reflect biologic differences between T-NHLs in Asian and Caucasian patients.

The most important observation of this study is the association of high circulation serum levels of sTNFRII with an adverse prognosis of patients with T-NHLs.

### Table 4. Multivariate analysis of serum levels of sTNFRII in relation to OS and EFS adjusted to the IPI factors (Cox model)

<table>
<thead>
<tr>
<th>Factor</th>
<th>RR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>2.581 (0.93–7.16)</td>
<td>0.068</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>1.579 (0.64–3.87)</td>
<td>0.320</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>0.932 (0.27–3.24)</td>
<td>0.910</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.898 (0.38–2.11)</td>
<td>0.800</td>
</tr>
<tr>
<td>Extranol involvement &gt; 1</td>
<td>3.101 (1.285–7.48)</td>
<td>0.012</td>
</tr>
<tr>
<td>Serum levels sTNFRII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2.16 ng/mL</td>
<td>2.073 (0.92–4.70)</td>
<td>0.081</td>
</tr>
<tr>
<td>EFS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDH &gt; N</td>
<td>1.793 (0.69–4.66)</td>
<td>0.230</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>1.274 (0.61–2.64)</td>
<td>0.520</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>0.935 (0.30–2.91)</td>
<td>0.910</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>0.667 (0.32–1.40)</td>
<td>0.280</td>
</tr>
<tr>
<td>Extranol involvement &gt; 1</td>
<td>1.747 (0.81–3.77)</td>
<td>0.150</td>
</tr>
<tr>
<td>Serum levels sTNFRII</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2.16 ng/mL</td>
<td>2.490 (1.22–5.08)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

NOTE: Significant P value of sTNFRII serum levels is shown in bold and italics.
observations are in line with previous studies on the influence of sTNFR levels on NHL and Hodgkin lymphoma outcome (18, 20, 24, 25) and highlight the importance of sTNFR levels for the small and very aggressive subgroup of T-NHLs, a subgroup that was underrepresented in earlier studies.

By comparing patients with elevated levels of sTNFRI or sTNFRII, we observed that approximately 80% of patients with elevated levels of sTNFRII also had elevated levels of TNFRI. This is probably because similar signals of the host lead to secretion of both receptors.

The molecular mechanisms behind the associations of polymorphisms and serum levels with disease outcome are not clear and have to be elucidated in more detail. TNF-α can act as cancer-promoting as well as cancer-inhibiting, either by acting as an auto- or paracrine growth factor on lymphoma cells modifying the microenvironment and distant organs, causing fever, weight loss, and other B-symptoms or by promoting apoptosis of malignant cells, leading to inhibition of tumor growth (15, 56). Therefore, the role of sTNFRs in the maintenance of malignant cell growth could be the stabilization of TNF-α, prolonging its half-life or the neutralization of its apoptotic effects by blocking TNF-α in the serum and thus preventing binding to the membrane-bound receptors in lymphomas. The understanding of the biologic role of elevated circulating levels of sTNFR in patients with lymphomas needs more detailed analysis in the future.

The balance between TNF-α and its 2 receptors is tightly regulated. Increasing levels of TNF-α in the serum induce shedding of TNFRII and especially TNFRII from the membrane. This can lead to a desensitization of the cell to TNF-α-mediated signaling (57). In healthy individuals, the shedding is limited to a maximum, which might permit the elimination of excess unbound TNF-α (57). This balance might be deregulated in patients with lymphomas, leading to the observation that elevated circulating levels of sTNFRI and especially sTNFRII are characteristic for NHLs and Hodgkin lymphomas independent from the origin of the lymphoma cells. However, it cannot not be ruled out that elevated levels of soluble cytokine receptors solely reflect an active immune response against malignant cells and that it is a sign for a chronic inflammation process not controlled by the host.

We are aware of the histologic heterogeneity of peripheral T-NHLs based on the wide variety of histologic subtypes included in our cohort, although all samples were obtained from prospective clinical trials. Therefore, the results obtained in this exploratory study need to be confirmed in additional prospective trials.

In summary, we provide strong evidence that elevated levels of sTNFRII and to some extent also sTNFRI are prognostic for the outcome of patients with T-NHLs. We further report here for the first time that the TNFRI–609G/T gene variation can be associated with the treatment outcome of patients with T-NHLs. Additional studies will have to prove whether the TNFRI–609G/T gene variation will indeed become a prognostic factor for T-NHL outcome. The results of this study encourage future studies to dissect the influence of components of the TNF-TNFR pathway onto the clinical outcome of patients with T-NHLs.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: C. Heemann, M. Schoof, M. Löfler, M. Pfreundschuh, L. Trumper, D. Kube
Development of methodology: C. Heemann, F. von Bonin, L. Trumper, D. Kube
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): I. Stoller, N. Schoof, M. Ziepert, W. Jung
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Heemann, M. Kreuz, I. Stoller, N. Schoof, M. Löfler, D. Kube
Writing, review, and/or revision of the manuscript: C. Heemann, M. Kreuz, I. Stoller, N. Schoof, M. Ziepert, L. Trumper, D. Kube
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Ziepert, W. Jung, L. Trumper
Study supervision: M. Pfreundschuh, L. Trumper, D. Kube

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References


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Christina Heemann, Markus Kreuz, Irene Stoller, et al.

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