Effect of *Interleukin-10* Gene Polymorphisms on Clinical Outcome of Patients with Aggressive Non-Hodgkin’s Lymphoma: An Exploratory Study

Dieter Kube, Thanh-Duc Hua, Frederike von Bonin, Nils Schoof, Samira Zeynalova, Marita Klöss, Daniela Gocht, Bernd Potthoff, Mladen Tzvetkov, Jürgen Brockmöller, Markus Lößler, Michael Pfreundschuh, and Lorenz Trümper

**Abstract**

*Purpose:* Current chemotherapy can achieve high response rates in aggressive non-Hodgkin’s lymphoma (NHL), but the factors that influence regression and survival remain unknown. The present exploratory study tested the hypothesis whether interleukin-10 (IL-10) polymorphisms predict clinical outcome, leukocytopenia, or infectivity during therapy. IL-10 was chosen because immune alterations are a major risk factor for NHL, and IL-10 is a cytokine involved in inflammatory processes associated with clinical outcome.

**Experimental Design:** Five hundred patients with aggressive NHL treated with CHOP/CHOEP were analyzed for IL-10 gene polymorphisms, including distal loci -7400InDel, -6752AT (rs6676671), and -6208CG (rs10494879) in comparison with proximal loci -3538AT (rs1800890), -1087AG (rs1800896), and -597AC (rs1800872) according to the incidence and outcome of the lymphoma.

**Results:** No differences in allele frequencies or haplotypes were found comparing a cohort of patients with aggressive NHL/diffuse large B-cell lymphoma with a healthy control group. Patients with aggressive NHL characterized by IL-10-7400DelDel had shorter overall survival periods compared with the other genotypes \((P = 0.004)\). The 3-year rate is 43.4% for IL-10-7400DelDel and 73.4% for IL-10-7400InIn and IL-10-7400InDel together. A significant increased risk for event-free survival is found for carriers of the genotype IL-10-6752TT-6208CC-3538AA \((P = 0.047)\). Multivariate analysis of IL-10-7400 gene variation in relation to overall survival adjusted to international prognostic index revealed a relative risk of 1.9 for carriers of IL-10-7400DelDel \((P = 0.037)\). No associations were found analyzing diffuse large B-cell lymphoma patients separately.

**Conclusion:** Our results indicate that IL-10 gene variations could be associated to the clinical course of aggressive NHL, which points out the importance of host factors and respective genetic elements for treatment response.
affect the risk and clinical outcome of NHL as well as side effects of therapy like infections or hematotoxicity (for review, see refs. 4–6).

The magnitude and profile of immune responses are regulated to a large extent by cytokines. The extent to which cytokine secretion varies between individuals with consequent variations in the intensity of a given immune response could be defined in part by regulatory gene variations. Several regulatory genetic elements associated with differences in cytokine secretion have been identified in genes coding for cytokines, in part also associated with disease outcome (for review, see refs. 7, 8). These regulatory polymorphisms are therefore thought to be partially responsible for interindividual differences to cope with a given challenge to the immune system. Specific cytokine genotypes may be beneficial by creating a ‘proinflammatory’ phenotype that may predispose to chronic inflammatory diseases or to a more severe form of inflammatory disease with a worse clinical outcome. However, the mechanisms underlying differences in immune response between individuals are complex but include inherited genetic variation.

Interleukin-10 (IL-10) is an important immunoregulatory cytokine in man. IL-10 is part of a balanced network of cytokines and can be cancer promoting (immunosuppressive; stimulation of cell proliferation) or cancer inhibiting (antiangiogenic; refs. 9–11). IL-10 is produced by several cells including normal and neoplastic B cells, stimulated monocytes/macrophages, and subsets of T cells. IL-10 has been implicated in certain infectious diseases, autoimmunity, transplantation tolerance, and tumorigenesis (for review, see also refs. 4, 9, 12, 13).

Polymorphisms in the IL-10 5′-flanking region genetically affect interindividual differences in IL-10 production (14–23). Variable associations between IL-10 production capacity and either the IL-10 microsatellite alleles, single nucleotide polymorphisms (SNP), or SNP haplotypes in the 7-kb IL-10 5′-flanking region have been reported (14, 16–18, 23). In most studies, the major proximal haplotypes GCC, ACC, or ATA formed by SNPs IL-10-1087AG, IL-10-824CT, and IL-10-597AC were found to be related to the in vitro IL-10 production capacity. The ATA haplotype was described as IL-10 low producer. (14, 16–18, 23) Several studies have reported that these proximal IL-10 promoter polymorphisms may be related with increased risk of a diverse range of diseases (reviewed in ref. 4). This indicates that genetic variations within the IL-10 gene locus are relevant in vivo.

Recent reports provided evidence that a risk to develop NHL or the clinical outcome of patients suffering from diffuse large B-cell lymphoma (DLBCL) might be related to certain IL-10 promoter gene variations. In one study, it was reported that proximal genotypes or haplotypes with low IL-10 expression are a risk factor for aggressive lymphoma, whereas a second study suggest that genotypes of high expression potential are a risk factor for developing lymphoma in patients with AIDS (24, 25). An InterLymph epidemiologic multicenter study described the IL-10-3538A regulatory SNP to be associated with for increased risk to develop NHL (26). This, however, was not verified in a subgroup from Germany/Heidelberg (27). In our study, allele frequencies of lymphoma patients are comparable with those of unmatched healthy controls (28). A French study (GELA) showed that in DLBCL patients the IL-10-1087T allele may be a risk factor for disease susceptibility, but this could not be verified in a cohort from Scandinavia (29, 30). In addition, no correlation to any clinical variables was found as described in the GELA study (29, 30). Analyzing the IL-10 gene loci at -3538 (A/T), -1354 (A/G), -824 (C/T), and -597 (A/C), we did not find any difference in overall survival (OS) or event-free survival (EFS) for NHL patients (28). However, these studies differed to some extent in terms of age range, lymphoma subtype, modest study size, and partially insufficient power.

The aim of this study was to analyze distal gene variations within the 5′-flanking region of the IL-10 gene in a large, representative, equally treated cohort of patients suffering

### Table 1. Clinical characteristics and diagnosis of patients with aggressive NHL

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>All patients in NHL-B1/B2 trials (N = 1,399)</th>
<th>All patients analyzed for IL-10 gene variations (n = 500)</th>
<th>DLBCL patients analyzed for IL10 gene variations (n = 319)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>789 (56)</td>
<td>280 (56)</td>
<td>180 (56)</td>
</tr>
<tr>
<td>Female</td>
<td>610 (44)</td>
<td>220 (44)</td>
<td>139 (44)</td>
</tr>
<tr>
<td>Age, median (min; max)</td>
<td>60 (18; 75)</td>
<td>62 (23; 75)</td>
<td>62 (23; 75)</td>
</tr>
<tr>
<td>Serum LDH &gt;N</td>
<td>316 (23)</td>
<td>123 (25)</td>
<td>85 (27)</td>
</tr>
<tr>
<td>Age &gt;60 y</td>
<td>689 (49)</td>
<td>273 (55)</td>
<td>178 (56)</td>
</tr>
<tr>
<td>Performance status ECOG &gt;1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ann Arbor stage III/IV</td>
<td>163 (12)</td>
<td>65 (13)</td>
<td>46 (14)</td>
</tr>
<tr>
<td>No. extranodal sites ≥2</td>
<td>567 (41)</td>
<td>202 (40)</td>
<td>122 (38)</td>
</tr>
<tr>
<td>IPI*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (IPI = 0, 1)</td>
<td>840 (60)</td>
<td>280 (56)</td>
<td>173 (54)</td>
</tr>
<tr>
<td>Low intermediate (IPI = 2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High intermediate (IPI = 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (IPI = 4, 5)</td>
<td>170 (12)</td>
<td>99 (20)</td>
<td>66 (21)</td>
</tr>
<tr>
<td>Bulky tumor (≥7.5 cm)</td>
<td>139 (10)</td>
<td>67 (13)</td>
<td>46 (14)</td>
</tr>
<tr>
<td>B symptoms</td>
<td>467 (33)</td>
<td>54 (11)</td>
<td>34 (11)</td>
</tr>
<tr>
<td>Extranodal involvement</td>
<td>402 (29)</td>
<td>164 (33)</td>
<td>109 (34)</td>
</tr>
<tr>
<td></td>
<td>86 (27)</td>
<td>54 (11)</td>
<td>46 (14)</td>
</tr>
<tr>
<td></td>
<td>252 (50)</td>
<td>139 (10)</td>
<td>86 (27)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62 (23)</td>
<td>66 (21)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34 (11)</td>
<td>34 (11)</td>
</tr>
</tbody>
</table>

**NOTE:** Values in table expressed as total number of patients (%), unless otherwise indicated. For additional information about the histology of patients with aggressive NHL, refer to Supplementary File 1.

*LDH >N, age >60 years, ECOG >1, stage III/IV, and number of extranodal sites ≥2.
from aggressive NHL and their role in predisposing an individual to lower remission rates, OS, or shorter periods of EFS and whether these associations are distinctive for DLBCL subtypes. The comparison of these gene variations with clinical variables such as EFS and OS revealed that distal regulatory gene variations of the IL-10 gene are related to some extent to poor prognosis of patients with aggressive NHL.

Materials and Methods

Patients and treatment. Lymphoma patients included into this study were from the NHL-B1/B2 study from the German NHL Study Group as described recently (Supplementary File 1; refs. 31, 32). The study was conducted in accordance with the Declaration of Helsinki. The protocol was approved by the ethics review committee of each participating center. All patients gave written informed consent. Patients were eligible if they had previously untreated, biopsy-confirmed aggressive NHL according to the Revised European-American Lymphoma Classification (translated into the WHO classification).

In this analysis, we included 500 patients from NHL-B1 and NHL-B2 studies. Three hundred and ninety-seven patients within this cohort were already genotyped for proximal SNPs at -1087A/G (rs1800896), -597A/C (rs1800872), and -3538A/T (rs1800890) as published recently (28). Clinical characteristics of the 500 patients eligible for this study are shown in Table 1 and are representative for all 1,399 NHL-B1 and NHL-B2 patients. The respective histology presented in Supplementary File 1 is based on a blinded central pathology review by a panel of expert hematopathologists.

The control group included 236 healthy blood donors. All samples were taken with no regard to sex or age, and donors were free of any chronic diseases.

Genotyping analyses. Blood samples and DNA isolation, multiplex PCR, and Taqman real-time PCR were done.

DNA from 236 unrelated healthy blood donors was included into this analysis as described previously (23, 33). DNA samples from 500 lymphoma patients were isolated by the same procedure and were described previously (23, 28, 34).

For the analysis of the genetic polymorphisms of the IL-10 5’-flanking region, a multiplex assay was used as described recently (23, 28, 33). Within the multiplex assay, the -7400InDel gene variation was analyzed as well as SNPs at -6752A/T (rs6676671), -6208C/G (rs10494879), -597A/C (rs1800872), and -3538A/T (rs1800890). In addition, the SNPs at -1087A/G (rs1800896), -6752A/T (rs6676671), -6208C/G (rs10494879), -597A/C (rs1800872), and -3538A/T (rs1800890) were analyzed by Taqman SNP genotyping assays (for details, see Supplementary File 2).

Statistical analysis. For the analysis of the IL-10 polymorphisms, 500 patients were selected from the NHL-B1/B2 study population, considering the factors of the international prognostic index (IPI; age >60 years, lactate dehydrogenase (LDH) >N, Eastern Cooperative Oncology Group (ECOG) >1, stage III/IV, >1 extranodal involvement), bulky disease, and B symptoms to be representative for the NHL-B1/B2 trial population.

Genetic data were analyzed using GENEPOP software. Analysis included tests for Hardy-Weinberg equilibrium and genotypic and allelic differentiation between healthy controls and lymphoma patients. Haplotype analysis was done using Arlequin software and http://www.bioinf.mdc-berlin.de/projects/hap/. WHO grades for leukocyteopnia and infection, genic, genotypic, and allelic differentiation between groups were analyzed using the χ² test and, if required, Fisher’s exact test.

EFS was defined as time from first day of therapy to progressive disease under therapy or failure to achieve complete remission or CR unconfirmed (that is, 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, whichever came first. OS was defined as time from first day of therapy 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 test.

Multivariate analyses were done with the use of Cox proportional hazards models to estimate hazard ratios 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 done with SPSS (version 11.5) software.

Results

IL-10 gene polymorphisms in patients with aggressive NHL and in healthy control subjects. The IL-10 5’-flanking gene variations at IL-10-7400InDel, IL-10-6752A/T, IL-10-6208C/G, IL-10-3538A/T, IL-10-1087A/G, and IL-10-597A/C were analyzed in 500 NHL patients. Allele frequencies, genotypes, and haplotypes were defined and compared with corresponding healthy controls. In Table 2, genic and genotypic data are summarized for the IL-10 gene variations. For the IL-10-7400InDel gene variation only, the genotype IL-10-7400InDel is less frequently present in the group of patients with NHL. However, this difference is not significant (P = 0.110). The testing showed that there are no significant differences between healthy controls and NHL patients in our study as well as for the other analyzed gene loci.

Our control group of 236 healthy blood donors was taken with no regard to sex or age, and donors were free of any chronic diseases. Therefore, this is not a classic case-control
study. However, a comparison of genotype distribution for IL-10-3538AT and IL-10-1087AG of our control group with published data from EPILymph Germany performing a classic case-control study revealed no relevant differences (Supplementary Table S1; refs. 26, 27). The Hardy-Weinberg test showed no significant differences between observed and suspected numbers of homozygotes or heterozygotes for the analyzed gene variations of the IL-10 gene in both groups (Supplementary Table S1). The same is found for the subgroup of 319 DLBCL patients within our study (Supplementary Table S2).

Based on the presented genotyping data, respective haplotypes of the 5′-flanking region of the IL-10 gene were estimated. All haplotypes with a frequency higher than 3% are shown in Fig. 1. Haplotype estimation was done for both healthy controls and NHL patients. Four major haplotypes are present: IAGTAA, IAGTAC, ITCAGC, and DTCAGC (IL-10 gene variations -7400, -6752, -6208, -3538, -1087, and -597). The IL-10-7400Del locus seems to be nearly exclusively linked with IL-10-1087G. The further analysis of haplotypes reveals no significant differences between healthy controls and lymphoma patients.

**IL-10 polymorphisms and aggressive NHL outcome.** The clinical prognostic features incorporated in the IPI, including age, LDH level, performance status, clinical stage and number of extranodal sites, mostly reflect the disease extension and the patient’s characteristics. Therefore, we compared gene variations of the 5′-flanking region of the IL-10 gene with these clinical variables, including hematotoxicity or infections according to WHO grades. Among 500 NHL patients, no associations were found between IL-10 gene variations and five single IPI prognostic factors (LDH >N, ECOG >1, extranodal involvement >1, stage >II, and age >60 years).

Infection WHO grade 3 or 4 during the first cycle of chemotherapy ranged between 0% for patients characterized by IL-10-7400DelDel and 5.6% carrying IL-10-3538AA. However, these differences were not significant between analyzed genotypes (data not shown). Similar results were obtained when taking into account all cycles of chemotherapeutic treatment. The rate of cycles with infection grade 3 or 4 was also not significant.

Leukocytopenia as a variable of hematotoxicity of chemotherapy was analyzed in relation to IL-10 gene variations. For the gene variation IL-10-597AC, we observed a risk to develop leukocytopenia with WHO grade 3 or 4 for heterozygous patients. Leukocytopenia of WHO grades 3 and 4 was observed in 37% and 39% cycles for homozygous AA and CC patients, respectively, and in 45% cycles for heterozygous AC patients (P = 0.002). The rates of patients with leukocytopenia grades 3 and 4 were 38%, 31%, and 47% for patients with AA, CC, and AC, respectively (P = 0.001).

Univariate analysis of OS and EFS of 500 NHL patients in comparison with IL-10 gene variations showed a significantly shorter OS for the IL-10 genotype IL-10-7400DelDel (Table 3; Fig. 2A). The respective 3-year survival rates were significantly reduced. Within this period, only 43.4% of patients carrying IL-10-7400DelDel survived [95% confidence interval (95% CI), 19%, 68%], whereas 72.3% (95% CI, 67%, 77%) or 75.3% (95% CI, 69%, 82%) of patients carrying IL-10-7400InDel and IL-10-7400InDel, respectively, had no event of death (P = 0.009). The OS rate for the IL-10-7400DelDel genotype is shown in more detail as a Kaplan-Meier plot in Fig. 2A. The OS difference between patients carrying IL-10-7400DelDel and patients with the other genotypes together was also significant (P = 0.004;
The role of inherited factors in the extent of IL-10 deregulation in malignant disorders is still controversial. The preliminary data obtained thus far indicate that additional larger studies of patients are required to confirm initial results in the understanding of the role of IL-10 in lymphoma development. We report one of the first analyses of the association between far distal gene variations of the IL-10 5′-flanking region and aggressive NHL within the thus far largest homogenously treated aggressive NHL patients group with 500 individuals representing the NHL-B1/B2 study from the DSHNHL study group. The results of the present exploratory study strongly support the hypothesis that genetic polymorphisms within the chromosomal locus 1q31/32 of the 5′-flanking region of the IL-10 gene are associated with adverse prognostic factors and predict poor outcome of aggressive NHL. In this study, we show that patients suffering from aggressive NHL carrying the IL-10 genotype

**Table 3.** OS and EFS of patients suffering from aggressive NHL in relation to gene variations of the IL-10 gene 5′-flanking region

<table>
<thead>
<tr>
<th>Genotype</th>
<th>3-y rate OS</th>
<th>P</th>
<th>3-y rate EFS</th>
<th>P</th>
<th>Genotype</th>
<th>3-y rate OS</th>
<th>P</th>
<th>3-y rate EFS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>-7400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>InIn (n = 305)</td>
<td>72.3</td>
<td>0.009</td>
<td>59.1</td>
<td>0.190</td>
<td>InIn; InDel</td>
<td>73.4</td>
<td>0.004</td>
<td>60.4</td>
<td>0.091</td>
</tr>
<tr>
<td>InDel (n = 178)</td>
<td>75.3</td>
<td>62.5</td>
<td>DelDel</td>
<td>43.4</td>
<td>DelDel</td>
<td>43.4</td>
<td>39.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DelDel (n = 17)</td>
<td>43.4</td>
<td>39.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6752</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (n = 176)</td>
<td>72.8</td>
<td>0.143</td>
<td>60.3</td>
<td>0.166</td>
<td>AA; AT</td>
<td>73.7</td>
<td>0.051</td>
<td>60.8</td>
<td>0.064</td>
</tr>
<tr>
<td>AT (n = 250)</td>
<td>74.4</td>
<td></td>
<td>61.2</td>
<td>53.2</td>
<td>TT</td>
<td>64.6</td>
<td>53.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (n = 74)</td>
<td>64.6</td>
<td>53.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-6208</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (n = 80)</td>
<td>68.8</td>
<td>0.448</td>
<td>59.4</td>
<td>0.768</td>
<td>GG; CG</td>
<td>73.1</td>
<td>0.365</td>
<td>59.7</td>
<td>0.499</td>
</tr>
<tr>
<td>CG (n = 262)</td>
<td>74.4</td>
<td></td>
<td>59.9</td>
<td>59.4</td>
<td>CC</td>
<td>68.8</td>
<td>59.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (n = 158)</td>
<td>70.9</td>
<td></td>
<td>59.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-3538</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (n = 74)</td>
<td>66.3</td>
<td>0.172</td>
<td>56.2</td>
<td>0.217</td>
<td>AT; TT</td>
<td>73.5</td>
<td>0.116</td>
<td>60.3</td>
<td>0.137</td>
</tr>
<tr>
<td>AT (n = 249)</td>
<td>75.2</td>
<td></td>
<td>61.4</td>
<td>58.7</td>
<td>AA</td>
<td>66.3</td>
<td>56.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT (n = 177)</td>
<td>71.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-1087</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (n = 134)</td>
<td>71.7</td>
<td>0.788</td>
<td>60.6</td>
<td>0.936</td>
<td>AA; AG</td>
<td>73.4</td>
<td>0.553</td>
<td>60.1</td>
<td>0.733</td>
</tr>
<tr>
<td>AG (n = 253)</td>
<td>74.2</td>
<td></td>
<td>59.8</td>
<td>58.5</td>
<td>GG</td>
<td>69.1</td>
<td>58.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG (n = 113)</td>
<td>69.1</td>
<td></td>
<td>58.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-597</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA (n = 26)</td>
<td>71.6</td>
<td>0.666</td>
<td>52.4</td>
<td>0.778</td>
<td>CC; AC</td>
<td>72.4</td>
<td>0.416</td>
<td>60.1</td>
<td>0.480</td>
</tr>
<tr>
<td>AC (n = 196)</td>
<td>72.7</td>
<td></td>
<td>59.5</td>
<td>52.4</td>
<td>AA</td>
<td>71.6</td>
<td>52.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (n = 278)</td>
<td>72.2</td>
<td></td>
<td>60.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Patients characterized by the genotypes IL-10-7400DelDel or IL-10-6752TT had a poorer prognosis compared with the other genotypes respectively. Italic P values are significant. For continuative description of significant results, see also Fig. 2.
IL-10-7400DelDel have a shorter cumulative OS. In addition, homozygous carriers for TCA haplotypes (IL-10 gene variations -6752, -6208, and -3538) have poor EFS. The multivariate analysis of the most distal gene variation an insertion/deletion mutation at -7400 revealed a 1.9-fold increased risk for carriers of the deletion on both alleles to have a poor prognosis (OS; Table 4). This observed relative risk is comparable with those estimated by analyzing clinical variables (age >60 years and ECOG >1), which have a relative risk of 2.0 or 2.1, respectively.

The increased relative risk of carriers of -7400DelDel has to be discussed also in the context of the IL-10 production capacity of respective healthy carriers. In a recent work, we showed that this genotype is characterized by an extremely high IL-10 expression capacity after in vitro stimulation with lipopolysaccharide (35). The IL-10-7400DelDel genotype is rare...
Table 4. Multivariate analysis of far distal IL-10. 7400 gene variations in relation to OS adjusted to IPI (Cox model)

<table>
<thead>
<tr>
<th>Factor</th>
<th>Relative risk (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDH &gt; N</td>
<td>1.7 (1.2-2.5)</td>
<td>0.005</td>
</tr>
<tr>
<td>Age &gt; 60 y</td>
<td>2.0 (1.3-3.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>ECOG &gt; 1</td>
<td>2.1 (1.4-3.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stage III/IV</td>
<td>1.5 (1.0-2.1)</td>
<td>0.029</td>
</tr>
<tr>
<td>Extranodal involvement &gt;1</td>
<td>1.0 (0.7-1.4)</td>
<td>0.888</td>
</tr>
<tr>
<td>DelDel vs InsIns, InsDel</td>
<td>1.9 (1.0-3.6)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

in Caucasians as well as in other thus far analyzed geographic regions from central Africa or Vietnam (23). This could suggest that additional gene variations probably in linkage disequilibrium to the described haplotype are important but not yet identified. Follow-up analysis will be needed to investigate additional gene variations across the chromosomal region 1q31/32 as suggested recently by Lan et al. (36). Several DНase 1–hypersensitive sites on a conserved 40-kb region between IL-19 and IL-10 genes revealed three functional enhancer elements, which reflect changes of the chromatin structure associated with IL-10 gene expression. Two of these enhancer elements with influence on IL-10 gene expression are located in comparable distances to the transcriptional start site of IL-10 as the analyzed far distal gene variations (37–43). Whether these chromatin changes are also affected by far distal IL-10 gene variations and influence therefore the differential IL-10 expression level remains to be analyzed in the future.

Promoter polymorphisms, including so-called regulatory SNPs, have been subject to the most scrutiny, particularly with regard to possible influence on regulation of gene transcription. The mechanism behind this for the IL-10 gene is still unknown probably because of the close proximity of -1087AG to the IL-10.G microsatellite; the IL-10.R microsatellite may affect IL-10 expression levels (17, 19). At this stage, the precise role of IL-10 promoter gene variations, individually or as part of defined proximal or distal haplotypes, in determining IL-10 expression is still a subject awaiting answers, including the question of allele-specific regulation (44). For example, the haplotype TCA (IL-10-6752T-6208C-3538A) could be a risk factor for poor clinical outcome for patients with aggressive NHL and respective DLBCL patients. Patients with IPI >2 are about twice more often (>20%) homozygous for IL-10-3538AA (for additional details, see Supplementary Table S3). However, after Bonferroni correction for multiple testing, these differences would be not longer significant. This observation that patients homozygous for IL-10-3538AA may have a higher risk for IPI >2 and that carriers of IL-10-7400DelDel and IL-10-6752TT are characterized by shorter cumulative OS may support the hypothesis that specific cytokine gene variations or polymorphisms in regulatory regions of cytokine genes are markers of tumor progression. Our finding of an increased risk to develop leukocytopenia WHO grades 3 and 4 when carrying both IL-10-5974A and IL-10-597C alleles adds some new aspect but does not fit to the current understanding of IL-10 production capacity and therefore remains to be elucidated by additional studies and in vitro experiments.

The clinical outcome of patients suffering from DLBCL was not related to proximal IL-10 promoter gene variations as described by other investigations. There was no significant difference in OS and EFS in the subgroup of DLBCL patients, but remarkably the genotype IL-10-7400DelDel had a clear trend in OS. Apparently these conflicting data may in part reflect the pleiotropic functions of IL-10 as cancer promoter or inhibitor in relation to the biological growth patterns of the lymphoma subgroups (11). Furthermore, differences in cancer karyotype complexity are also defining the disease state. In addition, antigenic and nonantigenic stimuli may be affecting the IL-10 expression by the lymphoma cells or their microenvironment (45).

Gene expression data have started to delineate the known molecular heterogeneity of aggressive NHL into distinct molecular entities but underlined the role of host response factors in some subtypes of these lymphomas (46–48). Bringing together molecular classifications, epidemiologic findings with well-designed clinical trials and respective in vitro analysis will help us understand the regulatory role of inherited IL-10 expression in the pathogenesis of lymphoma (49). Our major finding about an association between the IL-10 genotype IL-10-7400DelDel and the significant shorter cumulative OS or that of the haplotype TCA (IL-10-6752T-6208C-3538A) with poor EFS of NHL patients is, however, not yet suitable as a prognostic factor in routine use.

In this work, we have chosen to extract the most prominent clinical aspect and defined a new focused hypothesis for further validation study: IL-10-7400DelDel or the haplotype TCA (IL-10-6752T-6208C-3538A) could be a risk factor for poor clinical outcome for patients with aggressive NHL. This hypothesis can now be verified in independent patient cohort, for example, within the DSHNHl RICOVER study, or even in cohorts from other study groups with comparable expert hematopathologist review and treatment regimen.

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

References
6. Lossos IS, Morganstem D. Prognostic biomarkers

Downloaded from clincancerres.aacrjournals.org on April 13, 2017. © 2008 American Association for Cancer Research.
Imaging, Diagnosis, Prognosis


Effect of Interleukin-10 Gene Polymorphisms on Clinical Outcome of Patients with Aggressive Non-Hodgkin's Lymphoma: An Exploratory Study

Dieter Kube, Thanh-Duc Hua, Frederike von Bonin, et al.