Interleukin-4 Promoter Polymorphisms: A Genetic Prognostic Factor for Survival in Metastatic Renal Cell Carcinoma

Thomas Kleinrath, Christoph Gasner, Peter Lackner, Martin Thurnher, and Reinhold Ramoner

ABSTRACT

Purpose
Renal cell carcinoma (RCC) is considered a cytokine-responsive tumor. The clinical course of a patient may thus be influenced by the patient’s capacity to produce distinct cytokines. Therefore, cytokine gene polymorphisms in RCC patients were analyzed to determine haplotype combinations with prognostic significance.

Patients and Methods
A selection of 21 single nucleotide polymorphisms within the promoter regions of 13 cytokine genes were analyzed in a cross-sectional single-center study of 80 metastatic RCC patients. Univariate and multivariate analyses and the Cox forward-stepwise regression model were chosen to assess genetic risk factors.

Results
Multivariate Cox regression analysis confirmed by a bootstrap technique identified the heterozygous IL4 genotype $-$589T $-$33T/$-$589C $-$33C as an independent prognostic risk factor (risk ratio, 3.1; $P < .01$; 95% CI, 1.4 to 6.9; adjusted for age, sex, and nuclear grading) in metastatic RCC patients. IL4 haplotype $-$589T $-$33T and $-$589C $-$33C were found with a frequency of 0.069 and 0.025, respectively, which represents a two-fold decrease of IL4 haplotype $-$589T $-$33T ($P < .01$) and an increase of IL4 haplotype $-$589C $-$33C frequency ($P < .05$) in metastatic RCC compared with other white reference study populations. The median overall survival was decreased 3.5-fold ($P < .05$) in heterozygote patients carrying IL4 haplotype $-$589T $-$33T and $-$589C $-$33C (3.78 months) compared with patients homozygote for IL4 haplotype $-$589C $-$33C (13.44 months). In addition, a linkage disequilibrium between the IL4 gene and the KIF3A gene was detected.

Conclusion
Our findings indicate that IL4 promoter variants influence prognosis in patients with metastatic RCC and suggest that genetically determined interleukin-4 (IL-4) production affects the clinical course of the disease possibly through regulation of immune surveillance.


INTRODUCTION

Renal cell carcinoma (RCC) is resistant to conventional therapies such as radiation, hormone therapy, and chemotherapy. At diagnosis, 20% to 30% of patients with RCC already have metastatic disease and 20% to 40% of patients with organ-confined tumors at nephrectomy will develop metastatic disease.1 Metastatic disease is characterized by a poor prognosis, with a median survival of 13 months.2 RCC tissue frequently is infiltrated by lymphocytes,3 macrophages,4 and dendritic cells,5 reflecting the immunogenicity of RCC.

RCC has also been shown in vitro to respond to various cytokines including tumor necrosis factor alpha (TNF-$\alpha$),6 interleukin-6 (IL-6),7 type I interferon (IFN-$\alpha$),8 IFN gamma (IFN-$\gamma$),9 IL-4,10-13 and IL-13.14 The cytokine effects observed in these studies were either growth-promoting (TNF-$\alpha$, IL-6) or antiproliferative (type 1 IFN, IFN-$\gamma$, IL-4, and IL-13). For IL-4 and IL-13, growth-inhibitory effects on RCC in vitro via binding of the cytokines to their specific receptors have been reported.11,14 However, despite these pronounced preclinical observations, subsequent clinical trials of systemic IL-4 in patients with RCC and other tumors did not indicate significant beneficial effects.15-17 In contrast, treatment with type 1 IFN and IL-2 showed a modest survival benefit.2,18

Cytokines and their receptors are known to be highly conserved within their coding region.19,20 However, several examples of amino acid sequence
variations have been described in healthy individuals.\textsuperscript{21} Although polymorphisms outside of the coding region do not influence the amino acid sequence, they may affect protein expression by influencing alternative mRNA splicing, mRNA stability, or transcription levels.\textsuperscript{22,23} Especially polymorphisms within the 5'- and 3'-regulatory regions of cytokines are of crucial impact, given that they can determine transcription factor binding sites within the cytokine gene promoters as well as the structure of enhancers and silencers.\textsuperscript{21} In part, human diversity in immune responses may be explained by such polymorphisms because they lead to interindividual differences in cytokine-producing capacity, which results in a variety of biologic consequences.

For example, findings of different studies showed that the \textit{IL4} promoter variants \textit{IL4}−589T and \textit{IL4}−33T lead up to a three-fold higher transcriptional activity in vitro, which was subsequently confirmed in vivo.\textsuperscript{24-26} \textit{IL4}−589, \textit{IL4}−33, and other noncoding variations of \textit{IL4} were also shown to influence the risk of Th2-mediated diseases such as asthma and atopy, and to be involved in the regulation of serum immunoglobulin E levels, which is one of the major clinical manifestations of these diseases.\textsuperscript{25,27,28}

To elucidate further a potential role of endogenous cytokines in the course of metastatic RCC, a representative selection of interindividual cytokine and cytokine receptor gene promoter polymorphisms were investigated in 80 RCC patients.

**Table 1. SNPs Detected by Heidelberg Cytokine Genotyping Kit**

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP Position*</th>
<th>Trivial Name in Heidelberg Kit</th>
<th>SNP Alises, Used in Literature</th>
<th>MA (F)</th>
<th>Source of MA (F)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{IL1A}</td>
<td>C T</td>
<td>−1613</td>
<td>−889</td>
<td>MAF</td>
<td>A</td>
<td>39</td>
</tr>
<tr>
<td>\textit{IL1B}</td>
<td>T G</td>
<td>−307</td>
<td>3962</td>
<td>A</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>\textit{IL1R1}</td>
<td>C T</td>
<td>−1585</td>
<td>pst1 1970</td>
<td>NA</td>
<td>NA</td>
<td>37</td>
</tr>
<tr>
<td>\textit{IL1RN}</td>
<td>G T</td>
<td>1059</td>
<td>114</td>
<td>28</td>
<td>A</td>
<td>41</td>
</tr>
<tr>
<td>\textit{IL2}</td>
<td>G T</td>
<td>311</td>
<td>116</td>
<td>42</td>
<td>A</td>
<td>43</td>
</tr>
<tr>
<td>\textit{IL4}</td>
<td>G T</td>
<td>308</td>
<td>172</td>
<td>43</td>
<td>A</td>
<td>44</td>
</tr>
<tr>
<td>\textit{IL6}</td>
<td>G T</td>
<td>308</td>
<td>172</td>
<td>44</td>
<td>A</td>
<td>45</td>
</tr>
<tr>
<td>\textit{IL10}</td>
<td>C T</td>
<td>−57</td>
<td>−33</td>
<td>45</td>
<td>A</td>
<td>46</td>
</tr>
<tr>
<td>\textit{IL12B}</td>
<td>C A</td>
<td>10842</td>
<td>1188</td>
<td>46</td>
<td>A</td>
<td>47</td>
</tr>
<tr>
<td>\textit{IFNG}</td>
<td>T G</td>
<td>10842</td>
<td>1188</td>
<td>47</td>
<td>A</td>
<td>48</td>
</tr>
<tr>
<td>\textit{TGFB1}</td>
<td>C T</td>
<td>−307</td>
<td>3962</td>
<td>A</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>\textit{TNF}</td>
<td>G T</td>
<td>308</td>
<td>172</td>
<td>50</td>
<td>A</td>
<td>51</td>
</tr>
</tbody>
</table>

Abbreviations: db, database; SNP, single nucleotide polymorphism; MA \(F\), minor allele frequency; rs, reference SNP; NA, not available; NCB, National Center for Biotechnology Information.\textsuperscript{1}

\({}^*\)Position relative to ATG, including SNP nucleotide (positive or negative).

\(\dagger\)Sources for MA \(F\) data were from HapMap project\textsuperscript{25} or NCB\textsuperscript{1} (www.ncbi.nlm.nih.gov) for the white CEU- and CAUC panels, respectively.

\(\ddagger\)Typing results for SNP rs2243248 did not meet quality requirements for interpretation and were therefore excluded from additional analysis.

**Study Population**

Eighty-nine white patients were selected in a single-center study. Nine patients were excluded from analysis because of low DNA quality or poor DNA yield. All patients had RCC with clear-cell histology and had already developed bidimensionally measurable metastatic lesions. Furthermore, all patients were participants in the local dendritic cell vaccine program (1998 to the end of 2004).\textsuperscript{29,30} The primary tumor was removed in all patients and the start of follow-up was defined by the beginning of immunotherapy. Patients with solitary brain metastasis, other malignancies than RCC within the last 5 years, treatment with immunosuppressive drugs, other immunotherapies or chemotherapy within 4 weeks before treatment start, pregnancy or lactation, presence of acute or chronic infections, HIV or viral hepatitis, or a Karnofsky performance score less than 60 were excluded from the study. Furthermore, a computed tomography of brain, chest, and abdomen and a bone scan were performed. All patients were informed about the investigative character of the study and provided their written informed consent.

**Cytokine Genotyping and DNA Preparation**

Genomic DNA was isolated from frozen peripheral blood mononuclear cells using Nucleon BACC2 reagents (Amersham, Buckinghamshire, United Kingdom).

A selection of 21 single nucleotide polymorphisms (SNPs) within 13 genes (\textit{IFNG, IL1A, IL1B, IL1R1, IL1RN, IL2, IL4, IL4R, IL6, IL12B, TGF\textsubscript{B1}, and TNF}) was typed using the Cytokine CTS-PCR-SSP Tray Kit as described by the manufacturer (Department of Transplantation Immunology, University of Heidelberg, Heidelberg, Germany) according to procedures recommended by the 13th International Histocompatibility Workshop Cytokine Polymorphisms Component.\textsuperscript{31}

Details on specificities, primer sequences, and reaction conditions of the Cytokine CTS-PCR-SSP Tray Kit are available for \textit{IL10}, \textit{TGF\textsubscript{B1}}, \textit{TNF}, \textit{IL4R}, \textit{IL1B, IL1RN, IL1R1, IL1A, IL4}, and \textit{IL6}.\textsuperscript{32-34} Typing results for SNP rs2243248 did not meet quality requirements for interpretation and were therefore excluded from additional analysis.

Many different aliases for the same polymorphism are found in the literature. Some authors refer to the position relative to the transcriptional start site, whereas others use the position relative to the “A” of the start codon ATG.
Eighty patients were typed for all SNPs described above. For six of these genes (IL2, IL4, IL6, IL10, TGFB1, and TNF), a typing method also known as double amplification refractory mutation system–polymerase chain reaction was chosen, which allowed the detection of haplotypes instead of simply the analysis of SNPs. Hence, for each of the above-mentioned genes, four haplotypes could be defined.

**Linkage Disequilibrium Analysis**

For linkage disequilibrium (LD) analysis, SNP genotype data (CEU [CEPH: Utah residence with ancestry from Northern and Western Europe] population) of a 140-kbp region—containing the cytokine genes IL13 and IL4, as well as RAD50 homolog (RAD50) and kinesin family member 3A (KIF3A)—were imported from the HapMap project database and analyzed using the Haploview software version 3.32 (www.broad.mit.edu/mpg/haploview/). An LD map was created using the “Solid spine of LD” setting of the Haploview software.

**Statistical Analysis**

To identify genotyping errors, the Hardy-Weinberg equilibrium was calculated within population-based data sets. The false-discovery rate criterion, which has been proposed as an appropriate statistical method in genetics research, was used for controlling the errors in multiple comparisons. The Hardy-Weinberg equilibria for all polymorphisms (n = 13) were in the normal range (corrected P > .3). Subsequently, multivariate analysis was done using the Cox forward-stepwise regression model. In the stepwise procedure, a significance level of .05 for entering and .10 for removing the respective explanatory variables was used to determine the independent risk factors. A bootstrap technique was applied to confirm the choice of variables. One thousand bootstrap samples were created and stepwise Cox regression was applied to each sample. The percentage of samples for which each variable was included in the model from the 1,000 samples was calculated. After having identified the IL4 promoter polymorphisms to be the best independent risk factor predicting survival, gene allele frequency comparison was calculated using y2 test (two-tailed, significance level = .05). The IL4 heterozygote genotype 2/4 was observed in only one of the 80 patients and was therefore excluded from the survival analysis. The Kaplan-Meier method and log-rank test were used to assess the prognostic significance of the respective cytokine genotypes for overall survival.

**RESULTS**

This study was conducted during a 6-year period in 80 patients with metastatic RCC. All patients had clear cell histology. Median age of patients was 56 years at the beginning of the study, with a median overall survival of 12.6 months. Sixty-one percent of the study population was male and 36% was female. All patients were enrolled for dendritic cell vaccination protocols.

**Multivariate Analysis**

First, a multivariate analysis of the effects of genotype on survival was conducted using Cox proportional hazards models to adjust for confounding factors. Using a significance level of .05 for entering and .10 for removing a variable in a stepwise cox regression, four variables were selected: tumor grading, IL4, IL10, and transforming growth factor beta (TGFB1). However, when fitting the final model with these four variables, as well as sex and age as independent predictors, TGFB1 and IL10 showed a P value of more than .05. Therefore, TGFB1 and IL10 were not included in the final model. To confirm this decision, a bootstrapping technique was applied. The percentage of inclusion among the 1,000 samples created by the bootstrapping technique for...
tumor grading and *IL4* were 99.9% and 93.4%, respectively. In contrast, the percentage of inclusion for *TGFβ1* and *IL10* was lower than 60%. The results of the bootstrap procedure confirmed the variables chosen for the final model. Among these 13 promoter variations, the *IL4* promoter polymorphisms −589/−33 turned out to be the best independent risk factor predicting survival. The *IL4* genotype 1,4 was found in 11 patients, showing a 3.1-fold increased risk ratio ranging from 0.108 to 0.144 and 0.877 to 0.852, respectively.60,61 In our RCC patient cohort, the frequency of *IL4* haplotype 1 was decreased significantly (P < .01) adjusted for age, sex, and grading (Table 3).

**Frequencies of IL4 Haplotypes 1 and 4 in Metastatic RCC**

In white control populations, which include a locally matched control group, *IL4* haplotypes 1 and 4 were found with frequencies ranging from 0.108 to 0.144 and 0.877 to 0.852, respectively.63 In our RCC patient cohort, the frequency of *IL4* haplotype 1 was decreased significantly (0.069; P < .01), and at the same time, *IL4* haplotype 4 was increased significantly (0.925; P < .05). In line with the study by Beghè et al,60 a trend for a decreased *IL4* haplotype 4 frequency was also found in the locally matched control population analyzed with the same cytokine genotyping kit for *IL4* haplotype 4 (P = .095). Data are summarized in Table 4.24-26,60

**Univariate Survival Analysis**

In the next step, the influence of *IL4* haplotypes on patient survival was examined. Using the Kaplan-Meier method and log-rank test, the only significant association with survival was found for the *IL4* promoter polymorphism. Median overall survival was 3.5-fold increased (P = .027) in patients homozygote for *IL4* haplotype 4 (13.44 months) compared with heterozygote patients carrying *IL4* haplotypes 1 and 4 (3.78 months; Fig 1 and Table 5). Thus, patients expressing the *IL4* genotype 1,4 showed a significantly decreased survival. Given the haplotype frequencies observed, the rare *IL4* genotype 1,1 was not encountered in our study group (Table 5).

*IL4* haplotypes 1 and 2 both carrying the −589T allele represent two high *IL4*-expressing haplotypes, whereas low-expressing haplotypes 3 and 4 exhibit an *IL4*/−589C allele.24-26 Hence, the predicted functional phenotypes for the homozygous genotype *IL4* 4,4, the heterozygous genotype *IL4* 1,4, and the homozygous genotype *IL4* 1,1 are low (low/low), intermediate (high/low), and high (high/high) *IL4* producers, respectively (Tables 4 and 5).

Thus, patients with an expected low *IL4*-producing phenotype have a 9.7 median months survival benefit compared with patients with an intermediate *IL4*-producing phenotype (Fig 1).

**LD Analysis of the IL4 region**

Finally, we performed an analysis of the LD map for the *IL4* region, containing the genes *RAD50*, *IL13*, *IL4*, and *KIF3A*,

---

**Table 4. Comparison of *IL4* Haplotype Frequencies and Corresponding IL-4 Expression As Published Previously**

<table>
<thead>
<tr>
<th><em>IL4</em> Haplotype Identification</th>
<th><em>IL4</em> Expression in T Cells24-26</th>
<th>Previously Published60</th>
<th>Locally Matched Healthy Control4</th>
<th>Renal Cell Carcinoma Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2243250 −589*</td>
<td>rs2070874 −33*</td>
<td><em>No. (n = 1,310)</em> Frequency P</td>
<td><em>No. (n = 446)</em> Frequency P</td>
<td><em>No. (n = 160)</em> Frequency P</td>
</tr>
<tr>
<td>1 T T</td>
<td>High</td>
<td>189 0.144 0.009</td>
<td>48 0.108 0.155</td>
<td>11 0.069</td>
</tr>
<tr>
<td>2 T C</td>
<td>High</td>
<td>5 0.004 0.649</td>
<td>4 0.009 0.744</td>
<td>1 0.006</td>
</tr>
<tr>
<td>3 C T</td>
<td>Low</td>
<td>0 0.000 ND</td>
<td>3 0.007 ND</td>
<td>0 0.000</td>
</tr>
<tr>
<td>4 C C</td>
<td>Low</td>
<td>1,116 0.852 0.012</td>
<td>391 0.877 0.095</td>
<td>148 0.925</td>
</tr>
</tbody>
</table>

NOTE. Haplotype frequencies between renal cell carcinoma patients and the previously published cohort or the locally matched healthy control group respectively were compared using the *χ²* test.

Abbreviations: rs, reference SNP; ND, not determined; SNP, single nucleotide polymorphism.

*SNP position relative to ATG, including SNP nucleotide (positive or negative; see also Table 1).


**Table 5. Observed IL4 Genotypes, Frequencies, and Corresponding Median Overall Survival in Renal Cell Carcinoma Patients**

<table>
<thead>
<tr>
<th><em>IL4</em> Genotypes4</th>
<th>Observed Genotypes*</th>
<th>Median Survival in Renal Cell Carcinoma Patients (months)†</th>
<th>Predicted IL4 Expression in T Cells24-26</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,4</td>
<td>(n = 80) Frequency</td>
<td>3.78 High/low</td>
<td>Low/low</td>
</tr>
<tr>
<td>2,4</td>
<td>1 0.013 ND</td>
<td>High/low</td>
<td>Low/low</td>
</tr>
<tr>
<td>4,4</td>
<td>68 0.850 13.44</td>
<td>High/low</td>
<td>Low/low</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not determined.

*Haplotypes are described in Table 4.
†Figure 1.
which were found in four major linkage blocks. RAD50 was located in the first, IL13 in the second, and IL4 and KIF3A in the third — 58-kbp block, respectively. No gene was positioned in block four. All IL4-specific SNPs analyzed in this study, as well as most of the variations within the complete coding regions of the IL4 gene and the KIF3A gene, showed strong LDs within block three.

**DISCUSSION**

In this study, we demonstrate for the first time the prognostic role of IL4 promoter polymorphisms in patients with metastatic RCC. Median overall survival was 3.5-fold decreased in heterozygote patients carrying haplotypes 1 and 4 compared with patients homozygote for haplotype 4 (3.78 v 13.44 months; P = .027; Fig 1). The heterozygous IL4 genotype 1,4 (intermediate IL4-producer) is thus associated with an approximately three-fold increased risk of an unfavorable clinical course of RCC compared with genotype 4,4 (low IL-4-producer). Given that the rare IL4 genotype 1,1 (high IL-4 producer) was not encountered in our study group, no actual data on the outcome for these patients can be presented. However, it seems plausible that survival of such patients might even be shorter, compared with IL4 genotype 1,4 heterozygotes (intermediate IL-4 producers).

Another finding of this study was the reduced frequency of the IL4 haplotype 1, which is associated with decreased survival, and the concomitantly increased frequency of the IL4 haplotype 4 in metastatic RCC patients compared with other white study populations (Table 4). One possible interpretation of this finding is that rapid disease progression in patients with the IL4 haplotype 1 may prevent patient enrollment into study protocols due to insufficient performance status, and thus results in a relative paucity of IL4 haplotype 1 in RCC study populations. However, the issue is complicated further by the observation that the frequency of IL4 haplotype 1 is increased 5.4-fold in Asians (0.789) compared with whites (0.144). Intriguingly, the substantially increased frequency of the IL4 haplotype 1 in Asians is associated with a lower incidence of kidney cancer. These data, which may appear contradictory at first glance, can be interpreted to suggest that different mechanisms underlie the early development of RCC and the subsequent progression of the disease, and that genetically determined IL-4 may affect these two mechanisms differentially. Thus, a distinct capacity to produce IL-4 may be protective and prevent RCC development, for instance through anti-inflammatory activity, but may be detrimental once the disease is established, for instance by hampering tumor immune surveillance.

For additional exploration of the biologic effects of IL4 polymorphisms, an LD map (data not shown) was created to detect possible nearby functional variants in strong linkage with our investigated haplotypes. Analysis revealed a high LD throughout the genes IL4 and KIF3A, but no linkage to the nearby locus of IL13. Therefore, no linkage of IL4 haplotypes 1 to 4 to potentially functional IL13 variants may be expected. KIF3A is coding for a subunit of kinesin II, a microtubule-based motility protein. Because of its biologic function, a correlation of KIF3A with RCC may not be expected, but should not be excluded either, given that KIF3A expression levels are known to play a role in the metastatic ability of tumor cells; its genetic inactivation in mice inhibits renal ciliogenesis and produces polycystic kidney disease. To date, there are no reports of association studies of KIF3A polymorphisms. In contrast to KIF3A, however, association of specific polymorphisms of IL4 with biologic functions (eg, its transcriptional activity and circulating immunoglobulin E levels) have been shown. Additional detailed genetic investigations inside the LD block harboring IL4 and KIF3A will be needed to reveal and prove the causative polymorphisms correlated with survival in RCC.

IL-4 is a pleiotropic cytokine mainly produced by a subset of CD4⁺ T cells, designated T helper (TH) 2 cells, but also by basophils, mast cells, natural killer T cells, and γδ T cells in response to receptor-mediated activation events. IL-4 plays a central role in regulating the differentiation of antigen-stimulated naive T cells. IL-4 causes such cells to develop into cells capable of producing IL-4 and a series of other cytokines including IL-5, IL-10, and IL-13 (ie, TH2-like cells). Concomitantly, IL-4 powerfully suppresses the appearance of IFN-γ-producing CD4⁺ T cells (TH1 cells). In addition, IL-4 has been proposed as an anti-inflammatory agent with antitumoral activity. Along this line, previous work has demonstrated growth-inhibitory effects of IL-4 on human RCC in vitro. However, clinical administration of IL-4 to patients with RCC or other tumors failed to reproduce the promising in vitro findings in vivo. The discrepancy between the in vitro and in vivo effects of IL-4 may again reflect the different mechanisms operating during the early onset of RCC and during disease progression. Thus, enhanced IL-4 production in patients with IL4 haplotype 1 may inhibit proliferation of RCC in the early phase of the disease, but may cause TH2 deviation during disease progression and thereby prevent effective immune surveillance by TH1 cells.

Reliable prognostic factors in human RCC are Karnofsky performance score and the Eastern Cooperative Oncology Group performance status, which include histopathology of TNM staging and Fuhrman nuclear grading. Less specific and selective prognostic factors are high corrected level of serum calcium, high level of the serum lactate dehydrogenase, and low hemoglobin level. Moreover, increased serum IL-6 or serum vascular endothelial growth factor have been reported to influence negatively the prognosis of metastatic RCC. On the basis of the current study, we recommend IL4 promoter polymorphisms as an additional independent and routine-relevant genetic predictor for survival in metastatic RCC.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicated no potential conflicts of interest.

**AUTHOR CONTRIBUTIONS**

Conception and design: Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Financial support: Martin Thurnher, Christoph Gassner
Administrative support: Christoph Gassner, Reinhold Ramoner
Provision of study materials or patients: Martin Thurnher, Reinhold Ramoner
Collection and assembly of data: Thomas Kleinrath, Christoph Gassner, Martin Thurnher, Reinhold Ramoner
Data analysis and interpretation: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Manuscript writing: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
Final approval of manuscript: Thomas Kleinrath, Christoph Gassner, Peter Lackner, Martin Thurnher, Reinhold Ramoner
REFERENCES


2. Cohen HT, McGovern FJ: Renal cell carci-


Kleinrath et al

JOURNAL OF CLINICAL ONCOLOGY

Information downloaded from www.jco.org and provided by UNIV INNSBRUCK MED FACHBIB on February 27, 2007 from .

Copyright © 2007 by the American Society of Clinical Oncology. All rights reserved.

Acknowledgment
We thank Hubert Gander and Andrea Rahm for their efforts in sample collection. We also thank G. Bartsch, head of the Department of Urology, as well as D. Schönitzer, former head of the Central Institute for Blood Transfusion, for their continuous support.