Increased haematopoietic progenitor cells are associated with poor outcome in patients with metastatic renal cancer treated with sunitinib

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Background: Haematopoietic progenitor cells (HPCs) are present in blood in metastatic renal cell cancer (mRCC). We investigate their expression in mRCC patients treated with sunitinib and correlate their expression with plasma growth factor levels [insulin-like growth factor (IGF)-1].

Methods: Circulating HPCs (CD34+ and CD45+) and plasma IGF-1 levels were measured at specific sequential time points (0, 6, 18 and 28 weeks) in 43 untreated mRCC patients receiving sunitinib (50 mg for 28 days followed by 14-day off treatment). Univariate and multivariate analysis assessed the prognostic significance of HPCs and IGF-1.

Results: HPCs levels were raised in 40 of 43 (93%) of patients. IGF-1 levels were raised in 9 of 43 patients (21%). Univariate and multivariate analysis revealed that high HPCs before treatment were associated with a significantly shorter overall survival (hazard ratio 3.3, 95% confidence interval 1.23–8.8, \( P = 0.01 \)), which was not the case for IGF-1 levels. Both HPC and IGF-1 levels fell with sunitinib (61% and 14% fall, respectively, \( P < 0.05 \) for both). A positive correlation between the falls in HPC and IGF-1 occurred \( (P < 0.001) \).

Conclusions: HPCs are over expressed in the peripheral blood in the majority of patients with mRCC. Higher levels are associated with poor prognosis. A concurrent fall in HPCs and growth factor expression (IGF-1) with sunitinib occurs.

Key words: HPC, IGF-1, renal cancer, sunitinib

introduction

Targeted therapy had revolutionised the outcome for metastatic clear cell renal cancer patients [1–7]. Sunitinib, a multitargeted vascular endothelial growth factor (VEGF) tyrosine kinase inhibitor, is now established as first-line therapy in this disease [1]. Sunitinib’s mode of action is thought to be predominantly through its antiangiogenic effects, although it also targets a number of tyrosine kinases not specifically associated with angiogenesis. The importance of these ‘off target’ effects in the efficacy and toxic of the drug are unclear [8].

There is currently a lack of correlative biomarkers in this setting and it is not possible to identify patients who benefit from sunitinib.

Preliminary data suggest that abnormally high levels of circulating haematopoietic progenitor cell (HPC) (CD34+ and CD45+) are present in metastatic renal cell cancer (mRCC) [9]. The reason for their presence is unknown. We hypothesise that they may be a consequence of renal cancer-associated growth factor/cytokine production, stimulating the bone marrow and subsequent HPC production [10–12].

Insulin-like growth factor (IGF)-1 is one of these renal cancer-associated growth factors that stimulates HPC production from the bone marrow but is not directly targeted by sunitinib [11, 13]. It is therefore an attractive marker to investigate if a relationship between HPC expression and serum growth factor levels exists in patients treated with sunitinib therapy.

Therefore, in this work, sequential HPCs and plasma IGF-1 levels were measured before and during sunitinib therapy as a secondary end point of a prospective clinical trial. We investigated for a correlation between these potential biomarkers and measure their significance on outcome. This clinical trial incorporated an enforced 5-week break in sunitinib as part of the protocol allowing us to investigate if the effects were reversible.

patients and methods

From September 2007 to September 2009, 43 patients with metastatic clear cell renal cancer were enrolled onto an ethically approved clinical trial. Only......
patients with Memorial Sloan Kettering Cancer Centre (MSKCC) intermediate- and poor-risk disease were enrolled [14]. All patients received sunitinib therapy as first-line therapy for metastatic disease. Treatment was given in the standard 6 weekly cycles (50 mg daily for 28 days followed by 14 days off therapy). After the completion of three cycles, sunitinib was stopped and patients were offered a cytoreductive nephrectomy as part of the clinical trial. Once the patients recovered from this surgery, the sunitinib was restarted. The median treatment break was 5 weeks (range 4–8 weeks). The effects of the treatment on HPCs were a secondary end points of the study. Response evaluation was made according to RECIST criteria (v1.1).

All samples were obtained with informed consent and research ethics committee approval as part of the clinical trial. HPCs and IGF-1 were measured from the peripheral blood at specific time points. These included before sunitinib started (at diagnosis), after the first and third cycle of therapy (weeks 6 and 18) and after the treatment break (week 24). Analysis of the HPCs (CD34+ and CD45+ coexpression) was carried out with CD45-fluorescein isothiocyanate and CD34-phycoerythrin antibodies using standard operating procedures. The cells were analysed using an FACS\textsuperscript{C}anto flow cytometer and BD\textsuperscript{ACD} DIVA software. Circulating endothelial cells are CD45 negative therefore not included in this population. The presence of CD34-positive elements are representative of immature haematopoietic cells. Validated levels were used to define normal population. The presence of CD34-positive elements are representative of endothelial cells are CD45 negative therefore not included in this study. 

Univariate and multivariate analysis was carried out to address the prognostic significance of the circulating HPCs, plasma IGF-1, age, gender, MSKCC prognostic score, number of metastatic sites, neutrophil count, platelet count and serum albumin. Results were expressed as hazard ratios. Both progression-free survival (PFS) and overall survival (OS) were calculated using the Kaplan–Meier method. Analysis of the prognostic significance of a >50% fall in HPCs and IGF-1 levels and a fall above or below the median decrease in the parameters after 6 weeks of therapy also occurred. Descriptive statistics were used to compare groups. Pearson’s correlation coefficient was used to examine for a relationship between IGF-1 levels and HPC expressions.

This study was conducted with the appropriate ethical approval.

### Table 1. Patients characteristics

<table>
<thead>
<tr>
<th>Median age at treatment with sunitinib</th>
<th>62 (range 44–78)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSKCC prognosis for clear cell RCC, n (%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>10 (23)</td>
</tr>
<tr>
<td>Poor</td>
<td>33 (77)</td>
</tr>
<tr>
<td>Albumin</td>
<td>38 g/dl (range 20–48)</td>
</tr>
<tr>
<td>Number of metastatic sites</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>3 or more</td>
<td>13</td>
</tr>
<tr>
<td>Gender, n (%)</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>32 (74)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (26)</td>
</tr>
<tr>
<td>Response to sunitinib therapy at 18 weeks, n (%)</td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>8 (19)</td>
</tr>
<tr>
<td>SD</td>
<td>27 (67)</td>
</tr>
<tr>
<td>PD</td>
<td>8 (19)</td>
</tr>
<tr>
<td>TNM stage at diagnosis, n (%)</td>
<td></td>
</tr>
<tr>
<td>T1–2</td>
<td>4 (9)</td>
</tr>
<tr>
<td>T3–4</td>
<td>39 (91)</td>
</tr>
<tr>
<td>N0</td>
<td>13 (27)</td>
</tr>
<tr>
<td>N1/2</td>
<td>31 (72)</td>
</tr>
<tr>
<td>M0</td>
<td>0 (0)</td>
</tr>
<tr>
<td>M1</td>
<td>43 (100)</td>
</tr>
</tbody>
</table>

CR, complete response; PD, progressive disease; PR, partial response; RCC, renal cell cancer; SD, stable disease; TNM, tumour–node–metastasis.

(7%). Eight patients came off treatment before the 18-week time point due to progressive disease.

To date 27 (63%) patients have progressive disease. The median PFS is 7.5 months [95% confidence interval (CI) 5.4–12.8] and the median OS is 12.1 months (95% CI 8.4 to not achieved).

**effect of sunitinib on HPCs**

At baseline, HPCs levels were markedly elevated compared with healthy controls (upper limit of normal 0.4 [15–17]) in 40 of 43 (93%) patients (median 1.28; range 0.13–6.18) (Figure 1). A decline in the HPC count occurred in 37 of 43 (86%) at 6 weeks (P < 0.05). Sunitinib treatment was associated with a significant decline in the HPCs levels (41% fall at 6 weeks and a 61% fall at 18 weeks; P < 0.05). The study protocol had a pre-planned treatment break (during which time a nephrectomy took place). During this period, the HPC count recovered and was not significantly different from the baseline level. Patients with primary resistant disease (n = 8) did not have an increase in HPC levels during disease progression (mean 26% fall in HPC level during this period, P > 0.05).

**effect of sunitinib on IGF-1 levels**

Initial plasma IGF-1 levels varied from 25 to 512 ng/ml (median 146 ng/ml) (Figure 2). Nine patients (21%) had an
initial IGF-1 level above the normal range (200 ng/ml) [15].
After 6 weeks of sunitinib, IGF-1 levels fell in 29 patients and
increased in 7 (no change in 2) (sign-rank test: \( P = 0.003 \)). The
mean fall was 11.4\% (range +52\% to 59\%). A positive
correlation existed between the fall in CD34 count and the fall
in IGF-1 plasma levels on sunitinib (Pearson’s correlation
coefficient \( r = 0.69, P < 0.001 \) (Figure 2)).

Univariate and multivariate analysis for OS
Univariate analysis revealed MSKCC prognostic group
(intermediate versus poor, \( P = 0.002 \)), increased number of
metastatic sites (\( >2, P = 0.002 \)) and raised HPC count at
diagnosis (above versus below median, \( P = 0.02 \)) were
associated with a poor OS (Figures 3 and 4). In univariate
analysis, IGF-1 levels had no influence on survival (\( P = 0.47 \)).

The multivariate model showed that three prognostic
factors were all associated with a poor prognosis. Two or more
metastatic sites were associated with a hazard ratio (HR) of
death of 4.4 (95\% CI 1.59–12.3, \( P = 0.004 \)), while a raised HPC (above median) at diagnosis was resulted in
a HR of 3.3 (95\% CI 1.23–8.8, \( P = 0.01 \)) and MSKCC
poor prognostic group was associated with a HR of 2.79
(95\% CI 1.01–7.65, \( P = 0.04 \)). The prognostic significance
of HPC levels at diagnosis on PFS and OS is shown in
Figure 2a and b.

The prognostic significance of a fall in HPCs at the 6-week
time point was also investigated. As described earlier, 86\%
of patients experienced a fall from the baseline level.
We specifically compared patients with a fall of >50\%
with the remained of the population. Results showed that
a \( >50\% \) fall was not of prognostic significance in terms of
PFS or OS (\( P > 0.05 \) for both; Figure 2). There was also no
correlation between an HPC fall above or below the median
drop and outcome (data not shown). There was no
prognostic significance of a fall in IGF-1 levels with therapy
(log rank \( P > 0.05 \)).

discussion
Under normal physiological conditions, \( <0.05\% \) of
circulating cells are CD34\(^+\) [18]. Studies have shown
increased levels of circulating HPCs in response to systemic
injury such as surgical trauma and cardiac injury [19, 20].
There are, however, very few data available on the
mobilisation of HPCs from the bone marrow into the
peripheral blood in the context of nonhaematological
malignancy.

The work presented here is to our knowledge the first to
show a correlation between increased HPC expression and
outcome in patients with mRCC. The individuals in this study
were all treated uniformly with sunitinib as part of a prospective
clinical trial. Sunitinib is standard first-line therapy for this
disease, making these findings particularly relevant [1].

The presence of increased HPCs in mRCC and their
prognostic significance is intriguing. Sunitinib treatment
resulted in a fall in both IGF-1 and HPC expressions, which
supports our hypothesis that tumour related growth factors
may have a stimulatory effect on haematopoiesis and HPC
migration, which in turn results in overexpression of
circulating HPCs. IGF-1 is an attractive growth factor to use in
this setting as it is linked with mRCC and not a direct targeted
by sunitinib, making it independent of the process, unlike
VEGF [11, 21]. Additionally, IGF-1 stimulates CD34\(^+\) cell
production in the bone marrow, which strengthens our
hypothesis [22].

The identification of HPCs in the peripheral blood as an
adverse prognostic factor in the multivariate model adds a new
independent marker into this arena, which is worthy of
further investigation. It remains unclear whether their
presence is just prognostic or whether they are also
predictive of response to therapy. Theoretically dynamic
changes to HPC levels could be a marker of response and
resistance to sunitinib. Our data show that the fall in HPC seen
with sunitinib does not correlate with outcome, and
individuals with primary resistance to sunitinib did not
experience a rise in HPCs. Together, these findings suggest
that this correlation with treatment-related outcome may not
be present. However, further work is required in this setting.

One of the areas of potential concerns from this work is the
rapid rebound in HPCs during the enforced treatment break
(Figure 1). It remains unclear if treatment breaks with targeted
therapy in mRCC are safe as they have been associated with tumour regrowth in case reports [23, 24]. It is conceivable that the increase in HPCs during this treatment-free period represents this flare; however, the lack of correlation between fluctuation in HPCs and outcome in our data counters this argument.

This study included only MSKCC intermediate- and poor-risk patients and the PFS data are consistent with those figures seen in the larger randomised studies. However, the OS is relatively short, which may reflect the lack of availability of second-line therapy in the UK where this study was carried out (www.NICE.org).

This work has a number of shortcomings. Firstly, the data are limited by the relatively small size of the cohort, which results in less robust statistical power. Additionally, the HPC and IGF-1 measurements were taken after completion of a 6-week cycle of therapy. As each cycle consists of 4 weeks of sunitinib followed by 2-week off drug, it is possible that the effects on cell counts may be more marked after the 4 weeks on drug rather than at the end of the 6-week cycle. Finally, IGF-1 levels can be influenced by other systemic factor such as liver function and the presence of receptors such as VEGF on HPCs may have a direct influence on their expression [25, 26]. These factors may have confounded the results seen.

Nevertheless, this work has shown that raised HPCs in the peripheral blood are a prognostic marker in this setting and the fall with sunitinib correlates with growth factor expression. This link gives some insight into the pathogenesis of the process.

Figure 3. (A) Progression-free survival comparing patients with a high and low CD34 count at the start of therapy. (B) Overall survival comparing patients with a high and low CD34 count at the start of therapy.
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disclosure
Pfizer supplied an educational grant to the chief investigator (TP) for the running of this study.

references


Figure 4. (A) Overall survival comparing those patients who have a fall in CD34 count of > or ≤50% after 6 weeks of sunitinib. (B) Progression-free survival comparing those patients who have a fall in CD34 count of > or ≤50% after 6 weeks of sunitinib.