Efficacy of cetuximab in metastatic castration-resistant prostate cancer might depend on EGFR and PTEN expression: results from a phase II trial (SAKK 08/07)

Richard Cathomas¹, Christian Rothermundt², Dirk Klingbiel³, Lukas Bubendorf⁴, Rolf Jaggi⁵, Daniel C. Betticher⁶, Peter Brauchli³, Denise Cotting³, Cornelia Droege⁷, Ralph Winterhalder⁸, Daniele Siciliano⁹, Dominik R Berthold¹⁰, Miklos Pless¹¹, Ralph Schiess¹², Roger von Moos¹¹ and Silke Gillessen²* on behalf of the Swiss Group for Clinical Cancer Research (SAKK).

¹Kantonsspital, Graubünden, Switzerland, ²Kantonsspital St.Gallen, Switzerland, ³SAKK Coordinating Centre, Bern, Switzerland, ⁴Institute for Pathology, University Hospital Basel, Switzerland, ⁵University Bern, Switzerland, ⁶HFR Fribourg Kantonsspital, Fribourg, Switzerland, ⁷University Hospital Basel, Switzerland, ⁸Kantonsspital Luzern, Switzerland, ⁹Stadtpital Triemli, Zürich, Switzerland, ¹⁰Instituto di oncologia della Svizzera italiana Bellinzona, Switzerland, ¹¹Kantonsspital Winterthur, Switzerland, ¹²ProteoMediX AG, c/o ETH Zürich, Switzerland.

* These two authors contributed equally to the work and share the last authorship.

Corresponding author:

Richard Cathomas, MD
Medical Oncology
Kantonsspital Graubünden
Loëstrasse 170
7000 Chur, Switzerland
Phone: +41 81 256 6111
Fax: +41 81 256 6668
Email: richard.cathomas@ksgr.ch
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Statement of translational relevance:
So far the inhibition of the EGFR pathway was not perceived to be a valid target in the treatment of CRPC despite high expression of the target in advanced CRPC. Previous clinical trials with EGFR tyrosine kinase inhibitors such as gefitinib or erlotinib did not demonstrate any significant activity. In our trial however we report significantly improved efficacy for the monoclonal EGFR antibody cetuximab in patients with overexpression of EGFR and persistent PTEN. The immunohistochemical results were corroborated by molecular methods measuring gene expression and mRNA levels. Our finding is new and consistent with similar results in colorectal carcinoma and non-small cell lung cancer and is one step forward in the direction of creating target-driven subgroups in prostate cancer. This may represent a new approach to the treatment of prostate cancer, a cancer type in which in contrast to many other cancer types so far no predictive markers have been defined, and all patients are still treated uniformly.

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consultant role for Novartis, Sanofi Aventis, Janssen Cilag, Pfizer, Takeda-Millenium.

All other authors report no conflicts of interest.

**Trial Registration:** This study is registered with ClinicalTrials.gov with the identifier NCT00728663.

The protocol for this trial was developed at the 8th FECS/AACR/ASCO Workshop on Methods in Clinical Cancer Research in June 2006 in Flims, Switzerland.

Results partially presented in abstract form at the 46th Annual Meeting of the American Society of Clinical Oncology, June 4 – 8, 2010, Chicago IL and at the 35th Annual Congress of the European Society of Medical Oncology, October 8 – 12, 2010, Milan, Italy.
Abstract

**Purpose:** The epidermal growth factor receptor (EGFR) is overexpressed in the majority of metastatic castration-resistant prostate cancers (mCRPC) and might represent a valid therapeutic target. The combination of docetaxel and cetuximab, the monoclonal antibody against EGFR, has not been tested in prostate cancer patients.

**Experimental design:** Patients with mCRPC progressing during or within 90 days after at least 12 weeks of docetaxel were included in this phase II trial. Treatment consisted of docetaxel (75mg/m² q3w or 35mg/m² d1, 8, 15 q4w) in combination with cetuximab (400mg/m² d1, then 250mg/m² weekly). The primary endpoint was progression-free survival (PFS) at 12 weeks defined as absence of PSA, radiographic or clinical progression. Evaluation of known biomarkers of response and resistance to cetuximab (EGFR, PTEN, amphiregulin, epiregulin) was performed.

**Results:** 38 patients were enrolled at 15 Swiss centers. Median age was 68 years and median PSA 212 ng/ml. PFS at 12 weeks was 34% (95%CI 19-52%), PFS at 24 weeks was 20% and median overall survival (OS) was 13.3 months (95%CI 7.3 - 15.4). Seven patients (20%) had a confirmed ≥ 50% and eleven patients (31%) a confirmed ≥ 30% PSA decline. 47% of enrolled patients experienced grade 3 and 8% grade 4 toxicities. A significantly improved PFS was found in patients with overexpression of EGFR and persistent activity of PTEN.

**Conclusions:** EGFR inhibition with cetuximab might improve the outcome of patients with mCRPC. A potential correlation between EGFR overexpression, persistent expression of PTEN and EGFR inhibition should be investigated further.
Introduction

Docetaxel-based chemotherapy has become the standard treatment for patients with advanced prostate cancer whose disease has progressed on castration-based therapy (i.e. castration-resistant prostate cancer, CRPC) [1]. However, disease progression is inevitable and further treatment is necessary. At the time of the design of our trial no standard second line treatment for CRPC was available.

Activation of the epidermal growth factor receptor (EGFR) in prostate cancer contributes to metastatic progression as well as to disease relapse [2]. Immunohistochemical analysis has demonstrated an increase in expression of EGFR during the natural history of prostate cancer and this correlates with tumor recurrence, high Gleason score and advanced stage disease [3]. The chimeric IgG1 monoclonal antibody cetuximab is directed against the ligand binding site in the extracellular domain of the EGFR. It enhances antitumor effects of chemotherapy by inhibiting cell proliferation, angiogenesis and metastasis and by promoting apoptosis [4]. Cetuximab in combination with chemotherapy has demonstrated significant prolongation of survival in metastatic colorectal cancer and non-small cell lung cancer (NSCLC). The response to cetuximab has been associated with several factors: in colorectal cancer with the lack of KRAS mutation [5], overexpression of the EGFR ligands amphiregulin and epiregulin [5] and persistent PTEN [6], in NSCLC with EGFR overexpression [7].

Cetuximab has demonstrated preclinical activity in prostate cancer models: in DU145 cell line it inhibits growth by inducing G1 arrest [8]; in both androgen-responsive and –independent cell lines it blocks the EGF-induced receptor activation and induces internalization of the receptor [9]. Cetuximab has also demonstrated significant
antitumor activity in animal models of prostate cancer and this effect was enhanced by simultaneous administration of paclitaxel [10].

This trial tested the hypothesis whether adding cetuximab to chemotherapy with docetaxel can reinduce a tumor response and hence prolong progression-free survival (PFS) in patients with metastatic docetaxel-refractory CRPC. Moreover it explored the association of the efficacy of EGFR inhibition by cetuximab and known biomarkers of response/resistance to this monoclonal antibody.

Materials and methods

Experimental design

Patients with mCRPC progressing during or within 90 days after at least 12 weeks of docetaxel were included in this phase II trial. Treatment consisted of docetaxel (75mg/m² q3w or 35mg/m² d1, 8, 15 q4w) in combination with cetuximab (400mg/m² d1, then 250mg/m² weekly). The primary endpoint was progression-free survival (PFS) at 12 weeks defined as absence of PSA, radiographic or clinical progression. Evaluation of known biomarkers of response and resistance to cetuximab (EGFR, PTEN, amphiregulin, epiregulin) was performed.

Eligibility criteria

Eligibility criteria included histologically documented adenocarcinoma of the prostate, metastatic disease, proven disease progression while on androgen deprivation therapy, received at least 12 weeks of docetaxel chemotherapy and experienced progressive disease by PSA progression (confirmed PSA increase ≥25% above nadir) or progression of metastases during docetaxel or within 90 days after stopping docetaxel treatment, WHO performance status 0-2 and adequate hematological
(neutrophils ≥ 1500/mm³, platelets ≥ 100'000/mm³), hepatic (bilirubin ≤ 1.5 x ULN, ALAT ≤ 2.5 x ULN) and renal function (calculated creatinine clearance ≥ 30ml/min). Patients were excluded if they had neuropathy ≥ grade 2, known CNS metastases, radiotherapy within two weeks before inclusion, antiandrogen therapy not discontinued > 6 weeks before inclusion and if they had received prior treatment with drugs interacting with the EGFR pathway.

The trial was approved by the local ethics review boards and Swissmedic (Swiss agency for therapeutic products). It was registered at the National Institute of Health (www.clinicaltrial.gov; identifier number: NCT00728663). All patients gave informed consent.

**Treatment**

Patients were treated with the same docetaxel schedule they had received before registration into the trial. Hence two different docetaxel schedules were administered: the 3qw regimen consisted of docetaxel i.v. at a dose of 75mg/m² administered over one hour on day 1 of each 21-day cycle and the weekly regimen of docetaxel i.v. 35mg/m² on days 1, 8 and 15 every 28 days. Cetuximab was administered prior to docetaxel at an initial dose of 400mg/m² i.v. over 120 minutes on day 1 and then on a weekly basis at a dose of 250mg/m² i.v. over 60 minutes. Oral prednisone at a dose of 10mg/d was administered continuously from day 1. Ongoing castration with LHRH analogues was mandatory for all patients (if not surgically castrated). Treatment was administered for a maximum of 24 weeks, until progression, unacceptable toxicity or withdrawal of consent.

**Assessments and disease evaluations**

Screening assessments including computed tomography (CT) scan of the chest,
abdomen and pelvis and a bone scan were recorded within 28 days of registration.
All patients had repeated radiographic assessments every 12 weeks, PSA was measured every 4 weeks.

PFS was defined as absence of disease progression for biochemical PSA progression, progression of measurable disease, progression of bone lesions or clinical progression. PSA progression was defined as an increase of \( \geq 25\% \) above nadir or baseline. The increase had to be \( \geq 2 \) ng/ml and had to be confirmed \( \geq 3 \) weeks later. PSA response was defined as decrease in PSA of at least 50\% from baseline (PSA 50\%) or of at least 30\% from baseline (PSA 30\%).

Response of measurable disease was assessed by investigators according to the RECIST criteria 1.0. For evaluation of bone metastases in bone scans, the guidelines of Prostate Cancer Working Group 2 (PCWG2) were applied [11].

Adverse events (AE) were graded according to National Cancer Institute’s Common Terminology Criteria of Adverse Events, version 3.0.

**Translational research**

All patients who received at least one dose of trial treatment were eligible for inclusion into the translational research analysis. A separate informed consent was obtained; participation in the translational research part was not required for participation in the trial itself. Existing biopsies from the participating patients were collected at the end of the trial at Institute for pathology of University Hospital Basel.

**Immunohistochemistry**

All biopsies were fixed in 4% buffered formalin and were embedded in paraffin. Fresh sections (4 \( \mu m \)) of the paraffin embedded prostate biopsies were used for immunohistochemical staining with six different antibodies. We used standard indirect
immunoperoxidase procedures on the Ventana BenchMark XT autostainer (Roche Diagnostics). The following primary antibodies were used: Amphiregulin (R&D, goat polyclonal, dilution 1:20, microwave pretreatment for 60 minutes at 98 °C), epiregulin (R&D, goat polyclonal, dilution 1:50, pronase pretreatment for 15 minutes), EGFR (Ventana Medical Systems, clone 3C6, mouse monoclonal, prediluted, protease pretreatment), ERG (Biocare Med, clone 9FY, mouse monoclonal, dilution 1:10, pretreatment with Ventana cell conditioning 1 [CC1]), PTEN (Novocastra, clone 28H6, mouse monoclonal, dilution 1:200, pretreatment with CC1) and SPINK1 (Abcam, mouse monoclonal, dilution 1:800, pretreatment with CC1). One pathologist (L.B.), who was blinded to the clinical follow-up data, viewed all immunostained slides. A case was considered positive of ERG and SPINK1 when any tumor staining was present. The staining intensity of all other markers was visually scored and stratified into four groups: negative (absence of staining), weak (weak but distinct immunoreactivity), moderate (between weak and strong) and strong (apparent even at small magnification; x2.5 objective). A histoscore (H-score) was calculated as described earlier [12, 13] by multiplying the staining intensity (0, 1, 2, or 3) with the percentage of positive cells, which leads to an H-score ranging from 0 to 300. In case of EGFR only membranous staining was considered. Endothelial cells served as internal positive controls for ERG.

Molecular analysis

The RNA was isolated from paraffin cores (0.6 mm diameter) using a kit for archival material (AmpTec GmbH, Hamburg, Germany) as previously described [14]. RNA qualities were assessed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara CA., USA). The isolated RNA was tested for the following six genes: amphiregulin, epiregulin, EGFR, ERG, SPINK1, PTEN. Three control genes were
used for internal reference and normalization (GAPDH, RPL13A, UBC). The genes were measured by qRT-PCR using a one step protocol (Invitrogen, Basel, Switzerland) on a ViiA 7 instrument (Applied Biosystems, Foster City, CA., USA). Raw cycle threshold (C_t) values were normalized against the mean expression of GAPDH, RPL13A and UBC.

**Statistical Design and Analysis**

All patients who received at least one dose of trial treatment with cetuximab and docetaxel were considered evaluable for efficacy. A Simon’s optimal two-stage design was used for this single arm phase II multicenter trial [15]. The primary endpoint was PFS at 12 weeks. The trial therapy would be considered promising if the proportion of patients with a PFS at 12 weeks was 30% or more and uninteresting if it was 10% or less. With 90% power and 5% significance level, the total sample size was calculated to be 35 patients. Stage 1 accrued 18 evaluable patients: if two patients or less experienced PFS at 12 weeks the trial would be stopped early, otherwise another 17 patients were to be registered. At the end, if the total number reaching the primary endpoint was less than or equal to 6, the treatment would be rejected. Herndon’s modification was applied to continue accrual during the interim analysis [16].

Secondary endpoints included PFS at 24 weeks, PFS, overall survival (OS), PSA response with ≥ 50% decline and ≥ 30% decline, assessment of measurable disease according to RECIST, assessment of bone lesions and toxicity. AEIs were summarized by event type and grade over the total number of patients (worst recorded AE grade per patient).
Time-to-event endpoints were analyzed by Kaplan-Meier methods, groups were compared using the log-rank test. Numerical values were compared between groups using the Wilcoxon rank-sum test. For categorical data, Fisher's exact test was applied. For the biomarker data, patients with missing values were omitted from the analyses and cut-off values were determined using recursive partitioning and regression trees and confirmed by the maximally selected log-rank statistics method. P-values are two-sided, not adjusted for multiple testing, and considered significant if < 0.05. The data were analyzed in SAS (Statistical Analysis Systems Institute Inc, version 9.2) and the free statistical software package R version 2.13.1 or later [17].

Results

Patient characteristics

Between July 2008 and September 2009, 38 patients were enrolled. Three patients did not receive a complete dose of any trial treatment (2 patients experienced grade 3/4 hypersensitivity reaction within the first minutes of the first dose of cetuximab and one patient had to undergo surgery for spinal cord compression before start of trial medication) and were therefore not evaluable for efficacy as per protocol. Baseline characteristics of the evaluable patients are presented in Table 1. The median follow-up time was 25.4 months.

Treatment administration

A total of 157 cycles of trial treatment were given to 35 patients. Median number of cycles was 4 (range 1 – 8) and median treatment duration was 82 days (range 20 – 180). 26 patients (74%) received the 3qw and nine (26%) the weekly docetaxel schedule. 88% of docetaxel cycles and 82% of cetuximab cycles were given without
dose reduction, omission or delay.

**Activity of cetuximab in combination with docetaxel**

Confirmed PFS at 12 weeks according to definition was achieved in 12 of the 35 patients (34%, 95% CI 19 – 52%). Therefore the null hypothesis (PFS at 12 weeks < 10%) could be rejected. PFS at 24 weeks was achieved in seven patients (20%, 95% CI 8 – 37%). The median PFS was 2.8 months (95% CI 2.4 – 3.2 months). The median overall survival was 13.3 months (95% CI 7.3 – 15.4 months). The Kaplan-Meier curves for PFS and OS are shown in Figure 1a.

A confirmed ≥ 50% PSA decline was observed in 7 patients (20%) and a confirmed ≥ 30% PSA decline in 11 patients (31%). Full PSA response data (confirmed and unconfirmed) are summarized in the waterfall plots in Figure 2. For one patient there was only the PSA baseline measurement available, hence no change in PSA could be calculated.

In patients with measurable disease at baseline (N=24), one patient demonstrated a partial remission (4%), 13 patients had stable disease (54%), 6 patients were progressive (25%) and in 4 cases no reassessment was done (17%). Five patients (14%) had progressive bone metastases at the 12 week bone scan.

An unplanned subgroup analysis was performed looking at the efficacy of the combination of docetaxel and cetuximab in patients who developed any form of skin toxicity at any grade (N=29) compared to patients who did not (N=6). Figure 1b shows a statistically significant longer median PFS 2.8 (95% CI 2.5 – 4.4) vs. 1.8 (95% CI 0.9 – 2.8) months, (p=0.0002) and median OS 14.4 (95% CI 8.2 – 17.4) vs. 3.7 (95% CI 1.5 – 12.0) months, (p=0.0001) for patients developing any skin toxicity.

**Toxicity**
Toxicity observations are based on all enrolled patients that started trial treatment (N=37). 18 patients (47%) experienced a total of 23 serious adverse events (SAEs) during the trial. Eleven of the 23 SAEs were attributed to the trial treatment. Twenty of the 37 patients (53%) had treatment related grade 3 and three patients (8%) grade 4 toxicities. Table 2 lists all observed grade 3, grade 4 and grade 5 toxicities. Two patients died during trial treatment due to infections. One of the cases was associated with neutropenic sepsis.

**Translational research**

For an overview of the available samples and successfully performed tests with the respective cut-off values please refer to the flow diagram presented in Figure 3 (REMARK diagram) [18]. Confirmation of the cut-off values with the maximally selected log-rank statistics method found exactly the same cut-off in 6 out of 8 markers and in two cases (PCR for PTEN and EGFR) the cut-off was selected as neighbouring value.

**Immunohistochemical and molecular analysis**

In an exploratory analysis the expression of the biomarkers EGFR, PTEN, amphiregulin, epiregulin and SPINK1+/ERG- were correlated with the clinical efficacy parameters of PFS, OS and PSA response. Overexpression of EGFR or preserved/elevated expression of PTEN correlated significantly with PFS in molecular (for EGFR p=0.005; for PTEN p=0.0003) and immunohistochemical analysis (for EGFR p=0.01; for PTEN p=0.02). For patients with higher levels of both EGFR and PTEN (n=16 in IHC, n=13 in PCR) the PFS is even more significantly improved (Figure 4). For OS a statistically significant difference is only observed for preserved RNA expression of PTEN (p=0.02). Increased expression of EGFR and PTEN
appears positively associated with improved PSA response as demonstrated in the waterfall plots in Figure 2. Overexpression of amphiregulin showed a superior benefit for PFS and OS in the PCR measurements but not by IHC. Immunohistochemical or molecular expression of epiregulin or SPINK1+/ERG− did not correlate with efficacy parameters (data not shown).

Discussion

To the best of our knowledge this is the first trial testing the combination of the standard chemotherapy drug docetaxel with the monoclonal antibody cetuximab in patients with advanced prostate cancer. The trial was performed in a population of pretreated docetaxel-resistant mCRPC patients (defined as progression during docetaxel or within 90 days after docetaxel) at a time when no standard treatment options for these patients existed. Our results have to be distinguished from studies who looked at docetaxel-retreatment in patients who experienced response to prior docetaxel and would be considered docetaxel-sensitive [19]. Two papers have been published comparing different second line chemotherapy regimens in patients resistant to docetaxel [20, 21]: The treatments included monotherapies with mitoxantrone, ixabepilone or satraplatin or combination treatments including carboplatin/docetaxel or carboplatin/etoposide. In these trials a PSA response rate ≥ 50% of 17 – 23%, and median PFS between 2.1 – 3 months was reported, very similar to our trial. We also report a better OS for patients who develop any form or grade of skin toxicity. Of note however is the small number of only six patients without skin toxicity and these patients experienced an extremely short progression free and overall survival so that these results have to be interpreted with caution.
While there is no substantial advantage of using cetuximab in an unselected group of mCRPC patients, there might be subgroups that derive more benefit. The most interesting information in this trial comes from the immunohistochemical and molecular analyses with regard to known factors of response/resistance to EGFR inhibition. Our results suggest that patients whose tumors have EGFR overexpression and PTEN persistence had a better outcome when treated with the monoclonal antibody against EGFR, cetuximab. So far despite some promising preclinical results, EGFR inhibition in mCRPC has mainly resulted in negative experiences. Single agent trials with the EGFR tyrosine kinase inhibitors (TKI) gefitinib [22] and erlotinib [23, 24] or the monoclonal antibody panitumumab [25] did not achieve any significant PSA response. Two other trials with cetuximab in combination with chemotherapy for patients with advanced prostate cancer have been presented earlier [26, 27]: the combination of cetuximab with doxorubicin was associated with minimal PSA declines [26] and the combination of cetuximab with mitoxantrone did not show a benefit when compared to mitoxantrone alone [27]. When assessing the entire patient population, our trial showed similar results: although the null hypothesis could be rejected, neither the PFS nor the PSA response rates indicate clinical usefulness especially in the presence of considerable toxicity of the study treatment. However, it is well known that EGFR inhibition in an unselected patient population is not a useful approach: lack of KRAS mutation, overexpression of the ligands amphiregulin and epiregulin, persistence of PTEN and overexpression of EGFR have been shown to be predictive markers for response to cetuximab in colorectal or NSCLC, respectively [5 - 7].

While KRAS mutations are rare in prostate cancer [28], EGFR overexpression and the importance of PTEN is well documented [3, 29 - 31]. PTEN (phosphatase and tensin homologue deleted from chromosome 10) is a tumor suppressor gene that...
negatively modulates the phosphatidylinositol 3-kinase (PI3K)/Akt signaling transduction pathway that promotes tumor growth, proliferation and survival. Loss of PTEN is correlated in prostate cancer with development of castration-resistance, chemoresistance and poor prognosis [29 - 31]. Complete loss is found in 15 - 20% of localized disease and 30% of metastatic disease [32]. The influence of PTEN expression on cell response was tested in PC3 prostate cancer cells that are KRAS wild-type, PTEN-null and EGFR overexpressing: it was shown that reintroduction of PTEN could significantly reduce the constitutive overexpression of p-AKT downstream kinases (p-GSK3β and p-P70S6) and p-ERK 1/2 which led to significantly restored cetuximab-induced cell growth inhibition and induction of apoptosis [33]. Therefore patients with maintained PTEN expression might respond better to cetuximab based treatment, and our results support this preclinical hypothesis. Apart from PTEN persistence we demonstrate that the benefit from EGFR-targeted treatment is increased in presence of combination with higher EGFR levels. The question remains open, whether EGFR overexpression by itself would be a valid therapeutic target as might be suggested. In view of the discussed pathways and the importance of PTEN this appears unlikely but our sample size is too small to draw further conclusions. Recently a preclinical study reported attenuated tumor growth with cetuximab in a SPINK1+/ERG- prostate cancer mouse model [34], raising speculation about a possible new target for this subset of patients. Our results could not confirm this finding with no difference in PFS for patients with SPINK1+/ERG- prostate cancer according to immunhistochemical analysis.

Interestingly we could show the same correlations for PTEN and EGFR in the immunhistochemical as in the molecular analysis. This suggests that the methods used to extract RNA from prostate cancer tumor tissue as described and established...
earlier for breast cancer tissue by the group of Jaggi et al. [14] are also valid for prostate cancer. Provided that the tumor tissue is adequately sampled by an experienced pathologist the RNA isolated from the formalin-fixed, paraffin-embedded prostate cancer tissue is suitable for molecular profiling.

Since the completion of this trial in 2009 several different new treatment options have demonstrated a significant survival benefit for patients with mCRPC: they include the CYP-17 inhibitor abiraterone [35], the novel antiandrogen enzalutamide [36], the taxane cabazitaxel [37], the cellular immunotherapy with sipleucel-T [38] as well as the radionuclide radium-223 [39]. Clearly the new therapies targeting the androgen-receptor (AR) pathway will become widespread but eventually the disease progresses in the majority of patients and some patients are primarily refractory to these treatments. In these subgroups of patients inhibition of EGFR in combination with a cytotoxic drug might play an important role.

The major limitations of our analyses include the setting of a single arm phase II trial and the small sample size for translational research. No definitive conclusion can be drawn from these results regarding the predictive value of assessing PTEN function and EGFR overexpression for response to cetuximab in patients with mCRPC. PTEN is also known to be a prognostic factor for better outcome [30], and our results might simply be due to generally better prognosis or maintained chemosensitivity. With regards to EGFR overexpression however, this represents a negative prognostic factor at least in early stage disease: a recent study showed a significantly increased risk of biochemical relapse in patients with high expression of EGFR [40]. Another limitation is, that the translational research was performed on prostate biopsies obtained at the time of diagnosis. As outlined above, it is well known that the expression of EGFR can change with progression of disease [3] and occurrence of
complete PTEN loss increases [32]. Another point is the definition of docetaxel-resistant patients that is not agreed upon: by choosing a rather long 90-days window we may have introduced heterogeneity by including patients who would have responded to docetaxel-retreatment alone [19]. On the other hand we included PSA progression only in the primary endpoint and this could possibly have led to an underestimation of the benefit of the trial treatment.

In summary the results of this trial for the whole patient population are comparable to those of other second line phase II results with conventional chemotherapy in the treatment of docetaxel-refractory mCRPC. However, in the subgroup of patients with both EGFR overexpression and preserved PTEN a signal of efficacy could be observed from the addition of cetuximab. Our observation correlates well with recent preclinical research and with results from studies in patients with colorectal cancer or NSCLC and should therefore be investigated prospectively in order to clarify the clinical usefulness of the combination for the subgroup of patients with mCRPC selected by EGFR and PTEN status.
References


Table 1: Evaluable patient characteristics at baseline

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median Age: years (range)</td>
<td>68 (45 - 82)</td>
</tr>
<tr>
<td>Median PSA: ng/ml (range)</td>
<td>212 (4.4 – 8898)</td>
</tr>
<tr>
<td>WHO Performance status, N (%)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>9 (26)</td>
</tr>
<tr>
<td>1</td>
<td>21 (60)</td>
</tr>
<tr>
<td>2</td>
<td>5 (14)</td>
</tr>
<tr>
<td>Site of metastasis, N (%)</td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>31 (89)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>22 (63)</td>
</tr>
<tr>
<td>Visceral</td>
<td>12 (34)</td>
</tr>
<tr>
<td>Number of prior docetaxel regimens, N (%)</td>
<td></td>
</tr>
<tr>
<td>1 prior regimen</td>
<td>23 (65)</td>
</tr>
<tr>
<td>2 prior regimens</td>
<td>9 (26)</td>
</tr>
<tr>
<td>3 prior regimens</td>
<td>3 (9)</td>
</tr>
<tr>
<td>Best response to last docetaxel therapy, N (%)</td>
<td></td>
</tr>
<tr>
<td>Stable disease</td>
<td>18 (51)</td>
</tr>
<tr>
<td>Progression</td>
<td>9 (26)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8 (23)</td>
</tr>
</tbody>
</table>
### Table 2: Drug-related toxicities (worst grade observed per patient, N = 37)

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allergic reaction to cetuximab</td>
<td>1 (3)</td>
<td>2 (5)</td>
<td>-</td>
</tr>
<tr>
<td>Anemia</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bowel perforation and fistula formation</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac arrhythmia</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>2 (5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4 (11)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Leucopenia</td>
<td>2 (5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>3 (8)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Neutropenic fever</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pain</td>
<td>1 (3)</td>
<td>1 (3)</td>
<td>-</td>
</tr>
<tr>
<td>Pancytopenia</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>2 (5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pulmonary infection without leucopenia</td>
<td>-</td>
<td>-</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Rectal bleeding</td>
<td>1 (3)</td>
<td>-</td>
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<tr>
<td>Septicemia without leucopenia</td>
<td>-</td>
<td>-</td>
<td>1 (3)</td>
</tr>
<tr>
<td><strong>Skin toxicity (total)</strong></td>
<td>10 (27)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Acne</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Erysipelas</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Dermatitis exfoliativa</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Folliculitis</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Hand Foot syndrome</td>
<td>2 (5)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Periorbital dermatitis</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Pruritus</td>
<td>1 (3)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- Rash</td>
<td>2 (5)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. Overall survival and progression-free survival for all evaluable patients.

Figure 2. Waterfall plots demonstrating best response in PSA from baseline and association with EGFR overexpression and PTEN persistence according to immunohistochemical (A) or molecular (B) analysis, respectively.

Figure 3. REMARK diagram. Overview of available samples for translational research and performed immunohistochemical and molecular analyses including cut-off values.

Figure 4. Progression-free survival according to overexpression of EGFR and PTEN persistence according to immunohistochemical (A) and molecular (B) analysis, respectively.
Figure 1A.
Figure 1B.
Figure 2A.

EGFR & PTEN (IHC)

change in PSA from baseline (%)
Figure 3.

patients enrolled (n = 38)

not evaluable:
- hypersensitivity G3/4 (n=2)
- surgery before start of treatment (n=1)

evaluable patients (n = 35)

IHC (n = 29)

PTEN (n = 27)
> 0 (0–200)

EGFR (n = 29)
≥ 70 (0–300)

Amphiregulin (n = 28)
≥ 110 (10–300)

Epiregulin (n = 28)
≥ 65 (0–200)

ERG (n = 25)
pos vs. neg

SPINK1 (n = 29)
pos vs. neg

PCR (n = 28)

PTEN (n = 28)
≥ 0.455 (0.05–2.6)

EGFR (n = 28)
≥ 1.089 (0.51–3.74)

Amphiregulin (n = 26)
≥ 0.030 (0.01–4.74)

Epiregulin (n = 4)
n.a. vs rest

ERG (n = 28)
≥ 0.180 (0.03–3.67)

SPINK1 (n = 9)
n.a. vs rest
Figure 4A.

PFS (EGFR & PTEN, IHC)

- one high
- both high
- both low

Estimated survival probability

Time (months)

# at risk
- one high: 6, 5, 2, 1, 0, 0, 0, 0
- both high: 16, 15, 7, 5, 2, 1, 1
- both low: 5, 3, 0, 0, 0, 0, 0

p = 0.00874
Figure 4B.

PFS (EGFR & PTEN, PCR)

- **one high**
- **both high**
- **both low**

Estimated survival probability vs. time

# at risk
- one high: 8, 8, 3, 2, 0, 0, 0, 0
- both high: 13, 12, 7, 5, 3, 1, 1
- both low: 7, 4, 0, 0, 0, 0

p = 3.11e-05
Efficacy of cetuximab in metastatic castration-resistant prostate cancer might depend on EGFR and PTEN expression: results from a phase II trial (SAKK 08/07)

Richard Cathomas, Christian Rothermundt, Dirk Klingbiel, et al.

Clin Cancer Res  Published OnlineFirst September 12, 2012.