Gefitinib combined with endocrine manipulation in patients with hormone-refractory prostate cancer: quality of life and surrogate markers of activity

Giuseppe Curigliano[a,*], Filippo De Braud[a,*], Maria Teresa Sandri[b], Giuseppe Renne[b], Laura Zorzino[b], Epifanio Scardino[c], Bernardo Rocco[c], Gianluca Spitaleri[a], Tommaso De Pas[a], Cristina Noberasco[a], Franco Nolè[a], Fabrizio Verweij[c], Victor Matei[c] and Ottavio De Cobelli[c]

We investigated efficacy of gefitinib in hormone-refractory prostate cancer. Between March 2003 and December 2004, 23 patients with hormone-refractory prostate cancer were assigned to receive 250 mg oral gefitinib daily in addition to antiandrogen and luteinizing hormone-releasing hormone analogue for at least 2 months or until disease progression. Patients with progression stopped antiandrogen therapy, and received gefitinib and the luteinizing hormone-releasing hormone analogue. Serum HER2 and epidermal growth factor receptor extracellular domain were evaluated every 2 months. Gefitinib treatment did not result in any objective measurable response or responses in prostate-specific antigen. Median time to progression was 70 days (33–336). Median overall survival was 293 days (25–75 percentile: 235–349). HER2 extracellular domain mean value was 9.6 ng/ml (range 6.9–13.3) at basal time and was 10.1 (range 6.0–14.1) after 2 months. Epidermal growth factor receptor mean basal value was 51.0 ng/ml (range 41.4–75.3). After 2 months of treatment the mean value was 51.1 ng/ml (range 41.5–61.4). One patient had reduction in the pain score from baseline without an increase in the analgesic score. Four patients (17%) out of 23 had pain progression with an increase from baseline of at least 25% in the analgesic score. The study was discontinued before target accrual was reached owing to lack of efficacy of the drug. Our results do not support the efficacy of gefitinib in combination with endocrine treatment for hormone-refractory prostate cancer. *Anti-Cancer Drugs* 18:949–954

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[a]Division of Medical Oncology, Clinical Pharmacology and New Drugs Development Unit, [b]Division of Pathology and Laboratory Medicine and [c]Division of Urology, European Institute of Oncology, Milan, Italy

Correspondence to Giuseppe Curigliano, Division of Medical Oncology, Clinical Pharmacology and New Drugs Development Unit, European Institute of Oncology, Via Ripamonti 435, 20141 Milano, Italy
Tel: +39 02 57489482; fax: +39 02 57489581;
e-mail: Giuseppe.curigliano@ieo.it

*Giuseppe Curigliano and Filippo de Braud contributed equally to this work.

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Introduction

Prostate cancer is the most common male cancer and the second leading cause of cancer death in men, accounting for an estimated 33 000 deaths in western countries annually [1]. Androgen ablation with luteinizing hormone-releasing hormone (LH-RH) analogue in combination with flutamide or bicalutamide is standard first-line therapy for metastatic patients. Subsequent nonsteroidal antiandrogen therapies in combination with LH-RH analogue could be effective against prostate cancer even after relapse to first-line hormonal therapy. Second-line responders are androgen-independent but potentially still hormonally sensitive [2]. Patients who have progressed on complete androgen blockade may have tumour regression with cessation of the peripheral antiandrogen with withdrawal responses observed in approximately 20% [3]. Although chemotherapy has been shown to improve quality of life (QoL) and pain control, the real impact of such approach in patient care is still debatable [4]. Results of the phase 3 study TAX 327, comparing docetaxel and daily prednisone with mitoxantrone and prednisone, demonstrated that docetaxel led to superior survival and improved rates of response in terms of pain, serum prostate-specific antigen (PSA) level, and QoL, as compared with mitoxantrone and prednisone [5]. In-vitro proliferation of prostate epithelial cells cannot be induced by androgens alone but requires costimulation by a number of growth factors, including epidermal growth factor (EGF) [6,7]. Abnormal EGF receptor (EGFR) expression has been demonstrated in many malignancies including prostate cancer [6–10]. It has been shown that the EGFR family play an important role in the development of prostate cancer and, more specifically, in the progression to hormone-refractory clinical behaviour [11].

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Gefitinib coadministered with the antiandrogen bicalutamide results in concurrent dual inhibition of androgen receptor and EGFR/HER2 pathways. This causes a significant delay in the onset of EGFR-driven androgen independence [12]. In preclinical models and early clinical studies, inhibition of EGFR has resulted in antitumour activity [13,14]. Growth inhibition and tumour regression has been seen in human xenograft models in lung, prostate, breast and colorectal cancers [15–17]. Clinical activity of gefitinib was observed in patients with nonsmall-cell lung cancer (NSCLC), and head and neck cancer [18–24]. In cell lines expressing EGFR, gefitinib inhibits autophosphorylation of this receptor, resulting in the inhibition of downstream signaling molecules by formation of inactive unphosphorylated EGFR/HER2 and EGFR/HER3 heterodimers. The extracellular binding domains of EGFR and HER2 are proteolytically released from cell surface, and are detectable in conditioned media of carcinoma cell cultures and in serum of patients with cancers overexpressing these proteins. Serum EGFR levels seemed to predict response in women treated with trastuzumab, even if gefitinib did not affect both serum markers in patients with breast and NSCLC [25–27]. Considering the biological significance of an interaction between EGFR and Her-2/*neu* signalling in other human malignancies, we have investigated if serum soluble EGFR and HER2 extracellular domain (ECD) levels would predict clinical outcome in patients with hormone-refractory prostate cancer (HRPC) treated with gefitinib. Given the high expression of EGFR in HRPC and the need for new approaches in this disease, a phase II trial of single doses of oral gefitinib in patients who had progressed on complete androgen blockade was initiated at the European Institute of Oncology. Primary endpoint was to assess the activity of gefitinib by estimating the disease control rate [complete response, partial response and stable disease (SD)], on the basis of measurable and evaluable disease and/or PSA levels by 4 months after the start of treatment. We also evaluated the effects of gefitinib therapy on serum EGFR and HER2 ECD as surrogate markers of activity.

Methods

Patient eligibility

Patients with histological or cytological evidence of prostate adenocarcinoma, increasing PSA ≥ 4 ng/ml or increasing measurable disease while receiving LH-RH analogue (or orchiectomy) and androgen-ablative therapy were eligible. Increasing PSA was defined as $\geq 25\%$ increase in reference value of PSA (absolute value of increase, ≥ 5 ng/ml) a minimum of 1 week from the reference value and confirmed by a second increase in PSA at least 1 week later. To be considered measurable, a lesion must have measured at least 20 mm in one dimension with conventional techniques or at least 10 mm with spiral computed tomography according to Response Evaluation Criteria in Solid Tumors (RECIST criteria) [28]. The Institutional Review Board of the European Institute of Oncology approved the study protocol, and written informed consent was obtained before study entry. Other eligibility criteria included World Health Organisation performance status of 0–2, no prior chemotherapy (including extramustine) and no prior investigational agents. Patients must have been willing to complete monthly QoL assessments using the European Organisation for Research on the Treatment of Cancer (EORTC) QLQ-C30 questionnaire, QoL assessed on a three-point scale (good ± fair ± poor), pain score and analgesic consumption assessed daily by visual analogical scale as recorded in a patient diary and monthly pain intensity assessed using the McGill–Melzack questionnaire [29]. Pain was assessed by means of the present pain intensity (PPI) scale from the McGill–Melzack questionnaire. Patients recorded their daily PPI score and analgesic use in a diary. Patients were required to have stable levels of pain for at least 7 days before randomization, defined by a daily variation of no more than 1 in the PPI score or of no more than 25% in the analgesic score. Requirements for organ function were absolute granulocytes $\geq 1.0 \times 10^9$/l, platelets $\geq 100 \times 10^9$/l, serum creatinine > 2.0 mg/dl and bilirubin $\leq 1.5 \times$ upper normal limits, and aspartate aminotransferase and alanine aminotransferase (ALT) $\leq 2.5 \times$ upper normal limits ($\leq 5 \times$ upper normal limit if liver metastases were present.

Immunohistochemistry and serum detection of epidermal growth factor receptor and HER2

Immunohistochemistry

Patients with accessible tumour lesions or who did not undergo to radical prostatectomy underwent serial biopsy to determine EGFR and HER2 expression on tumours. All immunohistochemical analyses were performed on routinely processed, formalin-fixed, paraffin-embedded tissues using an avidin–biotin complex immunoperoxidase technique. Primary specific monoclonal antibodies to the following antigens were used at the specified dilutions: c-erbB-2 (Herceptest clone; DAKO, Cytomation, Carpinteria, California, USA, 1 : 600) and EGFR (clone 31G7; Zymed, Zymed Lab, California, USA, 1 : 20).

Detection of serum epidermal growth factor receptor and HER2

We collected serum samples from all patients for EGFR and cerbB2 ECD dosing every 2 months. Serum aliquots were assayed for the extracellular binding domain level of EGFR by a sandwich quantitative enzyme-linked immunosorbent assay (ELISA) using mouse monoclonal capture antibody against EGFR precoated onto a microtiter plate and an alkaline phosphatase-labeled mouse monoclonal antibody as detector specific for the electron capture detection of human EGFR, according to the

manufacturer's instructions (Oncogene Science, Bayer Corporation, Cambridge, UK). A reference range for HER1 was determined on 30 healthy subjects sera (45.7–71.3 ng/ml). Serum concentrations of HER2 ECD were determined using an ELISA kit (Oncogene Science) on the basis of the same test principle as the EGFR assay. The kit uses a mouse monoclonal antibody recognizing the ECD of HER2 protein.

Study design and treatment

This was a single-centre, open-label, noncomparative, single-arm, phase II trial in patients aged 18 years or older with histologically confirmed metastatic HRPC who have progressed on treatment with an LHRH analogue and antiandrogen (bicalutamide or flutamide). Initially, patients added gefitinib 250 mg to bicalutamide 50 mg (or flutamide 250 mg $\times$ 3 daily) orally once daily and goserelin 3.6 mg implant administered by subcutaneous injection every 28 days, for 2 months or until disease progression, unacceptable toxicity or withdrawal of consent. Patients who have not clinically progressed after 2 months of treatment continued treatment with gefitinib, antiandrogen and the LH-RH analogue until disease progression, unacceptable toxicity or withdrawal of consent. Patients with disease progression stopped antiandrogen therapy, and continued receiving gefitinib and the LH-RH analogue. Thirty-four patients were planned to be enrolled into the trial. Previous reports did not prove a better outcome when gefitinib was administered at higher doses in HRPC [35], thus we decided to treat patients at 250 mg/day for the better safety profile.

Statistical considerations

The aim of this trial was to evaluate the overall activity of the combination of gefitinib, antiandrogen (bicalutamide or flutamide) and an LHRH analogue in patients with metastatic HRPC who had evidence of disease progression following treatment with antiandrogen (or surgical castration) and an LHRH analogue [30,31]. The main endpoint of the trial was the percentage of patients with controlled disease (complete response, partial response or SD) at trial closure. In this setting, at least 30% of patients with controlled disease was considered a good result. An unacceptable result was defined as less than 10% of patients with controlled disease. A classic single-stage phase II design powered to choose between an acceptable disease control rate and an unacceptable one has been used. A sample size of 34 patients was needed based on acceptable and unacceptable disease control rates of 30 and 10%, respectively, at the 5% significance level and with 90% power. Seven or more patients with controlled disease in the 34 patients would indicate an active regimen. All patients that were enrolled and received trial treatment have been considered the intention-to-treat population. The analysis population for all efficacy endpoints have been the intention-to-treat population. The standard summary statistics for discrete variables were: count and proportion. The disease control rate have been summarized by proportions together with a 95% confidence interval (CI) and a 90% CI. Durations [of progression-free survival (PFS) and overall survival] have been summarized by Kaplan–Meier methods. QoL data have been reported by constructing overall and subscale scores as defined by the questionnaire manual, and these scores have been summarised by the appropriate summary statistics.

Results

Characteristics of the patients and treatment

From April 2003 to September 2004, a total of 23 patients with HRPC were enrolled. All patients were metastatic at study entry. Baseline demographic and clinical characteristics are listed in Table 1. Median age at study entry was 66 years (range 56–77). Three patients had measurable lesions according to RECIST criteria (15.7%). Median basal PSA at entry was 35.9 ng/ml (range 8.2–463.0). Eight out of 23 patients underwent no prior surgery for prostate cancer. Actual antiandrogen therapy on study entry was flutamide for 13 (57%) and bicalutamide for 10 (43%) patients. Eleven patients had Gleason grade 8–10 at diagnosis (47.8%). Karnofsky performance status was 100 for 16 (84.2%) patients and 70–90 for three (15.7%) patients. Median duration of treatment with gefitinib was 98 days (range 5–369).

Activity

Eleven patients (47.8%) out of 23 completed 4 months of treatment. Twelve patients (52%) did not complete planned treatment: two for protocol noncompliance, three for serious adverse events and seven for rapidly progressive disease. A PSA level drop $< 50\%$ compared with the baseline was observed in two patients after 2 months of treatment. A PSA level drop $> 50\%$ compared

Table 1 Clinico-pathological features of 23 patients included in the study

Clinico-pathological features	*N* (%)
Age (range years)	66 (56–77)
KPS 100/70–90	16 (84.2)/3 (15.7)
Measurable lesions[a]	3 (15.7)
No previous surgery	8 (20)
Stage	
Tx/T1/T2	3/1/4
T3/T4	14/1
Stage: Nx/N0/N1/N3	1/12/9/1
Gleason grade	
2–4	1 (4)
5	1 (4)
6	3 (13)
7	6 (26)
8–10	11 (48)
Unknown	1 (4)
Actual androgen therapy	
Flutamide	11 (57%)
Bicalutamide	8 (43%)

[a]According to Response Evaluation Criteria in Solid Tumors (RECIST) criteria. KPS, Karnosky performance status.

with day 57 (after antiandrogen withdrawal) was observed in one patient and < 50% in three patients after 4 months of treatment. Median time to progression was 70 days (33–336).

None of the three evaluable patients met criteria for objective responses according to RECIST criteria. One patient completed 369 days of treatment with a clinical SD and rising PSA at withdrawal; all other patients progressed. Four patients died owing to progression of disease (Table 2). Three patients had a basal PPI score of at least 2 and an analgesic score of at least 10 at baseline (13%). They have been assessed for the pain response at 4-week intervals. According to our rules one patient had a two-point reduction in the PPI score from baseline without an increase in the analgesic score. Four patients (17%) out of 23 had pain progression defined as an increase in the PPI score of at least one point from the nadir, an increase from baseline of at least 25% in the analgesic score or a requirement for palliative radiotherapy.

Safety

Grade 3–4 treatment related toxicity included increased ALT (10.5%), anaemia (5%) and fatigue (5%). One patient had ventricular tachicardia after 1 week of treatment (he was a cardiac pacemaker carrier). Two other patients were withdrawn from protocol owing to serious adverse events: increased ALT and skin necrosis owing to ulceration (Table 3).

Table 2 Clinical and biochemical responses

	Evaluable patients	After 2 months of treatment	After 4 months of treatment
Biochemical response	23		
Drop PSA >50%		0	1
Drop PSA <50%		2	3
Objective responses according to RECIST		0	0
Stable disease according to RECIST		1	1
Progression disease according to RECIST		2	–

PSA, prostate-specific antigen; RECIST, Response Evaluation Criteria in Solid Tumors.

Table 3 Serious adverse events of grade 3–4 registered during the study treatment

Toxicity	NCI-CTC score grading	*N* events
Ventricular tachicardia	4	1
ALT increase	3	1
AST increase	3	1
Skin necrosis with ulceration	4	1
Skin rash	3	1
Anaemia	3	1
Fatigue	3	1

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NCI-CTC, National Cancer Institute Common Toxicity Criteria.

Serum detection of epidermal growth factor receptor and HER2 extracellular domain

Six out of eight patients with no prior surgery underwent prostate biopsy. EGFR was immunohistochemically overexpressed in two patients, cerbB2 was negative in all tissue samples. Serum HER2 ECD was assessed in all patients. Mean basal value was 9.6 ng/ml (range 6.9–13.3). After 2 months mean value was 10.1 (range 6.0–14.1). Serum EGFR was assessed in all patients. Mean basal value was 51.0 ng/ml (range 41.4–75.3). Mean value after 2 months of treatment was 51.1 ng/ml (range 41.5–61.4). No statistical correlation has been described between drug activity and serum HER2 ECD or serum EGFR.

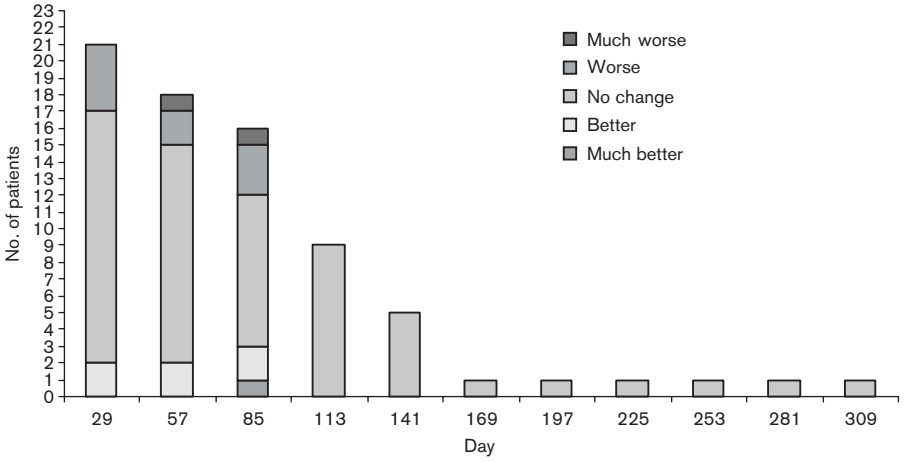
Quality of life

All eligible patients completed EORTC QLQ-C30 instruments, McGill–Melzack, monthly QoL scale form, daily clinical diary with visual analogical scale for pain scoring and report for analgesic consumption. The QoL was evaluated in all patients. Analysis of Global Health Status according to EORTC QLQ-C30 showed an improvement of the status in six patients (26%). Most of the patients had an increasing score in QoL after antiandrogen withdrawal. Twelve patients (52%) reported a worsening global health status related to progression of disease. All other patients reported no change in the QoL score assessment (Fig. 1). The greatest benefit in the patients was in the subscale representing prostate-specific concerns (including appetite, pain, physical comfort, and bowel and genitourinary function).

Discussion

Our study was designed to investigate activity of gefitinib in HRPC patients either when added to total androgenic block, either after subsequent antiandrogen withdrawal. After 2 months of treatment only two patients had a decreasing PSA (< 50%); level drop was ≤ 25% with respect to baseline, shorter than 4 weeks and not associated with clinical benefit. After the withdrawal of the antiandrogen (AAWD) given in combination with gefitinib, three patients showed a decreasing PSA > 25%, not associated with clinical response nor with improvement in QoL. For patients with progressive disease, despite androgen deprivation, withdrawal of antiandrogen has been reported to result in a decline in PSA level [32–34]. The rate of objective reduction in pain after treatment with gefitinib is low. Analysis of QoL according to EORTC QLQ-C30 showed an improvement of the status in six patients (26%), but most of the patients had an increasing score in QoL after antiandrogen withdrawal. Twelve patients (52%) reported a worsening global health status related to progression of disease. Characteristics of patients included in our study were negative: most of them had a high Gleason score at diagnosis and were nonresponsive to two lines on hormonal manipulation. Results reported here could be partly explained in

Fig. 1



Quality of life assessed according to quality of life questionnaire.

relation to the negative selection of patients. Similar results have been reported by other investigators in a study that evaluated activity of gefitinib in HRPC patients randomly assigned to 250 mg ($n = 19$) or 500 mg ($n = 21$) oral gefitinib daily continuously. None of the patients in this study demonstrated a PSA or objective measurable response. Authors concluded that gefitinib has minimal single-agent activity in HRPC [35]. In our study serum EGFR and HER2 levels were monitored in blood samples before, during and after treatment with gefitinib. We observed no modifications of serum markers that seems to reflect absence of gefitinib activity. To our knowledge this is the first study evaluating the relationship between gefitinib activity and serum EGFR and HER2 ECD levels in patients with prostate cancer. According to these results we cannot consider circulating EGFR and HER2 ECD as a surrogate marker of gefitinib treatment. A recent paper reported data indicating that basal EGFR concentrations and serum modifications in EGFR during therapy were associated to response to gefitinib and PFS in patients with NSCLC [27]. In our study we did not observe no evidence of a reduction or an increase in EGFR serum levels in relation to gefitinib therapy. Gefinitib, in our study, showed no clinical activity either on antiandrogen therapy nor after withdrawal from antiandrogen. The mechanism by which prostate cancer patients develop disease progression after AAWD is not understood. Several hypothesis have been raised to explain this event: (1) persistence of a clone of cells with partial or full sensitivity to testosterone testosterone with might be provided a growth advantage by androgen produced by the adrenal glands; (2) mutations in the androgen receptor resulting in resistance to the antiandrogens; and (3) proliferative advantage driven by other growth factors pathways such as EGF. Other mechanisms involved in disease progression after AAWD should be considered, e.g. activation of mammalian target of rapamycin (mTOR) cascade owing to loss or mutation of PTEN tumour-suppressor gene [36]. In the normal prostate, PTEN allows cells to undergo apoptosis, whereas in cancer cells, including androgen-refractory prostate cancer, mutation or loss of PTEN increases Akt activation and blocks apoptosis. The primary hypothesis of our study was the potential effect of gefitinib in HRPC cells owing to overexpression of EGFR, leading to tumour growth in this subgroup of patients. Failure of Iressa in achieving a clinical or PSA response should be related to alternative mechanisms of proliferation of prostate cancer cells as Akt-dependent pathways. The EGF signaling pathway remains an attractive target for cancer therapy. It is clear that a single targeted approach of EGFR pathway inhibition is inadequate to control tumour growth in HRPC, despite the frequent overexpression of EGFR that has been reported. The PTEN-dependent cascade is one of the pathways to be targeted in this multimodal strategy suggesting that Akt/phosphatidylinositol 3-kinase inhibitors (known as mTOR inhibitors) should be considered as an attractive target for prostate cancer treatment.

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