Phase II study on lapatinib in advanced EGFR-positive chordoma†

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Background: To report on a prospective, investigator-driven, phase II study on lapatinib in epidermal growth factor receptor (EGFR)-positive advanced chordoma patients.

Patients and methods: From December 2009 to January 2012, 18 advanced progressing chordoma patients entered this study (median age: 61 years; disease extent: metastatic 72% and locally advanced 28%). Epidermal growth factor receptor (EGFR) expression and activation were evaluated by immunohistochemistry and/or phospho-arrays, real-time polymerase chain reaction, fluorescence immunostaining. Fluorescence in situ hybridization analysis was also carried out. Patients received lapatinib 1500 mg/day (mean dose intensity = 1282 mg/day), until progression or toxicity. The primary study end point was response rate (RR) as per Choi criteria. Secondary end points were RR by Response Evaluation Criteria in Solid Tumor (RECIST), overall survival, progression-free survival (PFS) and clinical benefit rate (CBR; RECIST complete response + partial response (PR) + stable disease (SD) ≥ 6 months).

Results: All patients were evaluable for response. Six (33.3%) patients had PR and 7 (38.9%) SD, as their best Choi responses, corresponding to RECIST SD in all cases. Median PFS by Choi was 6 [interquartile (IQ) range 3–8] months. Median PFS by RECIST was 8 (IQ range 4–12) months, with a 22% CBR.

Conclusions: This phase II study showed a modest antitumor activity of lapatinib in chordoma. The clinical exploitation of EGFR targeting in chordoma needs to be further investigated, both clinically and preclinically.

Clinical trial Registration No: EU Clinical Trials Register trial no. 2009-014456-29.

Key words: chemotherapy, chordoma, EGFR, lapatinib, sarcoma, tyrosine kinase

introduction

Chordoma is a rare bone tumor [1]. The standard treatment is en bloc excision, but the site of origin of the disease often prevents complete surgery. For these patients, debulking surgery followed by radiation therapy (RT) or exclusive high-dose RT can be an alternative [2, 3]. However, local relapses occur in >50% of cases [3–6], while metastases affect >20% of patients [5–7]. Systemic therapy is needed in patients not amenable to surgery or RT.

A few data on chemotherapy are available. Anecdotal responses were reported to anthracyclines, cisplatin (Teva Pharmachemie B.V, Netherlands), alkylating agents and 9-nitrocamptothecin [8–11].

We described the activity of imatinib alone or in combination with the mammalian target of rapamycin (mTOR) inhibitors [12, 13]. Sunitinib showed also some clinical efficacy [14].

In 2005, Weinberger et al. [15] found the epidermal growth factor receptor (EGFR) expression in a series of 12 chordoma patients. This led to the report of a response to the EGFR inhibitors cetuximab and gefitinib, maintained for 9+ months [16]. Other reports on cetuximab plus gefitinib and erlotinib activities were described later [17, 18]. We also found EGFR expression/activation in 81% of 22 naïve chordomas, with HER2/neu receptor co-expression in 50% of cases, without mutations in the corresponding genes [19]. Shalaby et al. [20] evaluated EGFR status by immunohistochemistry (IHC) in 173 chordoma samples confirming its expression in 69% of cases, coupled with EGFR polysomy in a half of them.

Lapatinib is a tyrosine kinase inhibitor (TKI) active against both EGFR and HER2/neu [21]. This study was aimed at assessing its activity in EGFR-positive advanced chordoma.

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patients and methods

Adults with progressive, advanced chordoma, expressing an EGFR, were eligible for the study. Pathology, EGFR and HER2/neu status were centrally reviewed.

Main inclusion criteria were: locally advanced/metastatic disease; at least one measurable lesion; progression in the 6 months before entering the study and Eastern Cooperative Oncology Group performance status (ECOG PS) ≤4.

The study was conducted at two sites of the Italian Sarcoma Group and approved by the institutional review boards (all patients gave their written informed consent).

study design and statistical analysis

This is an Italian, multicenter, single-arm investigator initiated, phase II study. Primary end point was overall response rate (RR) according to Choi criteria (Choi) [22] in the intention-to-treat (ITT) population. We applied Choi as defined for GIST, and extended even to magnetic resonance imaging (MRI) [23], since Response Evaluation Criteria in Solid Tumor (RECIST) [24] were already found not to be fully adequate to detect response in chordoma treated with targeted therapy [12].

The trial was powered to detect a RR of ≥5% and to exclude a RR of ≤5%, alpha and beta error levels being fixed at 5% and 10%, respectively.

Secondary end points were: RR by RECIST (version 1.1) [24], RR by [18F]-fluorodeoxyglucose (FDG)-positron emission tomography (PET), overall survival (OS), progression-free survival (PFS) by Choi and RECIST, and clinical benefit rate (CBR) [RECIST complete response (CR) + PR + SD ≥ 6 months]. Both PFS and OS were estimated using the Kaplan–Meier method [25]. The time to occurrence of any event was computed from the date of treatment start to the date when the event was recorded, or censored at the date of last follow-up assessment in event-free patients. Death for any cause was accounted as an event for OS and PFS. Patients who progressed due to treatment interruption for any reasons and had a new response after restoring treatment were considered progressive disease (PD) at the time of the first progression.

EGFR and HER2/neu assessment

EGFR and HER2/neu activation was tested by biochemical analyses, immunofluorescence and/or real-time polymerase chain reaction (PCR) depending on the type of material available. EGFR and HER2/neu expression and gene copy number were assessed by formalin-fixed, paraffin-embedded (FFPE) material by IHC and fluorescence in situ hybridization (FISH) respectively.

analyses of FFPE material

EGFR and HER2/neu expression were assessed by IHC and scored as reported [19].

EGFR and HER2/neu gene status were investigated by FISH using commercial probes (EGFR spectrum orange CEP7 spectrum green; HER2/neu spectrum orange CEP17 spectrum green, Vysis, Downers Grove, IL) [19].

EGFR activation was investigated by immunofluorescence using anti-phospho-EGFR antibody (pEGFR1173, cat #4407, Cell Signaling Technology, Danvers, MA) [26].

The presence of EGF and transforming growth factor-alpha (EGF ligands) transcripts was investigated by real-time PCR [19].

analyses of frozen material

Phosphorylated receptor tyrosine kinases (RTKs) expression was detected using the phospho-RTK array kit (Proteome Profiler™ Array, ARY001, R&D Systems, Minneapolis). This approach could not be applied in two cases for the scarcity of the material. In both of them, EGFR immunoprecipitation (IP)/western blotting (WB) analysis was carried out.

treatment

Patients were to receive 1500 mg lapatinib once daily continuously till progression or toxicity. Treatment was withheld for hematologic toxicity grade ≥3 (G ≥ 3) and non-hematologic toxicity grade ≥2 (G ≥ 2).

Lapatinib was resumed after recovery to hematologic toxicity grade ≤2 or non-hematologic toxicity grade ≤1. Dose reductions to 1250 or 1000 mg/day were allowed in case of toxicity.

Patients had a regular physical examination and a complete blood count/serum chemistry evaluation. All patients were evaluated at baseline with whole-body computed tomography (CT), and MRI and/or CT of the tumor site. PET was carried out in a subgroup of patients.

Toxic effects were graded according to the US National Cancer Institute Common Toxicity Criteria, version 2.0.

efficacy and safety evaluation

ITT population included all enrolled patients who received at least one dose of lapatinib. In the ITT population, patients who went off-study due to adverse events or toxicity before the key response evaluation were considered treatment failures.

response assessment

Response was assessed after 4 weeks, then every 2 months by CT and/or MRI, according to Choi [22] and RECIST [24]. All patients had to be evaluated by using the same technique (MRI or CT). Images had to be available as DICOM files before/after contrast administration, in portal venous phase in case of CT, and/or as turbo spin-echo (TSE) T2-weighted/TSE T1-weighted (late phase) in case of MRI.

PET was recommended at baseline, after 4–6 weeks and after 3 months from treatment start. The maximum standard uptake value (SUV max) of primary tumor and/or metastases was calculated. PET response was assessed according to the European Organization for Research and Treatment of Cancer (EORTC) 1999 criteria [27].

As for Choi [22, 23], PR was defined as a ≥10% decrease in tumor size, a ≥15% decrease in tumor density (portal venous phase) on CT, or a ≥15% increase in TSE T2-weighted signal intensity, and ≥15% decrease TSE T1-weighted CE (late phase) at MRI compared with baseline, irrespective of any increase in tumor size, given the absence of new lesions. SD was classified when criteria for PR or PD were not met. PD was defined by the presence of a ≥10% increase in tumor size and no criteria for PR, or in case of a new lesion. CT, MRI and FDG-PET were reviewed centrally.

results

Between December 2009 and January 2012, 18 patients were enrolled. Supplementary Figure S1, available at Annals of Oncology online shows the CONSORT recruitment tracking flowchart. This analysis includes data from all 18 patients on an ITT basis. All patients who entered the study were evaluable for response. Median follow-up time was 10.5 [interquartile (IQ) range 6–18] months. Reasons for discontinuation were: PD (13 patients, 72.3%), adverse event (4, 22.2%) and patient decision (1, 5.6%). Table 1 summarizes patient characteristics.

EGFR and HER2/neu assessment

All patients who entered the study were screened for EGFR and were EGFR positive at least by one technique. Overall, of
25 screened patients, only 3 resulted EGFR negative and did not enter the study. Data on EGFR and HER2/neu expression/activation in treated patients are summarized in supplementary Table S1, available at *Annals of Oncology* online.

In addition to EGFR, the eight tumor samples tested by phospho-RTK arrays showed the co-activation of platelet-derived-growth-factor-receptor beta (PDGFRB), and of Tyro3 Axl Mer (TAM) family members in 6/8 cases (Axl/Dtk/Mer in 6/3/2, respectively), sustained by the expression of their cognate ligand growth arrest-specific gene (Gas) 6 (supplementary Figure S2, available at *Annals of Oncology* online).

All 18 patients who entered the study were evaluable for response by Choi and RECIST (supplementary Table S2, available at *Annals of Oncology* online).

**Choi assessment**
Best response was CR, PR, SD and PD in 0, 6 (33.3%), 7 (38.9%) and 5 (27.8%) patients. The ORR, that was the primary study end point, was 33.3% [95% confidence interval (CI) 13.3–59.0%], i.e. 6 of 18. Among three (16.7%) patients with Choi PR at 3 months, 1 (5.6%) showed no progression at 6 months. Figure 1 shows a Choi PR.

**RECIST assessment**
Best response was SD and PD in 15 (83.3%) and 3 (16.7%) patients, respectively. In six cases, RECIST SD corresponded to Choi PR; in all cases, RECIST PD corresponded to Choi PD.

The CBR by RECIST was 22.2% (95% CI 6.4–47.6%; 4 of 18) for the ITT population.

**FDG-PET assessment**
PET evaluation was available for 10 patients with evidence of four response, five progression and one SD. PET responses always corresponded to Choi PR. PET PD corresponded to Choi PD and SD in two and three cases, respectively.

**PFS and OS**
Median PFS for Choi was 6 (IQ range: 3–8) months (Figure 2). Median OS was 25 (IQ range: 23–26) months. Median PFS for patients non-progressive at 6 months were 11 (IQ range: 8–12) months by Choi and 12 (IQ range: 11–21) months by RECIST.

According to Choi, no patients were progression-free at >12 months. One patient progressed at 12 months. Another patient progressed at 4 months due to toxicity-related prolonged treatment interruption; he responded again after restarting lapatinib and is progression-free at 16 months. By RECIST, only one patient was non-progressing at 12 months, remaining free-from-progression at the last follow-up (18 months).

**discussion**
In this exploratory phase II study, 18 patients with advanced EGFR-positive chordoma were treated with lapatinib. The overall RR for Choi criteria (i.e. the study primary end point) was 33% (six cases). No patient achieved a PR as by RECIST. Median PFS according to Choi was 6 months. Median PFS as by RECIST was 8 months, with a 22% CBR (i.e. RECIST...
CR + PR + SD ≥ 6 months). A PET response was observed in 4 of 10 patients with a baseline assessment.

Treatment of chordoma is challenging. In fact, chordoma is a very rare tumor, with <25% patients disease-free at 10 years and in need for any medical therapy. No active drugs are approved to date. This investigator-driven study is indeed the second clinical study on targeted therapies in chordoma, the first in an academic, non-sponsored setting. It follows an Italian-Swiss explorative phase II study on imatinib in PDGFRB positive advanced cases [12]. The study presented here was conceived as an exploratory study, to preliminarily evaluate in a formal setting the activity of an EGFR inhibitor in this orphan tumor, given the evidence that EGFR is expressed in a proportion of chordomas and the anecdotal reports on responses to anti-EGFR agents [16–18]. We selected lapatinib, because it inhibits both EGFR and HER2/neu [21].

Since we realized that tumor responses to imatinib in chordoma can be non-dimensional [12], we chose RR according to Choi as our primary end point. By protocol, therefore, this study is positive with regard to its primary end point, i.e. Choi response. In fact, 33% of patients achieved a Choi PR, even if no RECIST responses were detected. We also observed a decrease in PET uptake in 40% of evaluable patients. However, responses were short lasting, with a median PFS of 6 months, a RECIST CBR of 22%, and only one patient progression-free at >12 months. This seems inferior to what observed in chordomas treated with imatinib. In the imatinib phase II study, median PFS as per Choi was 9 months, with a RECIST CBR of 64%, and 40% of patients being progression-free at 12 months. Of course, these are external comparisons between studies enrolling the limited numbers of patients.

Median PFS of 6 months may be viewed as interesting since >70% of our patients were metastatic and almost 90% of them had received at least one other medical therapy before lapatinib. In fact, chordoma is a potentially indolent disease, but it becomes more aggressive in the metastatic phase [5–7]. Furthermore, patients who entered this study progressed at 6 months before starting treatment. Unfortunately, tumor growth modulation assessment was not foreseen in the protocol. Interestingly, PFS according to Choi was lower than that as per...
The very complex molecular profile of chordoma could be probably better fought by combining different kinase inhibitors. We evaluated by phospho-RTK arrays the pretreatment tumor samples of eight patients who entered the inhibitors. We evaluated by phospho-RTK arrays the pretreatment tumor tissue for the molecular/biochemical analyses was limited, and we did not foresee a post-treatment biopsy to investigate EGFR status after lapatinib. It also remains unclear whether another EGFR inhibitors might be more active.

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In conclusion, this phase II prospective study confirms that lapatinib has some antitumor activity in chordoma. This is a useful piece of information in this orphan disease, devoid of any standard medical therapy, in which, however, anti-PDGFR agents were shown to have promising, and apparently higher, activity. Anti-EGFR agents, possibly combined with other kinase inhibitors, might find a place in the medical approach to chordoma. This is due to clarify by future translational and clinical research.

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disclosure

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references

Performance of multinomial designs in comparison with response-based designs in non-randomized phase II trials of targeted cancer agents

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Background: In phase II trials of cytotoxic agents, a multinomial phase II design incorporating early progression and response end points was shown to perform more efficiently than designs based only on response. We undertook a study to evaluate the performance of these designs in trials of targeted agents using the actual phase II data.

Patients and methods: Using best response data from sequentially enrolled patients in 15 NCIC Clinical Trials Group and 7 European Organization for Research and Treatment of Cancer trials of targeted agents, we determined that trials would have been stopped at the end of stage I of accrual by applying rules generated by the multinomial and Fleming designs. Two variants of the multinomial design were studied: to stop accrual after stage I of enrolment, Variant A required either response or progression criteria to be met, whereas Variant B required that both response and progression criteria to be met.

Results: Using early progression, null/alternate hypotheses of 60% and 40% (60/40), the multinomial A variant recommended early stopping more often than the Fleming design. In most of the cases, this recommendation was correct given the final trial outcome. In contrast, the multinomial B variant never led to recommendations for early stopping and changing progression hypotheses did not improve the performance of this design.

Conclusions: The multinomial A design using 60/40 hypotheses carried out better than the Fleming design in appropriately stopping trials of inactive targeted agents early. The multinomial B design was not useful for early stopping decisions. The multinomial A design may be favored over response-based designs in phase II trials of targeted agents.

Key words: clinical trials, multinomial design, phase II designs, targeted agents

introduction

Single-agent phase II studies of new cancer agents have traditionally employed objective tumor response as the primary end point to guide decisions on the activity of the agent. Such trials are often non-randomized and use a multistage design such as those of Gehan, Fleming and Simon [1–4] to terminate the trial after the first stage of accrual if too few responses are seen. The stopping rule and sample size of the trial are determined by the null (Ho) and alternate hypotheses (Ha) established for response and the alpha (false-positive) and beta (false-negative) error rates. These parameters will vary based on the tumor type, the patient population and the type of drug being evaluated.

Recently, early disease progression has been suggested to be an additional measure of inactivity in an innovative multinomial design [5]. This design presumes that a truly active treatment may be detected not only by evidence of response, but also by the observation of a low proportion of patients having early disease progression. Thus, information on both the number of observed responses and the number of early progression events is considered in decisions about early stopping and conclusions about activity. Zee et al. [5] defined early progression as a best overall response of progressive disease (PD), meaning that those patients had progressed at, or