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ORIGINAL ARTICLE

A phase II study of patients with metastatic or locoregionally recurrent nasopharyngeal carcinoma and evaluation of plasma Epstein–Barr virus DNA as a biomarker of efficacy

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Abstract
Background The epidermal growth factor receptor (EGFR) is commonly overexpressed in nasopharyngeal carcinoma (NPC) and gefitinib inhibits NPC growth in vitro.
Method Patients who progressed after prior platinum-based chemotherapy for recurrent NPC were given gefitinib orally at 500 mg/day at a 28-day cycle. Plasma Epstein–Barr virus (pEBV) DNA levels were obtained at specific intervals.
Results Sixteen patients enrolled and 15 were evaluable for response. The median age was 49 years (range 34–64 years), and most patients were males with metastatic NPC. No objective response was seen and three patients had stable disease (SD) for 2.8 to 8.5 months. Radiological progression of disease coincided with rising levels of pEBV DNA in most patients, while the level of a patient with the longest duration of SD fell to an undetectable level at study completion. The mean time to progression and overall survival was 2.7 (standard error, SE +/− 0.5 months) and 12 months (SE +/− 1.7 months), respectively. No unexpected drug-related toxicities were seen. The study was prematurely terminated because there was insufficient activity to warrant progression to the second stage of accrual.
Conclusion This study found limited activity of gefitinib in recurrent NPC. Further evaluation of pEBV DNA as a biomarker of response in clinical trials of target-based agents is warranted.

Keywords Gefitinib · Epidermal growth factor receptor · Plasma EBV DNA · Nasopharyngeal carcinoma

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Introduction

Nasopharyngeal carcinoma (NPC) occurring in the endemic regions of Southeast Asia is characterized by its association with the Epstein–Barr virus (EBV) and sensitivity to cytotoxic chemotherapy. Although platinum-based chemotherapy has been associated with rare incidences of prolonged survival in patients with metastatic NPC, the majority seldom survives beyond a median of 12 months [1]. The epidermal growth factor receptor (EGFR) represents a promising target against advanced NPC, as a growing body of evidence now supports an important role of EGFR in

NPC and the feasibility of anti-EGFR strategies. Preclinical studies have shown that the oncogenic EBV-encoded latent membrane protein-1 (LMP-1) can induce EGFR expression and proliferation in both EBV-positive human epithelial cells [2] and NPC cells [3]. EGFR overexpression is common in NPC and can be found in more than 80% of primary tumor biopsies of undifferentiated NPC. It is also a negative prognostic factor as patients with EGFR-overexpressing tumors are more likely to experience recurrent disease and die from NPC following radiotherapy [4, 5]. The targeting of EGFR with monoclonal antibodies and tyrosine kinase inhibitors can inhibit cell growth and enhance response to cytotoxic chemotherapy in EBV-positive NPC cells [6, 7], and induce cell cycle arrest in EBV-negative NPC cells [8]. As a proof of concept, the anti-EGFR monoclonal antibody cetuximab was evaluated in NPC in a multi-center trial of patients with recurrent or metastatic NPC, most of whom were heavily pre-treated with platinum-based chemotherapy [9]. The combination of cetuximab and carboplatin yielded an overall response and disease-control rate of 11.7 and 60%, respectively [9]. Gefitinib (ZD1839, Iressa™ AstraZeneca Ltd) is an oral anilinoquinazoline inhibitor of the EGFR tyrosine kinase domain that has activity against a variety of cancers including non-small cell lung cancer (NSCLC) and non-NPC squamous cell carcinoma of the head and neck (HNSCC) [10, 11]. Preclinical data suggest that gefitinib can inhibit growth and enhance the anti-cancer effect of certain cytotoxic agents in NPC cells [6]. The activity of gefitinib in NPC had not been evaluated, and we report the result of a phase II study of gefitinib in metastatic and recurrent NPC.

Plasma EBV DNA (pEBV DNA) is an important prognostic marker in NPC and its kinetics has been shown to reflect clinical response to cytotoxic therapies including radiotherapy [12], chemotherapy [13] and nasopharyngectomy [14]. Its role in monitoring response to non-cytotoxic, target-based therapies has not been investigated to date. This phase II study was designed to evaluate the response rate and time to progression following treatment with gefitinib in patients with recurrent or metastatic NPC. Serial levels of pEBV DNA were measured during treatment with the aim of investigating the role of pEBV DNA in monitoring response to gefitinib.

Patients

Eligible patients had to have histologically/cytologically confirmed recurrent or metastatic NPC who were previously treated with 1 to 2 lines of prior platinum-based chemotherapy. Patients had to be aged 18 years or above, have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2, and adequate organ and marrow function. Any prior chemotherapy, radiotherapy or surgery had to occur at least 4 weeks before enrollment into the study. The main exclusion criteria included patients who were lactating or pregnant, have a history of significant interstitial lung disease, serious medical co-morbidities or another malignancy within the past 5 years. Treatment with another investigational drug within 1 month prior to enrollment, and the concomitant use of phenytoin, carbamazepine, rifampicin or barbiturates were not allowed. This study was approved by the local Ethics Committee, and all patients gave written informed consent prior to enrollment.

Method

Gefitinib at 500 mg daily were given orally to eligible patients at a 28-day cycle for a maximum period of 8 months. Radiological assessment of response was performed every two cycles. Dose interruption of up to 14 days was allowed for poorly tolerated gefitinib-related skin toxicities, any grade 3 or 4 toxicity (e.g. diarrhea), or any toxicity of grade 1 to 2 in severity that was of clinical significance. If a patient required dose interruption for more than 14 days, dose reduction of gefitinib to 250 mg daily was allowed.

The primary objective of this study was response rate. Secondary objectives included the evaluation of pEBV DNA in monitoring response to treatment, time to progression, stable disease rate, toxicity and overall survival rates. Objective response was measured using the Response Evaluation Criteria in Solid Tumors (RECIST) criteria [15]. Bone metastases were assessed radiologically (e.g. bone scan) as either ‘present’ or ‘absent’. Toxicity was graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 3. Time to progression or overall survival was defined as the time from day 1 of each treatment cycle, to the time of disease progression or death due to any cause (or the final date of follow-up), respectively. The Fleming’s Two-Stage Phase II Design was used to calculate the sample size [16]. The study assumed that the response rate of gefitinib exceeds 20%, and the drug was considered inactive if response rate was equal to or lower than 5%. Therefore, a total sample size of 30 would give a type I error of 0.05 and power of 80%. During the first stage of accrual, 15 patients would be required and the study would proceed to stage II of accrual only if four or more responses were observed.

For the determination of pEBV DNA, 2 ml of plasma collected in EDTA tubes were collected from each patient at baseline, day 1 of cycles 2 and 3, and at study discontinuation. Real-time quantitative polymerase reaction (PCR) was used to determine the level of pEBV DNA in copies per ml using the technique as previously described [17].

Result

Sixteen patients were enrolled into the study and one patient died of disease-related cause shortly after enrollment, therefore 15 patients were evaluated for response and toxicity. The median age was 49 years (range 34 to 64 years), 13 patients were male and the majority had metastatic disease at more than one site. Information about their disease status at enrollment and previous treatment are summarized in Table 1. All patients had prior platinum-based chemotherapy regimen for recurrent disease, in combination with agents such as 5-fluorouracil, paclitaxel or gemcitabine. Following treatment with a mean of three cycles of gefitinib (range 2 to 8 cycles), no objective responses were seen. Of the three patients (with the codes of 'PW006', 'PW003' and 'PW014') whose best response was stable disease (SD), one had local recurrence at the base of skull ('PW006') and the others had distant recurrence. The duration of disease stabilization ranged from 3, 5 and 8.6 months in these patients and notably, one patient ('PW006') completed the planned eight cycles of gefitinib without experiencing disease progression. Overall, 90% of patients progressed shortly after two cycles of gefitinib, with a mean time to progression of 2.8 months (standard error, SE +/− 0.5 months) and a mean overall survival of 12 months (SE +/− 1.6 months). Gefitinib was well tolerated and no unexpected toxicities were seen. No interstitial lung toxicity occurred and there were no treatment-related deaths. The most commonly encountered toxicities related to gefitinib were grade 1 to 2 in severity, including dry skin (93%), diarrhea (73%) and acneiform rash (60% grade 1–2). Grade 3 to 4 toxicities were rare, with the commonest being grade 3 acneiform rash occurring in five patients (33%), requiring interruption and dose reduction of gefitinib to 250 mg daily.

The median pEBV DNA level before treatment was 11,233 copies/ml (range 0 to 2.9×10^6 copies/ml). The pEBV DNA levels taken before treatment and at end of study for all patients are outlined in Table 2. Of the 12 patients who did not respond to gefitinib, the serial levels of pEBV DNA clearly showed a rising trend that coincided with radiological evidence of progression in most patients except in three patients (study codes: PW005, PW007, PW015) who showed varying degree of declined levels (see Fig. 1a, b). It is noteworthy that the patient who had the longest duration of SD in the absence of disease progression (PW006, SD for 8.5 months) experienced a progressive decline in pEBV DNA to an undetectable level at completion of a planned treatment of eight cycles (Fig. 2). This patient had a minor shrinkage (RECIST, 22%) of his disease at the base of skull, which did not qualify as a partial response based on RECIST criteria. Another patient (PW003) who had SD that lasted around 5 months also had an initial fall in pEBV DNA followed by a sharp rise at the time of radiological disease progression (Fig. 2).

Table 1 Patients characteristics

Characteristics	Number of patients (%)
Age (median)	49 years (range 34 to 64)
Sex—male:female	13:2
Disease status at study entry	
Distant metastasis only	9 (60)
Locoregional recurrence only	4 (27)
Distant and local recurrence	2 (13)
For patients with distant recurrence	
No. of metastatic sites	
1 site	3
2–3 sites	8
Type of metastatic sites	
Liver	7
Lung	8
Bone	6
Number of prior chemotherapy	
1 line	13 (86)
2 lines	2 (14)
Prior radiotherapy to nasopharynx	
Yes	14 (93)
No	1 (7)

Table 2 pEBV DNA levels (copies/ml) obtained at baseline, day 1 of cycle 2 (C2D1), day 1 of cycle 3 (C3D1) or end of study

Patients study code	Baseline	C2D1	C3D1 or end of study
PW001	606539	1823625	Not done
PW002	548446	678049	1092252
PW003	5195	2563	31409
PW004	42796	269953	234261
PW005	2986054	5165564	1869225
PW006	499	168	0
PW007	35377	34583	23135
PW008	285330	1114242	342372
PW010	11233	78883	65283
PW011	4504	4874	7047
PW012	0	249	3889
PW013	12828	20132	23891
PW014	2239	11289	261
PW015	6129	2534	2874
PW016	12759	866	20225

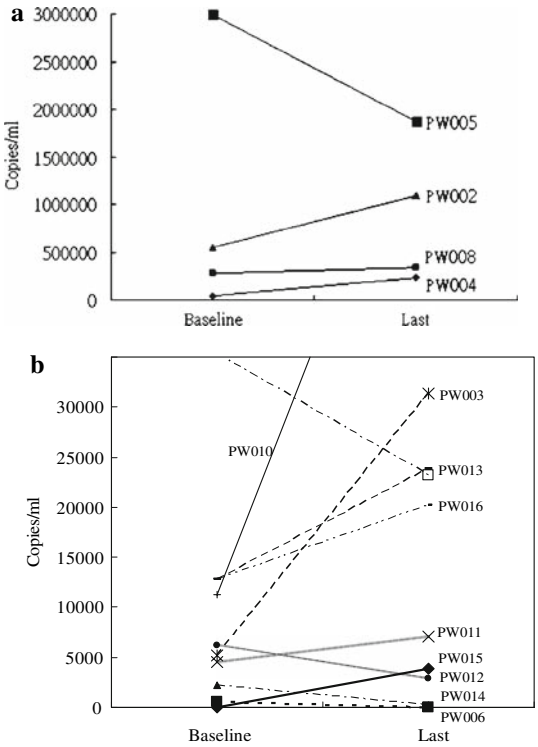

Fig. 1 **a**, **b** pEBV DNA of patients who progressed obtained at baseline and end of study (or day 1 of cycle 3)

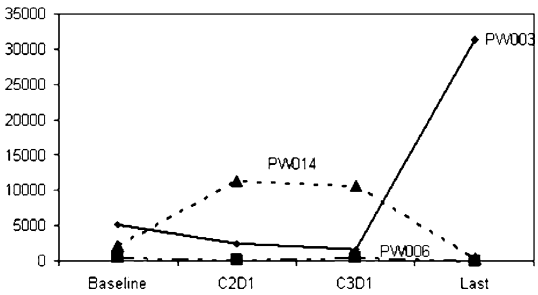

Fig. 2 Serial pEBV DNA levels of patients who achieved stable disease. Legend: *C2D1* cycle 2 day 1, *C3D1* cycle 3 day 1, *Last* end of study

Discussion

Gefitinib had little activity as monotherapy in this cohort of patients with predominantly metastatic NPC who had prior platinum-based chemotherapy. No objective response was observed during the first stage of accrual, where 90% of patients experienced disease progression after receiving only two cycles of gefitinib. The time to progression observed in this study did not compare favorably with that reported with agents such as gemcitabine [18], capecitabine [19] or ifosfamide [20] in a similar setting. Therefore, the study was prematurely terminated after 15 patients were accrued. Only one patient with local recurrence had disease stabilization over 6 months while receiving gefitinib.

The discovery of activating mutation in the EGFR tyrosine kinase (TK) domain as a predictor of response to EGFR tyrosine TK inhibitors in NSCLC is a major breakthrough in understanding the mechanism of kinase inhibition in oncology [21–23]. Monotherapy of gefitinib has minimal to modest activity in epithelial cancers where the incidence of EGFR mutation is low or negligible, such as in cancers of the breasts [24, 25], ovaries [26, 27], colorectum [28, 29], stomach [30, 31] and non-NPC HNSCC [10, 32]. In NPC, EGFR TK mutations have not been reported to date, and one retrospective study failed to detect any EGFR mutations in 102 samples of NPC [33]. Although benefit from gefitinib does not limit to patients who carry activating EGFR mutations in NSCLC [34], our study supports the overall impression from studies in other epithelial cancers that significant tumor response from gefitinib is unlikely to occur in the absence of such mutations [23].

The association between pEBV DNA and tumor burden [25] makes this a promising test in monitoring response to drug therapy. One of the more useful indications of pEBV DNA is that it allows early detection of disease progression during therapy, as shown in a study where serial levels were analyzed during chemotherapy in metastatic NPC [13]. However, this test may not accurately discriminate patients with objective tumor shrinkage from those who have disease stabilization only [13]. A similar pattern is shown in this study of gefitinib, where a progressive rise in pEBV DNA was observed in most patients prior to radiological evidence of disease progression. Interestingly, the pEBV DNA level became undetectable in one patient with local recurrence who experienced a minor tumor shrinkage and disease stabilization beyond 6 months.

There are some potential limitations of pEBV DNA as a biomarker of therapeutic response. Around 5% of patients with NPC do not have detectable level of pEBV DNA at diagnosis, and that less than 10% of healthy people may have low level of pEBV DNA [17, 35]. It is now known that EBV DNA exist as short fragments in the circulation instead of within intact virions, suggesting that the release of pEBV DNA level might be related to apoptosis of cancer cells instead of viral replication or lytic activation [36]. However, little is known about the factors that control the rate of release of pEBV DNA into the circulation, such as prior irradiation to the tumor bed, or the degree of tumor invasion into the adjacent vascular plexus(es). This knowledge is especially relevant to understanding why pEBV DNA is more sensitive in detecting distant metastases than local recurrence [37] and likewise, why pEBV DNA does not reflect tumor burden in a consistent manner. Overall, the result of this study suggests that pEBV DNA may also be a useful early indicator of disease progression during

target-based drug therapy, thus validation in larger studies is warranted.

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