UNEXPECTED RAPID PROGRESSION OF METASTATIC ADENOID CYSTIC CARCINOMA DURING TREATMENT WITH IMATINIB MESYLA TE

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Adenoid cystic carcinoma (ACC) is an uncommon cancer that arises from the salivary glands of the upper aerodigestive tract. This tumor is unique in its protracted course, even after development of local recurrence or distant metastasis. Some patients are known to live 10 to 20 years despite pulmonary metastasis, the most frequent manifestation of distant spread.

For local disease, radical surgery is the mainstay of treatment. The actual cure rate of ACC is poorly defined, because some studies with 10 to 20 years of follow-up have shown disease-related deaths continuing to occur throughout the follow-up period.1 Locoregional recurrence is common, and distant metastases occur in nearly half of patients. The optimal treatment for metastatic ACC remains undefined because of the lack of prospective trials with adequate patient numbers. Cisplatin-based combinations, with response rates of approximately 30%, are most commonly used.2,3 In the 1990s, four new agents were tested in clinical trials, but none showed an improve-
ment in response rate. All patients should be considered candidates for trials with investigational drugs.

Holst et al found KIT protein expressions in 27 of 30 ACCs by immunohistochemical study. No mutations of c-kit in exon 11 or exon 17 were identified in any of the tumors examined. Two other studies confirmed expressions of KIT in nine of nine and 15 of 15 ACCs, respectively.

Imatinib mesylate is a selective small-molecule inhibitor that targets a small family of protein tyrosine kinases (PTKs), including ABL, KIT, and platelet-derived growth factor receptor (PDGFR). On the basis of the exciting results of imatinib in gastrointestinal stromal tumors (GISTs), the therapeutic value of imatinib for metastatic ACC is worthy of investigation. Herein we report the results of a pilot study. The common mutational sites of c-kit and PDGFR-α were also analyzed.

PATIENTS AND METHODS

Patient Eligibility. Patients with immunohistochemically documented KIT-positive ACCs and measurable metastatic lesions were enrolled. Additional eligibility requirements included an Eastern Cooperative Oncology Group (ECOG) performance score of 0 to 2, age ≥18 years, white blood cell (WBC) count ≥3000/µL, platelets ≥75,000/µL, hemoglobin ≥8 g/dL, creatinine ≤2 mg/dL, bilirubin ≤1.5 mg/dL, and serum glutamate pyruvate transaminase (SGPT) ≤5× the upper limit of normal. Exclusion criteria included active infections, pregnancy, uncontrolled brain metastasis, concurrent treatment with other experimental drugs, and uncontrolled congestive heart failure or myocardial infarction in the previous 3 months. This trial was approved by the institutional review board, and written informed consent was obtained from all patients.

Treatment and Dose Modification. Imatinib mesylate (Glivec, Novartis, East Hanover, NJ) supplied by Novartis Pharmaceuticals was administered orally at the dose of 400 mg twice daily (bid). If the WBC count became less than 2000/µL or the platelet count was <50,000/µL, treatment was withheld until recovery to the eligibility criteria level and then restarted at a daily total dose of 600 mg. If the toxicity recurred, a further dose reduction to 400 mg daily was permitted. For any grade 2 or higher nonhematologic toxicity, the drug was withheld until recovery to grade 1 or baseline. In patients with grade 2 toxicity, the same dose was used. In patients with grade 3 or 4 toxicity, the dose was reduced to 400 mg daily. If grade 2 nonhematologic toxicity recurred, imatinib mesylate was again withheld, and on recovery, the dose was reduced to 600 mg daily or (if necessary) further reduced to 400 mg daily. If grade 3 or 4 nonhematologic toxicity recurred, the therapy was discontinued. All patients were scheduled to receive 8 weeks of treatment, unless disease progression or unacceptable toxicity occurred.

Patient Evaluation. Prestudy evaluations included a complete history, physical examination, and routine blood test. The staging workup included chest radiography, CT, or MRI of the affected sites. After treatment, history taking, physical examination, blood cell count, renal function test, liver function test, urinalysis, and chest radiograph were checked every 4 weeks.

Treatment response was assessed with chest x-ray, CT, or MRI of the affected area after 8 weeks of treatment. Patients were evaluated earlier for adverse reactions or disease progression, if clinically indicated. Objective tumor response was determined by Response Evaluation Criteria for Solid tumors (RECIST) criteria. Standard [18F]fluoro-2-deoxy-D-glucose positron-emission tomography (18FDG-PET) scanning was also performed before and after treatment to correlate with traditional images.

Statistical Considerations. Because there had been no report of imatinib mesylate in this uncommon cancer before initiating the study, we designed an open-label pilot trial with a planned sample size of six patients to evaluate this new indication. The primary objective was to determine the objective response rate of single-agent imatinib mesylate in ACC. Secondary objectives were to evaluate the duration of response, the correlation of response and c-kit or PDGFR-α mutations, safety, and tolerability of imatinib in this population.

Mutational Analysis of c-kit and PDGFR-α. DNA was extracted from formalin-fixed tumors using the Puregene DNA isolation kit (Gentra, Minneapolis, MN). Eight pairs of oligonucleotide prim...
ers were used to amplify exons 9, 11, 13, and 17 of the c-kit gene and exons 10, 12, 14, and 18 of the PDGFRA gene. The primer pairs used to amplify the c-kit exon 9, were 9R (5'-TGA CAT GGT TGT TGG AA-3') and 9L (5'-AGC CAG GGC TTC TGT TTT CT-3'); for exon 11, 11R (5'-TGG AAA GCC CCT GTT TCA TA-3') and 11L (5'-CGT AAT CGT AGC TGG CAT GA-3'); for exon 13, 13R (5'-GCA AGA GAG AAC AAC AGT CTG G-3') and 13L (5'-CAT GCG CTT GAC ATC AGT TT-3'); and for exon 17, 17R (5'-TGA ACA TCA TTC AAG GGT ACT TTT G-3') and 17L (5'-TTG AAA CTA AAA ATC CTT TGC AGG AC-3').

To amplify PDGFRA, the primer pairs for exon 10 were 10R (5'-AGA TGG TTC GAG AGA TGG TAC TGC-3') and 10L (5'-GGA CAC AGT AGA GTC CAA GGT GGT C-3'); for exon 12, 12F (5'-TCC AGT CAC TGT CGCT GCT TC-3') and 12R (5'-GCA AGG GAA AAG GGA GTC TT-3'); for exon 14, 14R (5'-CTC ACT CTC ATT CAA ACC TAT CAG C-3') and 14L (5'-TC ATA CCC ATC TCC TAA CGG C-3'); and for exon 18, 18F (5'-ACC ATG GAT CAG CCA GTC TT-3') and 18R (5'-TGA AGG AGG ATG AGC CTG ACC-3').

Polymerase chain reaction was carried out in a DNA thermal cycler, and the products were sequenced using ABI Prism 377 genetic analyzer (PE Applied Biosystems, Foster City, CA).

RESULTS

Patient Characteristics. Five patients were enrolled into the study, and all had received a complete resection of the primary tumor with or without adjuvant radiotherapy or concurrent chemoradiotherapy before distant metastasis occurred. Metastatic sites included lung (five of five), skull (two of five), brain (one of five), and subcutaneous tissue (one of five). All patients had received cisplatin-based combination chemotherapy before enrollment (Table 1).

Treatment Outcome. Four of five patients were evaluable for efficacy (Table 1). One had stable disease (patient 1). Disease in the other three progressed during study treatment, as evidenced by increased lung tumor sizes (patients 2, 3, and 4). Serial chest radiographs are shown in Figure 1 (patient 2) and Figure 2 (patients 3 and 4). The corresponding changes in 18FDG-PETs of patient 2 are shown in Figure 1. Before treatment, pleural effusion was negative or minimal in all patients. Unfortunately, patients 3, 4, and 5 had rapid accumulation of bloody pleural effusion with positive cytologic findings during study treatment. The tumor sizes of patients 3 and 4 also increased. The tumor size of patient 5 was stable after 4 weeks of imatinib. Because the pleural effusion of patient 5 was too minimal to do cytologic examination before treatment and rapid accumulation of pleural effusion could be due to disease progression or imatinib-related fluid retention, patient 5 was considered not evaluable.

The tumors of patient 2 stopped growing after imatinib was discontinued (Figure 1). His disease remained stable at 17.7 months without further salvage chemotherapy. Patient 3 had received three cycles of paclitaxel plus ifosfamide, but lung and subcutaneous metastasis progressed. Patients 4 and 5 did not receive further treatment after discontinuation of imatinib. The survival of patients 3, 4, and 5 were 6.1, 5.6, and 5.6 months, respectively. They all died of lung metastasis progression.

Toxicity. Side effects were mild to moderate (Table 2). The most frequent side effects were anemia, edema, nausea, and elevations of serum glutamic-oxaloacetic transaminase (SGOT)/SGPT. One patient (patient 1) had protracted grade 2 dizziness, so imatinib was reduced to

<table>
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<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Primary site</th>
<th>Local treatment</th>
<th>Metastatic site</th>
<th>Treatment duration</th>
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<th>Pleural effusion</th>
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Abbreviations: OP, operation; XRT, radiotherapy; SD, stable disease; CCRT, concurrent chemoradiotherapy; PD, progressive disease; NE, not evaluable; DOD, died of disease.
400 mg daily. Another patient (patient 5) had recurrent grade 3 elevation of SGOT/SGPT despite dose reduction to 400 mg daily, so imatinib was discontinued after 4 weeks of treatment.

**Results of c-kit and PDGFR-α Mutational Analysis.** In all five tumors, there were no detectable mutations in exons 9, 11, 13, and 17 of the c-kit gene or in exons 10, 12, 14, and 18 of the PDGFR-α gene.

**DISCUSSION**

Because ACC is usually slowly growing, chemotherapy is reserved for patients whose symptoms are not controlled by other means, but the response has been disappointing. On the basis of the successful molecular targeting treatments in breast cancer, lung cancer, and GIST, ACCs have also been examined immunohistochemically for expression of HER2/neu, epidermal growth factor receptor (EGFR), and KIT. The low prevalence of HER2/neu overexpression and equivocal results of EGFR expression in ACC limit the clinical usefulness of trastuzumab and gefitinib.13–15

KIT overexpression has been consistently reported in 90% to 100% of ACCs.8–10 Regrettably, imatinib had no antitumor activity in our study. In contrast, Alcedo et al16 reported that two patients with unresectable ACC (one recurrent and one locally advanced disease) achieved significant tumor regression after 3 weeks of imatinib (600 mg daily). However, Hotte et al17 recently reported that none of 16 patients with unresectable or metastatic ACC responded to imatinib, and nine had disease progression after 8 weeks of imatinib. In these two reports, c-kit or PDGFR-α mutations were not studied.

The response rate to imatinib in GIST was highest in patients harboring exon 11 c-kit mutations, whereas none of the tumors without mutations of c-kit or PDGFR-α responded.18 Similarly, Lynch et al19 reported that somatic mutations were identified in the tyrosine kinase domain of the EGFR gene in eight of nine patients...
with gefitinib-responsive lung cancer compared with none of the seven patients with no response. There were no c-kit or PDGFR-α mutations detectable in all tumors of our patients. This may partly explain why our patients did not respond to imatinib.

Because this disease is indolent, the observation of high progression rate during a short treatment period could not be incidental. So far, there is no clinical report of imatinib-enhanced tumor growth. However, this possibility cannot be excluded; because the growth rate of GIST and other cancers is usually more rapid than that of ACC, disease progression during treatment could be regarded as part of the natural course. We found that tumor growth rate was more rapid after treatment (Figures 1 and 2). Besides, the disease of patient 2 became stable again without further therapy after going off imatinib treatment (Figure 1).

What is the mechanism if imatinib, indeed, enhanced tumor growth of ACC? Imatinib mesylate in vitro inhibits ABL, ARG (abl-related gene), KIT, PDGFRA, and PDGFRB tyrosine kinases.20 Could the inhibition of wild-type KIT or other PTKs enhance the growth of ACC? There are two main classes of PTKs: receptor PTKs and cellular, or non-receptor, PTKs. Unregulated activation of these enzymes can lead to various forms of cancer. In contrast, ABL PTKs are involved in growth inhibition.21 KIT function plays a critical role in

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Abbreviations: NCI-CTC, National Cancer Institute-Common Toxicity Criteria; SGOT, serum glutamic-oxaloacetic transaminase; SGPT, serum glutamate pyruvate transaminase.
the differentiation of mesenchymal progenitor cells toward the interstitial cells of the Cajal phenotype during embryonic development.22 The role of KIT in ACC, which derives form the differentiation of myoepithelial cells, is still unknown. In addition, genes involved in KIT signaling were differently expressed among wild-type and mutant GISTs.23 Inhibition of mutant KIT may slow down tumor cell growth, but the biologic relevance of the inhibition of wild-type KIT has not been well studied.

Imatinib can act on normal CD34+ hematopoietic cells and inhibits the differentiation and function of dendritic cells in vitro.24 It also inhibits Flt3L-mediated dendritic cell expansion and reduces dendritic cell-dependent T and NK cell–mediated antitumor effects in vivo.25 However, this undesirable effect cannot explain the rapid change in tumor growth rate and why the observation so far seems limited to ACC.

We may hypothesize that imatinib, probably through the inhibition of wild-type KIT or impairment of dendritic cell–mediated antitumor effects, or both, promotes tumor growth of ACC. We should be cautious in using imatinib to treat cancers with KIT overexpression but lack of c-kit mutation. Further studies on molecular pathways are necessary to clarify the mechanism.

REFERENCES