Abstract. Background: Adenoid cystic carcinomas (ACCs) are rare tumors which most often arise in the salivary glands. They have a propensity for local relapse and tend to metastasize, frequently with a protracted clinical course. A substantial fraction of the tumors expresses c-Kit or the platelet-derived growth factor receptor β (PDGFRβ), both targets for imatinib mesylate. No standard systemic therapy is known for these neoplasms. Patients and Methods: c-kit and PDGFRβ-expression were determined by immunohistochemistry. Four patients with distant metastases and with at least one positive result were treated with 400 mg imatinib mesylate once daily and their response assessed. Results: c-Kit and PDGFRβ expressions were variable among the tumor samples. Toxicity was mild. No remissions were observed. Conclusion: These data support that c-Kit or PDGFRβ expression per se are not prognostic for the therapeutic response of metastasized ACCs to imatinib mesylate.

Adenoid cystic carcinomas represent approximately one quarter of all cancers developing in the salivary glands (1). However, ACCs were also described in several other organs like the trachea, bronchial tree or the breast. In the past, these tumors were sometimes classified as semi-malignant. However, the dismal clinical course of patients inflicted by these neoplasias supports the current view of a clearly malignant tumor. These malignancies typically progress comparably slowly, but have a marked propensity to relapse locally and to metastasize to the lung and the bones. Early perineural spread seems to be one of the contributing factors.

The initial therapy of these tumors is surgery, followed by radiation in certain cases (2). Indications for locoregional radiotherapy, usually following an operative intervention, are close margin, R1/2-resection, deep parotideal lobe involvement, infiltration of the seventh cranial nerve and local recurrence.

The biology of these tumors is poorly understood, although recently some progress was made through the application of modern microarray-based methods (3). No clear pharmacological target is known so far. Hence, current medicinal approaches rely on the application of conventional cytotoxic agents. However, due to low response rates, cytotoxic drugs with activity towards other cancers usually fail, and no standard drug therapy is accepted for ACCs (4-7).

Gastrointestinal stromal tumors are malignant neoplasias of the digestive tract, which express the c-Kit tyrosine-kinase transmembrane receptor. They share resistance towards all known cytostatic therapeutics with the ACCs. The discovery of imatinib mesylate as a specific inhibitor of only a few kinases, including c-Kit, resulted in its introduction into clinical trials. These demonstrated marked antitumor activity, which led to the recognition of imatinib mesylate as the standard medical treatment for this condition.

The observation that the majority of ACCs express the c-Kit receptor (8-11), in conjunction with the fact that treatment with imatinib mesylate causes only mild side-effects, motivated us to investigate whether this drug exerted antineoplastic activity against ACCs. We offered this treatment option to a small number of patients based on individualized clinical decision making.

Patients and Methods

Patients were considered eligible, if they fulfilled the following criteria: i. a histopathologically confirmed diagnosis of ACC no matter where the tumor originated; ii. performance status sufficient to tolerate mild toxicity; iii. presence of distant, measurable metastases; iv. positive staining of tissue samples for either c-Kit or platelet-derived growth factor receptor β.

All biopsy and resection specimens were fixed in 10% buffered-formalin and embedded in paraffin. Deparaffinized serial sections were stained using hematoxylin and eosin stain. For immunohistochemistry, serial sections were cut at 3 μm and placed

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on Superfrost Plus glass slides. Immunostaining was performed with antibodies directed against CD117 (polyclonal; dilution 1:2000; WAK-Chemie Medical GmbH, Steinbach, Germany) and PDGFR-β (monoclonal; 1:20; R&D Systems; Wiesbaden, Germany). For immunostaining, sections were deparaffinized in xylene and rehydrated in an alcohol series. Immunostaining with anti-CD117 was performed using the Ventana Basic DAB Detection Kit and the Ventana Nexus-immunostainer. Endogenous peroxidase was blocked for 4 min at 37°C, according to the manufacturer’s instructions. The primary antibody was incubated for 24 min at 37°C. The biotinylated secondary antibody, the avidin-horseradish-peroxidase conjugate and the basic DAB solution were applied, according to the manufacturer’s instructions. The reaction was enhanced with copper sulfate solution (4 min at 37°C).

For immunostaining with anti-PDGF-β, sections were incubated with primary antibody for 1 h at 37°C in a moist chamber, followed by incubation with biotinylated anti-mouse IgG/anti-rabbit IgG (1:200; Vector Laboratories; distributed by Camon, Wiesbaden, Germany) and ABC alkaline phosphatase reagent, each for 30 min at room temperature. Immunoreactions were visualized with the avidin biotin complex method, applying a Vectastain ABC alkaline phosphatase kit (distributed by Camon). Fast Red (Zytomed, Berlin, Germany) and ABC alkaline phosphatase reagent, each for 30 min according to the manufacturer’s instructions. The primary antibody was incubated for 24 min at 37°C.

All specimens were counterstained with hematoxylin. Primary antibodies were omitted for negative controls and tissue specimens recommended by the manufacturers were used as positive controls.

All patients gave written informed consent after all aspects of the intended therapy, including its experimental nature and possible side-effects, had been explained in-depth. For the first days of therapy, the patients were admitted to a patient ward to monitor potential adverse effects. After this, drug therapy continued on an ambulatory basis. Follow-up visits including imaging were scheduled after 4 weeks, 3 months and further, as needed.

Results

We treated 4 patients with ACCs with imatinib mesylate, and the results from immunohistochemical stainings of archival biopsy-samples are provided in Table I. The patients’ characteristics and outcomes are summarized in Table II.

Case Reports

First patient. At initiation of therapy the first patient was 41 years old. Her tumor originated from the right parotid gland. The tympanic cavity and the outer auditory meatus were infiltrated. The ipsilateral seventh cranial nerve was partially paralyzed. At the time of diagnosis, multiple, bilateral lung metastases were present. Her clinical stage was T4N2M1. Initially, a large biopsy (R2) from the deep parotideal lobe was taken and an ACC, solid-variant, diagnosed, followed by radiotherapy of the base of skull (50.4 Gy + boost 20 Gy). Six months after the first diagnosis, the lung metastases were enlarging, the patient experienced more cough and combination chemotherapy with cisplatin, doxorubicin and fluorouracil was begun and continued for 6 cycles. Oral chemotherapy with fluorouracil ensued for 9 months. Chemotherapy with carboplatin and paclitaxel followed, but had to be discontinued due to rhabdomyolysis. Thereafter, single-agent palliative therapy with gemcitabine was initiated. After the addition of vinorelbine, the lung metastases responded transiently. However, all of these systemic treatments were unable to stop the progressive course of the disease. Two years and 9 months after the initial diagnosis, whole lung radiotherapy was applied (18 Gy), followed by a boost to the lower mediastinum and both hili (28 Gy). Transiently, some metastases appeared smaller after this intervention. The further course was characterized by the occurrence of asymptomatic brain metastases, symptomatic bone filiae and a locoregional relapse. Radiotherapy was initiated for the bone manifestations and the local recurrence. Paraffin-sections from this patient revealed negativity for CD117. However, additional analysis showed expression of the PDGFB-receptor, another potential target of imatinib mesylate. Therapy with imatinib mesylate was begun but, although her disease status and the general condition, albeit limited, were stable during the weeks before, the patient died 3 days later – 3 years and 9 months after the first diagnosis of her malignant disease. No experimental treatment would have been initiated could the short prospective lifetime have been foreseen with sufficient probability.

Second patient. At diagnosis the patient was 29 years old. He presented with an inoperable ACC of the trachea. Primary megavoltage therapy was delivered to a total dose of 70 Gy.

Table I. Immunohistochemical results.

<table>
<thead>
<tr>
<th>Patient number</th>
<th>c-Kit-receptor (CD117)</th>
<th>PDGFB-receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>negative</td>
<td>positive (nuclear and cytoplasmic)</td>
</tr>
<tr>
<td>2</td>
<td>10-20% positive</td>
<td>strongly-positive in &gt;50%</td>
</tr>
<tr>
<td>3</td>
<td>positive</td>
<td>weakly-positive in &lt;5%</td>
</tr>
<tr>
<td>4</td>
<td>strongly-positive</td>
<td>moderately-positive in approx. 50% (cytoplasmic)</td>
</tr>
<tr>
<td>(membrane and in part cytoplasmic)</td>
<td></td>
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</tr>
</tbody>
</table>
The tumor showed a major response and, for the remaining course of the disease, did not produce any other symptoms. Seven months later, CT-imaging was suggestive of lung metastases – a suspicion that was confirmed through further controls. One month later, a single bone metastasis developed in the right humerus, which was treated by radiation therapy (39 Gy). More than a year after the first appearance of the lung filiae, they progressed. In addition, a bone scan performed 3 months later confirmed the presence of multiple bone metastases. Palliative radiation to the 12th thoracic vertebra, the lumbar spine and os sacrum was necessary (39 Gy). At this time additional immunohistochemical studies on his archival paraffin-embedded samples were undertaken. Ten to 20% of the tumor cells were positive for CD117 (c-Kit), but more than 50% stained strongly-positive for the PDGFβ-receptor. Imatinib mesylate therapy was initiated. During the following days, the patient reacted with sweating and slightly more pronounced bone pain. Both symptoms may have been related to the medication, although it is equally possible that they were mainly caused by the malignant disease. Six weeks after the start of imatinib mesylate, i.e. 3 and a half years after the diagnosis, the patient died from respiratory failure due to diffuse, progressive involvement of both lungs.

Third patient. The 61-year-old, male patient presented with a locally advanced ACC of the floor of the mouth and the mandible. Computed tomography (CT) and sonography showed hepatic metastases in both liver lobes. Initially, a biopsy extra mural yielded a low-grade mucoepidermoid carcinoma. A repeat biopsy, done as part of the local staging, demonstrated an ACC. The liver metastases were confirmed by liver biopsy. After tracheotomy, the tumor was resected en bloc with the suprahyoidal soft tissues and the continuity of the mandible restored with a titan-plate. The pathological stage was pT4pN0M1. Three months after surgery, CT-imaging indicated progression of the liver metastases and the occurrence of lung filiae.

Immunohistochemical analysis of metastatic cells obtained through liver biopsy established that more than 50% of the cells were positive for CD117 (c-Kit), while only a weak signal could be obtained for the PDGFβ-receptor. Imatinib mesylate therapy was started approximately 4 months after the initial diagnosis and was well tolerated. On readmission 1 month later, the patient complained about bone pain and mild constipation. These symptoms were probably related to the progression of his disease and the pain medication, respectively. They were treated symptomatically. A control CT-scan of the whole body unambiguously demonstrated a massive further growth of the hepatic tumor manifestations, slight progression of the pulmonary nodules, a small pleural effusion and newly developed bone metastases, predominantly in the spine. Medication with imatinib mesylate was discontinued. Six months after the diagnosis of his cancer, the patient is still alive.

Fourth patient. The patient was radically operated for an ACC of the left maxilla. Five years later, a locoregional lymph node metastasis was excised from the left submandibular region. Two years after this, a locoregional relapse developed, which, due to its extent, was resected incompletely. Postoperatively, the involved area was treated by radiotherapy (60 Gy). Pulmonary metastases occurred, so that 2 years later, 12 cycles of polychemotherapy with gemcitabine/vinorelbine were administered. During this time the lung filiae remained stable. Two years and 4 months later the lung metastases progressed and the patient again received 6 cycles of the same polychemotherapy. Transient disease stabilization could be achieved. However, 12 years after the first diagnosis of ACC and 5 years after the local recurrence, CT-imaging of the left maxillary sinus strongly suggested a second local relapse. This was supported by increased isotope-uptake on bone scintigraphy. At this time, the lung metastases had been
stable in size for roughly half a year. Immunohistochemical analysis of archival sections for CD117 (c-Kit) showed a marked membrane-bound reaction of the tumor cells (Figure 1). Therapy with imatinib mesylate was begun. On follow-up 4 months later, the patient reported fatigue, intermittent headache and an increased frequency of hematomas. A CT of the lungs at this time revealed a no change-status of the lung metastases as did another control 3 months later. She continues to be treated.

Discussion

The experience of the Princess Margaret Hospital in London, UK, with imatinib mesylate treatment of patients with mainly metastasized (at least 88%) ACCs expressing c-Kit, has recently been published (12). The researchers observed no responses, even though the dose was twice as high (400 mg bid) than the one given to our patients (400 mg once daily). Nine out of 16 patients showed stable disease as best response. Our results are in line with these data. No patient responded and only one exhibited a stable course of lung metastases. However, it is important to keep in mind that it is not unusual that ACCs exhibit a protracted course. In particular, the one patient with stable lung metastases has a disease history exceeding 12 years. In this case, the constant lung involvement during follow-up may well be due to the slow natural history of the disease and cannot, with any certainty, be attributed to the experimental therapy.

On the other hand, two Panamanian patients with ACC were reported as having achieved major remissions through

Figure 1. The adenoid-cystic carcinoma of patient No. 4 shows a membranous expression of c-Kit. Hematoxylin and eosin (H&E); anti-CD117 (CD117), hematoxylin counterstain; Original magnifications: x200 (H&E) and x400 (CD117).
treatment with imatinib mesylate (13). These two patients differ from those at the Princess Margaret Hospital and from ours in that their tumors were locally advanced either at initial presentation or at local relapse after prior treatment, but were reported as being free from distant metastases.

Several possibilities could explain the lack of activity of imatinib mesylate in patients with metastatic ACC: for example, among 127 examined gastrointestinal stromal tumors, all of the responding tumors carried a mutation in the gene for the c-Kit receptor (14). These authors also reported that the response rate to imatinib depended on the exact type of mutation present: exon 11 (corresponding to the cytoplasmic part of the protein adjacent to the cell membrane): 83.5%, exon 9 (external part also adjacent to the cell membrane): 47.8%, no mutation of c-Kit or platelet-derived growth factor receptor α: 0%. These differences translate into similar differences in event-free survival and overall survival as revealed by Kaplan-Meier analysis.

The tumors of the patients described here were not tested for potential mutations. However, the lack of clinical responsiveness suggests that no comparable, drug-sensitizing alterations were present. No mutations in exons 11 or 17 of the c-Kit gene were identified in two series of ACC-specimens comprising 27 and 20 samples, respectively (8, 9).

The treatment results reported here are reminiscent of those published for small cell lung cancer (SCLC). SCLCs express c-Kit – and its ligand, stem cell factor –, but no mutations of c-Kit have been described so far (15). In a small phase II study, therapy with imatinib mesylate was ineffective (16). A case report highlights the different reactions of mutated versus non-mutated c-Kit-receptors towards imatinib mesylate: a patient with chronic myeloid leukemia achieved a molecular complete remission through treatment with imatinib mesylate (17). While on continuous therapy with 600 mg/day of the drug, the patient developed reactions of mutated versus non-mutated c-Kit receptors.

An alternative explanation for the failure of imatinib mesylate to improve the outcome of patients with distant metastases – better able to reconcile the cited reports – assumes a spontaneous disease evolution. A well documented example is chronic myelocytic leukemia (CML). Initially, the BCR-ABL fusion protein is essential for the proliferation of the malignant clone. However, during the progression of CML through accelerated and blastic phases, additional genomic alterations develop, the sensitivity towards imatinib mesylate decreases and, ultimately, the disease becomes entirely resistant. If this model should hold true for ACCs, then the lack of response in patients with metastatic ACC would come as no surprise.

The clinical course of the patients with ACC treated in London and by us speaks against a therapeutic role of imatinib mesylate for patients with distant metastases from this rare, but frequently fatal cancer. Nonetheless, the positive report from Panama may indicate that neoadjuvant or adjuvant imatinib mesylate therapy should be evaluated for patients with localized disease.

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References


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