New agents for treatment of advanced transitional cell carcinoma

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Received 8 February 2006; revised 4 August 2006; accepted 10 August 2006

The prognosis for any patient with progressive or recurrent invasive transitional cell carcinoma remains poor. In this context, the focus of clinical research in these invasive cancers concentrates on identifying systemic treatment options and new agents in order to improve survival of patients. Cisplatin-based chemotherapy is standard treatment of patients with metastatic urothelial cancer; however, despite regimens as the cisplatin–gemcitabine combination, the overall response rates vary between 40% and 65%, with complete response in 15%–25% with survivals up to 16 months. This survival is frequently achieved with severe and life-threatening side effects. Nonetheless, almost all responding patients relapse within the first year; therefore, the need for development of new and tolerable agents is urgent. This review highlights some new active chemotherapeutic as new platinum compounds (oxaliplatin, lobaplatin), gallium nitrate, ifosfamide, the antifolates piritrexim and pemetrexed (Alimta/C226, LY231514), vinflunine and molecular targeting agents such as farnesyltransferase inhibitors (lonafarnib, R115777, SCH66336), ribozyme (RPI.4610), histone deacetylase inhibitor (CI-994) and monoclonal antibodies (epidermal growth factor receptor, Her 2/neu).

Key words: transitional cell carcinoma, gallium nitrate, vinflunine, platinum compounds, antifolates, molecular targeting agents

Introduction

In Europe, bladder cancer is the fourth most frequent cancer among men accounting for ~7% of total cancers. A total of 136 300 new cases are estimated each year. The annual incidence rate is 32 of 100 000 in men and nine of 100 000 in women, while the annual mortality rate is 11 of 100 000 for men and four of 100 000 for women. There are ~49 000 deaths/year from bladder cancer in all European countries [1]. For the majority of patients with deeply invasive tumors and regional or distant metastases, cure is not possible. In Europe and North America, the standard treatment of patients with invasive bladder cancers is radical cystectomy and urinary diversion followed or preceded by chemotherapy. Traditionally, surgery alone has resulted in <50% survival in patients with muscle-invasive bladder cancer. In this context, the focus of clinical research in invasive bladder cancer concentrated on identifying systemic treatment options in order to improve survival of patients. Metastatic transitional cell carcinoma (TCC) is moderately sensitive to chemotherapy and there is a huge variation in reported response and survival rates in bladder cancer patients treated in different studies using either single or multidrug regimen. Although survival differences reflect the different levels of activity of individual regimens, the relevance of pretreatment factors (e.g. patient selection) on outcome might be another reason for the differences in survival. Combination chemotherapy has been considered the standard care for patients with metastatic bladder cancer for decades because it has been found to be superior to the use of single agents [2]. Cisplatin-based combination chemotherapy regimens, such as methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC) and cisplatin, methotrexate and vinblastine, were standard treatment of patients with metastatic urothelial cancer [3–7]. The overall response rates (ORR) to ’standard’ cisplatin-based combination regimen have varied between 39% and 65%, with complete response (CR) in 15%–25% and median survivals up to 16 months [4, 8–11]. None the less, almost all responding patients relapse within the first year with a median survival of ~12 months [12]. The M-VAC combination continued to be the most common treatment regimen for advanced urothelial cancer until in a study by von der Maase et al. [13] the cisplatin–gemcitabine (GC) combination was compared with classical M-VAC as first-line therapy in a multicenter randomized phase III trial. Six cycles of each of the regimens were given to 405 patients with T4b and/or node-positive and/or distant metastatic disease. At a median follow-up of 19 months, the regimens were not statistically different in terms of overall survival, time to disease progression or response rate. The GC combination, however, was superior in terms of tolerability and effects on quality of life. In particular, GC resulted in a lower incidence of
treatment-related mortality and fewer episodes of complicated myelosuppression, oral mucositis and alopecia (resulting in fewer dose adjustments) and lesser effects on weight, performance status and fatigue compared to the MVAC group. As a result, the GC combination is now widely considered to be a (second) standard of care, along with M-VAC, for patients with advanced bladder cancer, though there is some disagreement regarding the reported benefits [14]. To compare long-term survival, the authors evaluated the efficacy data of the GC versus M-VAC study after 5 years. Time-to-event analyses were performed on the observed distributions of overall and progression-free survival. At the time of analysis, 347 out of 405 patients had died (GC arm: 176 patients; M-VAC arm: 171 patients). Overall survival was similar in both arms with a median survival of 14.0 months for GC and 15.2 months for M-VAC. The 5-year overall survival rates were 13.0% and 15.3%, respectively. The median progression-free survival was 7.7 months for GC and 8.3 months for M-VAC, with a hazard ratio of 1.09. The 5-year progression-free survival rates were 9.8% and 11.3%, respectively. Significant prognostic factors favoring overall survival included performance score (>70), TNM staging (M0 versus M1), low/normal alkaline phosphatase level, number of disease sites and the absence of visceral metastases. By adjusting for these prognostic factors, the hazard ratio was 0.99 for overall survival and 1.01 for progression-free survival. The 5-year overall survival rates for patients with and without visceral metastases were 6.8% and 20.9%, respectively [15]. Still, the discussion if GC is the new standard treatment is ongoing, and the more favorable risk-benefit ratio seems to promote this. The ongoing current European Organization for Research and Treatment of Cancer (EORTC) trial 30987 (Taxotere; Bristol-Myers Squibb, Ruell-Malmaison, France)–GC versus GC in advanced urothelial cancer might, however, add new information to the situation.

The prognosis for any patient with progressive or recurrent invasive bladder cancer, however, remains poor [16, 17]. Even with chemotherapeutic treatment, the overall median survival is ~1 year. This survival is frequently achieved with severe and life-threatening side effects. Therefore, the need for development of new and tolerable agents is urgent. This review highlights some new active chemotherapeutic and molecular targeting agents.

**platinum compounds (oxaliplatin, lobaplatin)**

Given the established efficacy of carboplatin for patients with advanced/metastatic bladder cancer, other platinum-based cytotoxic drugs may also find their place.

Oxaliplatin has been shown to have efficacy in a variety of phase II trials and to be well tolerated in other solid tumors less associated with the nephrotoxicity of cisplatin and myelosuppression of carboplatin. There is some published data on urothelial carcinoma. A study by Moore et al. [18] demonstrated a disappointing single-agent response rate in a largely pretreated patient group, with a partial response (PR) rate of only 6%. In a combination study with gemcitabine, a considerable toxicity and poor response (nine of 13 patients had progressive disease under treatment) were found [19]. In another study presented at the 2004 American Society of Clinical Oncology (ASCO) annual meeting, Font et al. [20] studied gemcitabine and oxaliplatin in 36 pretreated ‘ unfit’ (mainly due to renal function) bladder cancer patients. The ORR was 16.6% (six PR). The same combination was evaluated in a pilot trial by Culine et al. [19], 20 patients received bimonthly cycles of 1500 mg/m² gemcitabine and 85 mg/m² oxaliplatin. Thirteen patients were treated with the gemcitabine-oxaliplatin (GO) combination as first-line chemotherapy because of a poor performance status or a creatinine clearance <1 ml/s. The median number of cycles of GO was five (range one to seven). The median number of days between cycles was 14 throughout the treatment. Seven (8%) out of 87 cycles had to be delayed because of neutropenia or asthenia. A 25% dose reduction in the doses of cytotoxic drugs was necessary in two patients. One possible treatment-related death (myocardial infarction), one grade 3 neuropathy and progressive disease in nine patients occurred. Efficacy was not evaluated.

Winquist et al. [21] from London, Ontario, Canada, evaluated in a double-arm two-stage phase II study, patients with previously treated measurable unresectable or metastatic TCC stratified in ‘cisplatin sensitive’ or ‘cisplatin resistant’ on the basis of prior cisplatin treatment. Twenty patients were treated with 130 mg/m² oxaliplatin every 3 weeks. A median of two treatment cycles (range one to six) were administered. One PR was observed in 10 cisplatin-sensitive patients, and no responses occurred in eight cisplatin-resistant patients. The most common side effects were fatigue, neuropathy and nausea. Hematologic toxic effects were grade 2 or less. Eleven patients (55%) experienced non-hematologic toxicity of grade 3 or 4, and one patient died of a pulmonary embolism after the first cycle.

Lobaplatin is a third-generation platinum complex with DNA-alkylating activity originally developed by ASTA Medica (Degussa; Frankfurt, Germany), and then by Zentaris AG (AÉterna Laboratoires; AÉterna Zentaris Inc., Québec, Canada), which licensed lobaplatin to Hainan Tianwang in China (Hainan Tianwang International Pharmaceutical; Tianwang, Haikou, China). Lobaplatin has been approved in China for the treatment of chronic myelogenous leukemia and inoperable metastatic breast and small-cell lung cancer. It has, however, not yet been launched there. The EORTC assessed lobaplatin in a trial with pretreated bladder cancer patients with a disappointing response rate of 10% [22].

**gallium nitrate**

Although early clinical trials indicated that gallium nitrate, a ‘near metal’ compound, had activity against bladder cancer, its subsequent development centered primarily on its effect on bone metabolism and not on its antineoplastic activity. As a result, the drug was approved for the treatment of hypocalcaemia of malignancy. Pharmaceutical production of gallium nitrate, however, ceased during the late 1990s, stopping several gallium-based clinical trials. Gallium nitrate has recently awakened commercial interest and an oral formulation of gallium is in development. Gallium’s mechanisms of action include its targeting and binding transferrin and
transferrin receptors and inhibiting ribonucleotide reductase. Recent investigations show that gallium activates caspases and induces apoptosis through the mitochondrial pathway [23]. McCaffrey et al. [24] at the Memorial Sloan-Kettering Cancer Centre, New York (MSKCC) conducted a randomized phase II trial of gallium nitrate (350 mg/m², days 1–5) plus fluorouracil (5-FU) (1000 mg/m², days 1–5) repeated every 28 days versus gallium nitrate 700 mg/m²: for RTx, radiotherapy.

CR, complete response; CSF, cerebrospinal fluid; CTx, chemotherapy; n, number; OR, overall response rate; PR, partial response; pts, patients; RTx, radiotherapy.

**Table 1. Clinical phase II studies with single-agent gallium nitrate and in combination with other agents**

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Pretreated ORR and response</th>
<th>Toxicity</th>
<th>Reference</th>
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<tr>
<td>Gallium nitrate 700 mg/m²; initially every 2 weeks. Due to nephrotoxicity in four of the first 10 patients, the remaining 24 patients every 3 weeks with dose escalation to 1000 mg/m² (if no toxicity occurred)</td>
<td>34 pts, 28 pts</td>
<td>Mixed, prior CTx (17 pts)</td>
<td>27% (one CR, six PR), 4 months</td>
<td>Dose-limiting toxicity: nephrotoxicity, gastrointestinal toxicity and myelosuppression minimal. [25]</td>
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<td>Gallium nitrate 350 mg/m²: for five consecutive days every 3 weeks. Phase I: Dose finding with starting dose of 150 mg/m² for five consecutive days every 3 weeks escalated in 50 mg/m²</td>
<td>23 pts (phase II), 17 pts (phase I)</td>
<td>Yes</td>
<td>17.4% (one CR, 3 PR), 4 months</td>
<td>Myelosuppression minimal. Dose-limiting toxicity: reversible optic neuropathy. [26]</td>
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<tr>
<td>Gallium nitrate 350 mg/m²: for seven consecutive days every 3 weeks</td>
<td>eight pts</td>
<td>Yes</td>
<td>63% (four CR lasting 4 month to 3 years)</td>
<td>Hypocalcemia, nausea, anemia. [27]</td>
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<tr>
<td>Gallium nitrate 300 mg/m², days 1–5 with calcium 0.5 μg/day starting 3 days before each course (except the first); vinblastine 0.11 mg/kg, days 1 and 2; ifosfamide 1.2 g/m², days 1–5 (with Mesna); rhG-CSF 5 μg/kg/day, days 7–16; cycle repeated every 21 days</td>
<td>27 pts</td>
<td>Mixed (four pts CTx/RTx)</td>
<td>67% (11 CR, five with vinblastine, ifosfamide, and gallium nitrate (VIG) alone and six with subsequent surgery), lasting 5 months</td>
<td>Hypocalcemia, nausea, anemia. [28]</td>
</tr>
<tr>
<td>Gallium nitrate 300 mg/m², days 1–5 with calcium 0.25 μg/day; vinblastine 0.11 mg/kg, days 1 and 2; ifosfamide 1.2 g/m², days 1–5 (with Mesna); rhG-CSF 5 μg/kg/day, days 7–16; repeated every 21 days for a maximum of six cycles</td>
<td>45 pts</td>
<td>No</td>
<td>44% (six CR, 14 PR), duration of response 11.7 months, survival 10 months</td>
<td>Two early deaths. [29]</td>
</tr>
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Ifosfamide is an alkylating agent with modest activity in advanced TCC. The Eastern Cooperative Oncology Group evaluated single-agent ifosfamide in a group of patients who had progressive disease after receiving systemic chemotherapy. Of the 56 eligible patients entered into the study, there were five CR and six PR with an ORR of 20%. Major toxic effects were gastrointestinal, hematologic, renal and in the central nervous system (CNS). Concerns about significant renal and CNS toxicity led to a change of ifosfamide dosing halfway through this study. There were four early deaths to which the treatment probably contributed [30]. Nevertheless, ifosfamide has been evaluated as combination partner to other active regimen [31–35]. It, however, is too early to determine the activity of this agent in polychemotherapy regimen, but dosage is difficult and adds to toxicity.
antifolates piritrexim and pemetrexed (Alimta®, LY231514)

Oral piritrexim, a second-generation antimetabolite is a lipid-soluble inhibitor of dihydrofolate reductase that enters tumor cells rapidly by passive diffusion. Bioavailability after oral dosing is ~75%. The clinical studies are summarized in Table 2 [36–38]. Piritrexim has a low activity and high toxicity in patients with previously treated TCC of the bladder. Pemetrexed (Alimta®, LY231514) is a novel antifolate antimetabolite with multiple enzyme targets involved in both pyrimidine and purine synthesis. The agent has also been demonstrated broad antitumor activity in phase II trials in a wide variety of solid tumors. Clinical activity has also been demonstrated when pemetrexed is combined with cisplatin and gemcitabine. The most significant toxic effects, myelosuppression and mucositis, can be ameliorated by folate and vitamin B12 supplementation [39]. The first study of pemetrexed in patients with urothelial cancer was presented by Sweeney et al. [40] for the Hoosier Oncology Group, at the 39th ASCO annual meeting in 2003. The presentation summarized preliminary data of a multi-institutional phase II trial of pemetrexed in patients in whom one first-line regimen for metastatic disease had failed or relapsed within 1 year after adjuvant/neo-adjuvant treatment. Pemetrexed 500 mg/m² was administered as a 10-min infusion on day 1 of a 21-day cycle. Patients received oral low dose (400 µg) daily folic acid and vitamin B12 (1000 µg) administered intramuscularly by approximately every 9 weeks beginning 1–2 weeks before the first pemetrexed dose. Dose adjustments and delays in subsequent cycles of 25%–50% were based on grade 3 or 4 nadir granulocyte, platelet counts or non-hematologic toxicity. Dexamethasone 4 mg was administered for 3 days peri-treatment as prophylaxis of cutaneous reactions. Thirty-one of 43 planned patients entered the study with 17 assessable for efficacy. The ORR following treatment was 27%. The average time to progression was 2.4 months and the average overall survival was nearly 10 months. At 1 year following therapy, ~35% of patients were still alive {one CR, five PR [95% confidence interval (CI) 14.2–61.7%]. The most frequent grade 3/4 events in 22 patients assessable for toxicity included fatigue (four/zero patients), thrombocytopenia (two/zero patients) and hypertension (one/one patients). One patient each experienced grade 3 depression, constipation, syncope, coronary artery disease, hypotension, seizure, elevated alanine aminotransferase (ALT), febrile neutropenia or urinary tract infection. Dose adjustment or delay was required in four of the 16 (25%) patients.

At the 41st ASCO annual meeting in 2005, von der Maase et al. [41] presented data of a combined treatment with pemetrexed–gemcitabine in patients with locally advanced or metastatic bladder cancer wherein the majority of patients had poor prognostic factors (59% visceral metastases) but had not received prior chemotherapy. The patients received 1500 mg/m² gemcitabine (days 1 and 8) and 500 mg/m² pemetrexed on day 8 after the gemcitabine application. All patients received supplementation of folic acid, vitamin B12 and dexamethasone. The 62 enrolled patients were given a median of four cycles (range one to nine), for a total of 191 cycles. There were 20 dose reductions (nine pemetrexed, 11 gemcitabine), 51 dose delays (31 pemetrexed, 20 gemcitabine) and four dose omissions (two pemetrexed, two gemcitabine). The ORR was 26.5% (95% CI 14.2% to 38.9%) with three CR and 10 PR. The median overall survival was 10.1 months (95% CI 7.7–14.0 months). In all, 60 patients were analyzed for toxicity; grade 3/4 hematologic toxic effects included anemia (13.3%), thrombocytopenia (10.0%), neutropenia (36.7%), febrile neutropenia (18.3%) and neutropenic sepsis (3.3%). Grade 3/4 non-hematologic toxic effects included elevated aspartate aminotransferase (4.0%) and ALT (8.4%), fatigue (8.4%), stomatitis (4.0%), skin toxicity (2.2%) and dyspnea (8.3%). There was one toxic death. Further data collection and analyses including efficacy analyses of the 13 nonassessable patients in the intention-to-treat population are ongoing. The final data on efficacy and safety will have to be awaited. To date, it is not obvious that the combination is superior to single-agent gemcitabine as similar results.

Table 2. Clinical phase II studies with piritrexim

<table>
<thead>
<tr>
<th>Drug</th>
<th>n</th>
<th>Pretreated</th>
<th>ORR and response</th>
<th>Toxicity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piritrexim 3 × 25 mg: five consecutive days/week for three consecutive weeks followed by a 1-week rest; repeated every 28 days</td>
<td>35 pts, 28 pts assessable</td>
<td>Yes</td>
<td>7% (two PR lasting median 4.2 months)</td>
<td>Grade 3 and 4 myelosuppression and neuropathy hepatotoxicity (two), nausea (two), rash (one), pulmonary toxicity (one)</td>
<td>[36]</td>
</tr>
<tr>
<td>Piritrexim 3 × 25 mg: five consecutive days/week for three consecutive weeks followed by a 1-week rest; if tolerated, dose was increased to 3 × 50 mg</td>
<td>17 pts, 13 pts assessable</td>
<td>Yes</td>
<td>23% (three PR lasting 2, 8 and 14 months)</td>
<td>Myelosuppression, two pts died on treatment</td>
<td>[37]</td>
</tr>
<tr>
<td>Piritrexim 3 × 25 mg: five consecutive days/week for three consecutive weeks followed by a 1-week rest</td>
<td>33 pts, 29 pts assessable</td>
<td>Yes</td>
<td>33.3% (one CR, 10 PR lasting 4–12 months)</td>
<td>Myelosuppression requiring dose modification</td>
<td>[38]</td>
</tr>
</tbody>
</table>

CR, complete response; n, number; ORR, overall response rate; PR, partial response; pts, patients.
may have been obtained by gemcitabine alone. Unfortunately, these trials and others [‘Pemetrexed disodium in treating patients with locally advanced or metastatic recurrent cancer of the urothelium’ (study ID numbers: CDR0000069367, UCLA-0112033, LILLY-H3E-MC-)MEU, NCI-G02-2072) and ‘Phase 2 trial of ALIMTA (pemetrexed) plus gemcitabine in locally advanced or metastatic TCC of the urothelium’ (study ID number: NCT00034593)] have not been published yet.

**vinflunine (VFL)**

Vinflunine (VFL) is a novel semisynthetic vinca alkaloid showing in vivo antitumor activity in an orthotopic murine model of TCC of the bladder [42]. In a phase II trial, 53 pretreated patients with advanced TCC were given 350 mg/m² VFL every 21 days. Due to three episodes of fatal neutropenic sepsis and other toxicity, dose reduction and dose delays were necessary. Grade 3/4 neutropenia occurred in 37% of patients. There was no CR but nine PR. At present, the drug seems difficult to dose and has considerable toxicity with limited efficacy [43].

**molecular targeting agents**

In the following, some of the first clinical trials in urothelial cancers using a molecular-targeting approach are described.

**farnesyltransferase inhibitors (lonafarnib, R115777, SCH66336)**

Protein farnesylation by farnesyltransferase (FTase) is required for membrane localization and effective signal transduction by G-proteins, including Ras. Lonafarnib inhibits FTase and has shown antitumor activity in both preclinical and clinical settings [44–46]. As disturbances in Ras-signaling pathways have been implicated in the pathogenesis of TCC [47], the antitumor activity of lonafarnib was studied in a National Cancer Institute of Canada Clinical Trials Group phase II trial in 19 patients with previously treated TCC. The patients had at least one prior chemotherapy regimen for advanced unresectable or metastatic TCC or recurrence <1 year after adjuvant or neo-adjuvant chemotherapy. Lonafarnib was given at an oral dose of 200 mg twice daily continuously, with cycles repeated every 4 weeks. Median time on treatment was 7.1 weeks (range 0.6–23.9 weeks). Drug-related grade 3 toxic effects included fatigue, anorexia, nausea, confusion, dehydration, muscle weakness, depression, headache and dyspnea. Five patients discontinued the study protocol due to toxicity. No responses were observed in 10 patients who were assessable. Of nine patients not assessable for response, five had symptomatic progression, fulfilling the criteria to stop the study [48].

In a multicenter EORTC study, the pharmacokinetics and activity of a combined therapy with FTase inhibitor SCH66336 and gemcitabine in patients with advanced urothelial tract cancer were evaluated. Patients were treated with SCH66336 (150 mg in the morning and 100 mg in the evening) and gemcitabine (1000 mg/m² on days 1, 8 and 15 per 28-day cycle). A total of 152 cycles were administered in 33 patients (median 3, range 1–15). Toxicity was acceptable with no severe hematologic effects, nine PR and one CR were achieved in 31 assessable patients and corresponded to an ORR of 32.3% (95% CI 17% to 51%). There was no influence of exposure to SCH66336 on the plasma level of gemcitabine or 2’-difluorodeoxyuridine (dFdU) in 11 assessable patients [49].

In another phase II trial, FTase inhibitor R115777 at a dose of 300 mg orally given twice daily for 21 days followed by 7 days of rest for every 4-week cycle was examined in 34 patients with metastatic TCC. Patients were allowed to have one prior systemic chemotherapy regimen, not chemoradiation or neo-adjuvant chemotherapy. The results showed that R115777 was absorbed rapidly after oral administration and was generally tolerated well. Grade 3/4 neutropenia was observed in five patients (15%). Grade 3/4 non-hematologic toxicity was rare, consisting of rash and diarrhea in one patient each. Two patients (6%) without prior chemotherapy demonstrated PRs. Thirteen patients (38%) achieved disease stabilization that lasted a median of 4 months. No CRs were observed [50]. To date, no further studies in TCC with FTase inhibitor as single agent or in combination are reported.

**ribozyme (RPI.4610)**

RPI.4610 (Angiozyme) is a chemically stabilized ribozyme-targeting vascular endothelial growth factor receptor 1 (VEGF-R1). A study by Kobayashi et al. [51] evaluated the safety and pharmacokinetics of RPI.4610 in combination with carboplatin and paclitaxel in 12 patients with advanced solid tumors (including patients with TCC). The study used a sequential treatment design evaluating a single dose level for all three drugs: paclitaxel 175 mg/m² and carboplatin area under the curve (AUC) = 6 on day 1 of a 21-day cycle together with RPI.4610 100 mg/m² per day beginning on day 8 and continuing daily thereafter. The toxic effects were found to be grade 3/4 neutropenia, thrombocytopenia, pain (each three patients) and anemia and fatigue (each two patients). The ratio of the mean maximum plasma concentration (Cmax) for carboplatin when administered with paclitaxel alone versus when administered with paclitaxel and RPI.4610 was 1.07 (90% CI 0.77–1.37). For paclitaxel, the ratio of the mean Cmax when administered with carboplatin alone versus with carboplatin and RPI.4610 was 1.17 (1.03–1.31). One complete tumor response was observed in a patient with bladder cancer; one patient with an esophageal cancer achieved a PR. This principal evaluation showed that a ribozyme-targeting VEGF-R (RPI.4610) can be administered safely in combination without substantial pharmacokinetic interactions with carboplatin and paclitaxel. Further efficacy or safety data are to date not available.

**histone deacetylase inhibitor (CI-994)**

Histones are small basic proteins that, by complexing with DNA, form the nucleosome core. Repetitive units of this nucleosome led to the chromatin in which all the human genome is packaged. Histones can be in one of the two antagonist forms, acetylated or deacetylated, equilibrium regulated by the corresponding enzymes, histone acetylases and histone deacetylases (HDACs). Inhibition of HDACs represents a new strategy in human cancer therapy since these enzymes...
play a fundamental role in regulating gene expression and chromatin assembly. They are potent inducers of growth arrest, differentiation and apoptosis of tumor cells. A second generation of HDACs, synthetic benzamide-containing HDACs such as CI-994, have reached phase I and II clinical trials [52, 53]. In a phase I study by Pauer et al. [54], the maximum tolerated dose of CI-994 was determined in combination with carboplatin and paclitaxel in 30 patients with advanced solid tumors, including patients with TCC. Five cohorts of patients were treated with escalating doses (4–6 mg/m²) and alternative schedules (7 or 14 days) of CI-994. Dose escalation of paclitaxel was performed to achieve tolerability of CI-994 with a paclitaxel dose of 225 mg/m² when administered in combination with carboplatin. Maximum tolerated dose of CI-994 was determined to be 4 mg/m² administered for seven consecutive days following paclitaxel at a dose of 225 mg/m² and carboplatin at an AUC of 6 every 21 days. Neutropenia, thrombocytopenia and grade 3 respiratory insufficiency limited further dose escalation of CI-994. Pharmacokinetics showed that CI-994 absorption and disposition were unaffected by carboplatin and paclitaxel coadministration. Association between histone H3 acetylation levels and disease response was suggested. A subset of patients with lymphocyte H3 acetylation levels at least 1.5-fold times baseline achieved either a clinical response or stable disease. All assessable patients with progressive disease had H3 acetylation levels <1.5-fold times baseline. Twenty-four of the 30 patients received greater than one cycle of treatment. Five of these patients achieved a PR [three non-small-cell lung cancer (NSCLC), one colorectal cancer and one unknown primary] and two patients achieved a CR (esophageal and bladder cancer). A phase II study in patients with TCC is not available.

combination trials with monoclonal antibodies (epidermal growth factor receptor, Her 2/neo)

Evaluation of the therapeutic potential of the epidermal growth factor receptor (EGF-R) tyrosine kinase inhibitor (gefitinib, Iressa®; AstraZeneca Pharmaceuticals, London, UK) has been performed in preclinical models of bladder cancer [55–60]. To date, the Cancer and Leukemia Group B is conducting a phase II trial of gemcitabine, cisplatin and gefitinib to evaluate its clinical impact. Iressa, an orally administered EGF-R tyrosine kinase inhibitor, was approved for marketing in May 2003 for patients with NSCLC. The response rate in patients taking the drug was ~10%. The approved indication was for the treatment of patients who were refractory to established cancer treatments [both a platinum drug and docetaxel (Taxotere; Hoffmann-La Roche AG, Switzerland)]. Since the initial approval of Iressa, however, the Food and Drug Administration has carefully reviewed data from two failed clinical studies of Iressa, one of which was required by the agency as part of the drug’s accelerated approval. This trial enrolled patients with regionally advanced or metastatic NSCLC in whom one or two prior treatment regimens had failed. In this large study, 1692 patients were randomized to gefitinib or placebo. There was no significant survival benefit in the overall study population or in patients who had high levels of a surface marker called ‘EGF-R’. It is likely that optimized therapy approaches in bladder cancer will also require an accurate ‘molecular’ diagnosis allowing effective, selective, tailored therapeutic strategies to be designed.

Investigators at the University of Michigan have recently completed a phase II trial of trastuzumab (Herceptin®, Genentech, Inc., South San Francisco, CA), which targets the Her 2/neo receptor that is frequently overexpressed in invasive bladder cancer [61]. Patients with advanced TCC or squamous cell carcinoma that are Her 2/neo positive by immunohistochemistry, serology or fluorescence in situ hybridization in primary or metastatic site are treated with trastuzumab in combination with paclitaxel, carboplatin and gemcitabine. Results reported at the 2005 annual meeting of the ASCO indicated a 73% objective response rate in 44 Her 2-positive patients and a phase III trial may be considered on the basis of the data. [62]

**Conclusion**

Treatment goals for bladder cancer of any stage are complete removal of the initial tumor, prevention of disease recurrence and effective inhibition of progression to advanced disease with the ultimate aim of reducing mortality. Today, the optimal therapy for advanced urothelial carcinoma beyond cystectomy and urinary diversion remains a challenge. To increase the percentage of patients who achieve disease stabilization and prolonged survival and improved quality of life during treatment, numerous novel agents many of which are still in clinical trials are being developed. A new generation of drugs might be applied orally on an outpatient basis with low toxicity. To date, however, the clinical trials with new anticancer agents are limited in their impact on long-term survival and safety. Unfortunately, it has become increasingly clear that the tumor entity bladder cancer is a tumor where reasonable and long-lasting treatment results will not be achieved soon. Especially patients with recurrent or progredient tumor have, to date, no defined second-line chemotherapy option. The prognosis for patients with a progressive cancer is poor. One of the future goals of cancer therapy is to identify the molecular basis of bladder cancer genesis and progression to better define risk and response probabilities for the individual patient and to develop new therapeutic strategies aimed at the specific defects which characterize the specific bladder cancer. Accurate selection of a subgroup of patients most likely to benefit may become possible in a predictable manner in near future. During malignant transformation, tumor cells acquire a series of molecular characteristics resulting in growth factor independence, insensitivity to antiproliferative signals, escape from apoptosis, (neo)angiogenesis, proliferative capacity and the ability for invasion and metastasis. The knowledge of the molecular pathways allows the identification of novel molecular targets. There are enormous potential opportunities for discovery of new molecules therapeutically relevant in bladder cancer. Translational research plays a major role in taking the results of research from the laboratory bench to the bedside. Data pouring forth from high-throughput genomics and proteomics lead to the development of new technologies, ultimately resulting in rational drug design for cancer therapy.
One of the promising molecular-targeted approaches includes antisense therapy. Thereby, antisense oligonucleotides are directed against various molecular targets in bladder cancer cells. These include telomerase, the ribonucleoprotein enzyme that compensates for the progressive erosion of chromosomal telomeres and is often aberrantly expressed in bladder cancers [63–65]; apoptosis-regulating proteins such as Bcl-2, Bcl-X and other Bcl-2 family members [66–72]; VEGF [73, 74]; interleukin-8 [75]; Ki-67 [76]; survivin [77]; transforming growth factor-β [78] and p53 [79]. Clinical data, however, are still lacking. Other approaches target angiogenic mechanisms. Endostatin, an endogenously produced inhibitor of angiogenesis, has been tested as a potential agent for the inhibition of bladder cancer growth. In studies of nude mice, subcutaneous endostatin blocked angiogenesis by decreasing VEGF expression and induced apoptosis in bladder cancer cells [80]. Kikuchi et al. [81] reported that lentiviral-mediated endostatin gene transfer to various human bladder tumor cell lines was associated with decreased vascularization and inhibition of tumor growth when the tumor cells were instilled into a murine model. TNP-470, a compound derived from Aspergillus fumigatus, is known to be another potent inhibitor of angiogenesis and has been shown to inhibit the development of lymph node metastases in a murine model of human bladder cancer and to inhibit tumor growth in a rat bladder cancer model [82, 83]. In a study by Sejima et al. [84], tumor-bearing rats were treated by AGM-1470 or M-VAC, and the tumor volume was significantly reduced in both treatment groups compared with a control group.

The relatively low cytostatic activity of molecular-targeted agents makes it necessary to identify reliable, reproducible and accurate surrogate markers of outcome and biologic activity. Both existing and new therapies need to be tailored to benefit a select group of patients whose risk of disease progression can be predicted on the basis of the molecular alterations, i.e. surrogate markers, specific for their tumors. A profound understanding of the molecular biology of bladder cancer is crucial for the selection of new therapeutic modalities. Association of specific molecular alteration with chemoresistance can stratify patients on the basis of their individual ability to benefit from chemotherapy. A targeted clinical trial in bladder cancer must take into account molecular characteristics and known prognostic variables of bladder tumors and employ agents that are based on mechanism, risk of progression and chemoresistance. Individualization of both established and investigational treatment options based on molecular characteristics of the tumor is the future of bladder cancer therapy.

Novel drug agents for bladder cancer are few, but the anti-EGF-R agents and antiangiogenic agents may have promise; the development of antiapoptotic agents and antisense gene therapy may also become a component of the future multimodality management of this tumor.

The tremendous amount of data accumulated through genomics, proteomics and metabolomic technologies have not led to a definitive understanding of the mechanisms underlying cancer. The challenge remains as to how to integrate all of the relevant knowledge and data in a systematic manner so that researchers can gain the knowledge needed to devise the best therapeutic and diagnostic strategies. Human TCC of the bladder is genetically heterogeneous, and it is surrounded by a complex tissue microenvironment involving vasculature, stromal cells and connective tissue. One of the most challenging problems facing cancer researchers is the lack of correlation between in vitro cell lines and animal tumor models and human in vivo tumors. A few promising approaches are being devised that will help address this issue in the coming years. One such approach is the measurements of molecular levels of receptors, ligands, pathways components, etc., directly in human tumors through, for example, vivo imaging, proteomic and genetic profiling.

references


