Cancer Chemother Pharmacol (2002) 49: 342–345
DOI 10.1007/s00280-001-0417-z

CLINICAL TRIAL REPORT

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A phase II trial of methotrexate-human serum albumin (MTX-HSA) in patients with metastatic renal cell carcinoma who progressed under immunotherapy

Received: 3 August 2001 / Accepted: 12 December 2001 / Published online: 30 January 2002

Abstract *Introduction:* Renal cell carcinoma (RCC) has a poor prognosis when metastasized to distant sites, although immunotherapy may offer a prolongation of survival in selected patient groups. Unfortunately, no treatment options remain when immunotherapy fails. In this phase IIa trial the tolerability and efficacy of the antifolate drug methotrexate-human serum albumin (MTX-HSA) were evaluated in patients with metastatic RCC who progressed after first-line immunotherapy. *Patients and methods:* A total of 17 patients started treatment, and 14 (12 men, 2 women) were evaluable for response according to the phase IIa Gehan design. Patients had prior tumor nephrectomy, were in relatively good general condition, had no impairment of renal, liver or bone marrow function, and had progressive metastatic disease after treatment with interferon-α (IFN-α) with or without *cis*-retinoic acid (EORTC protocols 30951 and 30947). MTX-HSA was given once a week intravenously on an outpatient basis at a dose of 50 mg/m^2. The treatment interval was prolonged in those patients who had not yet recovered from previous toxicities. *Results:* Toxicity was manageable, relatively mild to moderate and reversible in most cases. Grade 2/3 mucositis (10/17) and grade 3 elevated transaminase levels (4/17) were most frequent, and in only one patient was a grade 4 thrombocytopenia reported. Of three inevaluable patients, one discontinued treatment due to drug-related toxicities. The mean administration interval was 12.1 days, and 7 of 14 evaluable patients had treatment intervals of 1 or 2 weeks. No objective responses were seen, although eight patients had stable disease (stabilization >2 months) for up to 8 months (median 121 days). *Conclusion:* MTX-HSA was generally well tolerated and can be given on an outpatient basis, but no objective responses were seen in patients with metastatic RCC who had progressed after previous immunotherapy.

Keywords Chemotherapy · Methotrexate · Safety · Efficacy · Renal cell carcinoma

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Introduction

Renal cell carcinoma (RCC) has a poor prognosis when metastasized, and the 5-year survival rate is less than 10% if distant metastases are present [1, 2]. It is acknowledged that immunotherapy with interferon-α (IFN-α) or interleukin-2 (IL-2) may be effective in some patients with metastatic disease, and may prolong disease-specific survival, especially after previous tumor nephrectomy [3, 4, 5, 6, 7]. Unfortunately, no appropriate treatment is available for those in whom immunotherapy has failed, and in these patients second-line treatment is warranted.

Favorable tolerability and cytostatic activity of the antifolate chemotherapeutic drug methotrexate (MTX) have been reported in patients with metastatic cancer originating from the breast, head and neck, and the urothelial epithelia, as well in patients with leukemias and lymphomas [8]. The exact mechanism by which MTX produces its cytotoxicity is not completely resolved at present, but its cytostatic activity is thought to result from an inhibitory effect mostly on folate-dependent enzymes. MTX-human serum albumin (MTX-HSA) is MTX covalently bound to human serum albumin. It has a significantly longer serum half-life than standard MTX,

and an increased uptake in solid tumors with an enhanced cytostatic effect [9, 10, 11]. Three phase I studies evaluating the dose-limiting toxicity (DLT) and the maximum tolerated dose (MTD) included 17 patients with metastasized RCC, and showed antitumor activity in a number of the RCC patients at concurrent acceptable toxicity levels (unpublished data; [12]).

In the current phase II trial the efficacy and safety of intravenously administered MTX-HSA were determined in patients with metastasized RCC who had progressed after tumor nephrectomy and first-line immunotherapy with IFN-α with or without *cis*-retinoic acid (EORTC protocols 30951 and 30947).

Patients and methods

Study objectives and eligibility

The primary end-point of this phase II trial of MTX-HSA was the tumor response rate to the investigative therapy. Secondary end-points were the time to progression, the time to response, and the duration of survival. The tolerability and safety of the drug were also evaluated in detail. The trial was approved by the institutional Medical Ethical Committee, and was performed in accordance with the ICH-GCP guidelines, and the Declaration of Helsinki. The Departments of Urology and Oncology of the Academic Hospital of Rotterdam, Dijkzigt, were responsible for conducting the clinical trial, data collection, adequate drug accountability, and the reporting of adverse events. The Department of Statistics and Data Management, Klinge Pharma GmbH analyzed the data.

Inclusion and exclusions criteria, case selection

All participants were informed in detail about the design of the study, and a written informed consent for trial participation was signed by each participant prior to study inclusion. Included patients had to have attained the age of majority, histologically confirmed RCC, prior tumor nephrectomy, progressive disease after immunotherapy with IFN-α, and no major impairments of renal, liver or bone marrow function. According to Gehan's design on the evaluation of new chemotherapeutic agents, at least 14 patients needed to be enrolled into this phase IIa trial for a possible effective tumor response rate of 20% [13]. These phase IIa trials allow early discontinuation if the drug is unlikely to be effective. If no objective tumor remissions were observed at the end of the projected treatment duration, the trial was not to be continued into the phase IIb setting to estimate the true response rate. To be able to comply with these objectives, 17 patients were initially included in this phase IIa trial. Evaluable patients were only those who did not have a substantial deterioration in their clinical condition (e.g. death, WHO performance status 3–4) for reasons other than adverse drug reactions before the completion of 8 weeks of treatment. The projected period of treatment was 6 months, or until progressive disease, or until unacceptable drug-related toxicity. The treatment could be continued in individuals who responded favorably to treatment or did not have progression of disease at 6 months. No patient received surgery or radiotherapy during the 2 to 4 weeks before initiation of the MTX-HSA drug therapy. Immunotherapy (i.e. IFN-α) had to be discontinued for 4 weeks at least.

Administration schedule and evaluation of treatment

All patients were seen and received treatment on an outpatient basis. At baseline, a complete evaluation of the medical and disease-specific history, concomitant disease, and concomitant medication was obtained for each patient. A detailed assessment of the tumor extent was performed by recording the presence, size and location of metastatic lesions on chest and abdominal computed tomography (CT). Intentionally, MTX-HSA was administered weekly intravenously at a dose of 50 mg/m^2 for a period of 8 weeks (one treatment cycle). Prior to each drug administration, patients were asked about side effects and complaints, their general well-being, pain status, and the use of concurrent medication (analgesics). A physical examination was performed to assess the vital functions (e.g. blood pressure, pulse, weight, WHO performance status).

Venous blood was collected for hematological and chemical laboratory analyses before each drug administration. All drug-related toxicities were recorded using the common toxicity criteria (CTC) for international studies, version 2.0 [14]. In patients with drug-related toxicity, the treatment interval was prolonged until the toxicity returned to CTC grade 1 or less, or to normal or baseline values. From earlier phase I trials it was clear that the administration intervals in some patients would have to be prolonged due to accumulation of MTX-HSA. If unacceptable drug-related toxicity (e.g. grade 3 or 4 nonhematological and/or grade 4 hematological toxicity, according to CTC) was reported, if the treatment was delayed for more than 4 weeks due to insufficient recovery from drug-related toxicity, or if the patient refused further investigations, the treatment was discontinued. After each treatment cycle of 8 weeks, the tumor status of the patients was reassessed by a CT scan of the thorax and abdomen. Enlargements (≥25%) of previously present and evaluable metastatic lesions, or any new lesions were considered progressive disease. In patients with an objective response (i.e. partial or complete regression of previously present metastatic lesions) and stable disease (i.e. $<50\%$ decrease to $<25\%$ increase), the next treatment cycle was started.

Pharmacokinetics

Venous blood samples were collected for therapeutic drug level monitoring. Both ELISA specific for MTX-HSA and the TDx assay (specific for free MTX plus MTX-HSA) were used to assess the steady-state level and the serum half-life of MTX-HSA.

Results

Toxicity and efficacy

A total of 17 patients met the inclusion criteria for study participation, and started treatment with the investigative drug. In these patients, the drug-related toxicity was assessed. Two patients were ineligible for response due to rapidly progressing disease that interfered with continuation of the study medication. No relationship with the investigative drug was reported. One patient had a grade 4 drug-related thrombocytopenia with epistaxis occurring after the second drug administration. This patient was hospitalized, successfully supported by thrombocyte infusions, and further chemotherapy was withheld. Ultimately, 14 patients (12 males, 2 females) completed one treatment cycle of 8 weeks and were evaluable for response (Table 1). Their mean age was 59 years (range 36–72 years). All patients had initially been treated in the EORTC protocol 30951 or 30947 (IFN-α with or without *cis*-retinoic acid) on which they eventually progressed. Most patients had progressive disease due to enlargement of lung metastases or paraaortic lymph nodes (or a combination of these), whereas

Table 1 The characteristics of patients included in the phase II trial investigating the tolerability, safety and efficacy of MTX-HSA in patients with metastasized RCC who failed tumor nephrectomy and under immunotherapy with interferon-α with or without *cis*-retinoic acid, and their individual responses to treatment (*SD* stable disease, i.e. stabilization >2 months; *PD* progressive disease; *I* ineligible for treatment response)

Patient no.	Age at entry (years)	Gender	WHO status	Initial metastatic site	Response to treatment
1	36	M	1	Lymph nodes	SD
2	60	M	0	Lung + lymph nodes	SD
3	72	M	0	Lung + liver + lymph nodes	SD
4	44	M	2	Lung + kidney + lymph nodes	SD
5	72	M	0	Lung + bone + lymph nodes	PD
6	58	M	1	Bone	I
7	54	M	0	Lung + liver + lymph nodes	PD
8	66	F	1	Lung + lymph nodes	SD
9	66	F	2	Lung + bone + lymph nodes	I
10	56	M	0	Lung + bone	SD
11	57	F	1	Lung + lymph nodes	PD
12	53	M	2	Lung + bone	PD
13	57	M	0	Lung	PD
14	46	M	1	Lung + adrenal	PD
15	62	M	0	Lung	SD
16	60	M	1	Lung + skin + adrenal	I
17	68	M	1	Lung	SD

Table 2 Toxicity of weekly MTX-HSA 50 mg/m^2 in 17 patients with metastatic RCC who failed tumor nephrectomy and treatment with interferon-α with or without *cis*-retinoic acid. The results are presented as the number (%) of patients with each toxicity and CTC toxicity grade (*ALAT* alanine aminotransferase, *ASAT* asparagine aminotransferase)

Toxicity	Grade 0–1	Grade 2	Grade 3	Grade 4
Mucositis/stomatitis	7 (41.2)	8 (47.1)	2 (11.8)	0 (0.0)
Nausea/vomiting	15 (88.2)	2 (11.8)	0 (0.0)	0 (0.0)
Diarrhea	17 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Anemia	15 (88.2)	2 (11.8)	0 (0.0)	0 (0.0)
Leukocytopenia	16 (94.1)	1 (5.9)	0 (0.0)	0 (0.0)
Thrombocytopenia	14 (82.4)	1 (5.9)	1 (5.9)	1 (5.9)
Elevated bilirubin	15 (88.2)	2 (11.8)	0 (0.0)	0 (0.0)
Elevated ASAT/ALAT	7 (41.2)	6 (35.3)	4 (23.5)	0 (0.0)
Nephrotoxicity	16 (94.1)	1 (5.9)	0 (0.0)	0 (0.0)
Drug-related allergy	17 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue	15 (88.2)	2 (11.8)	0 (0.0)	0 (0.0)

a minority of patients showed an increase in the size or number of bone and/or liver metastases. Despite these unfavorable prognostic indicators, 14 of 17 patients had a WHO performance status 0 or 1.

In general, MTX-HSA was well tolerated in the 17 included patients, and showed acceptable and reversible drug-related toxicity. Of the 17 patients, 2 (11.8%) reported immediate drug-related side effects including nausea or vomiting. Grade 2 and 3 mucositis/stomatitis occurred in eight patients (47.1%) and two patients (11.8%), respectively, and in four patients (23.5%) the transaminase levels were transiently increased (grade 3 CTC, nonhematological). In these patients, the administration interval was successfully prolonged for 1 to 2 weeks. The mean interval between two consecutive outpatient visits was 12.1 days, and 7 of 14 evaluable patients (50.0%) had an administration interval of 1 or 2 weeks. Other toxicities are listed in Table 2.

No objective tumor responses were reported in any of the evaluable patients. Of 14 patients, 8 (57.1%) had stable disease (stabilization >2 months) for a median of 121 days (Table 1). Tumor progression included radiological enlargement of lung metastases ($n=7$), para-aortic lymph nodes ($n=5$), liver metastases ($n=1$), bone metastases ($n=1$) and the development of new metastatic lesions ($n=4$). At the time this report was prepared, two patients had been under treatment for more than 8 months and 3 months, respectively.

Pharmacokinetics

ELISA (specific for MTX-HSA) and the TDx assay (free MTX plus MTX-HSA) showed a high correlation ($R=0.92$, slope 0.44). The steady-state concentrations of MTX-HSA administered weekly at 50 mg/m^2 was 18 μ*M* in the ELISA and 8 μ*M* in the TDx assay. Four to five doses were needed to reach a plateau. The half-lives were 11.4 days in the ELISA (MTX-HSA) and 9.3 days in the TDx (MTX/MTX-HSA).

Discussion

Patients with metastasized RCC fare poorly regardless of treatment modality and they may suffer from rapid

disease progression early after diagnosis [1, 2]. Current treatment options include systemic immunotherapy alone, or immunotherapy prior to, or followed by, cytoreductive surgery (i.e. nephrectomy or metastasectomy) albeit with limited efficacy. After failure of immunotherapy, the outcome may be even worse, and more than 80% of patients die within 1 year of treatment failure. No safe and effective second-line therapies are presently available from which these patients may eventually benefit, and trials on new chemotherapeutic drugs are warranted. We report here a non-controlled phase II trial in which the efficacy and tolerability of intravenously administered MTX-HSA were investigated in patients with metastasized RCC who had failed first-line immunotherapy with IFN-α. Three previous phase I trials in which the DLT and MTD were evaluated have demonstrated that this drug can be safely administered in an outpatient setting at intervals of 1 to 4 weeks.

The primary drug-related toxicities of standard MTX therapy are myelosuppression and gastrointestinal mucositis/stomatitis [8]. The side effects of MTX-HSA may be less severe than those induced by free MTX. Our findings indicate that MTX-HSA can indeed be safely administered on a weekly or two-weekly basis in the outpatient setting in most cases. The drug is generally well tolerated and the toxicities are manageable. No new toxicities were seen in the present study compared to standard MTX therapy. Grade 2/3 toxicity (mucositis, elevated transaminase levels) were relatively common, but in most of patients the toxicities were reversible and could be further managed by a prolongation of the treatment interval by 1 week.

Unfortunately, no tumor regressions were seen in any of the patients during the study, although in more than half the patients (8/14) the disease stabilized (stabilization >2 months) for a median of 4 months after the initiation of therapy. After 6 months, all but three patients eventually progressed, and at the last follow-up 7 of 14 evaluable patients (and 8 of 17 included patients) had died from metastatic RCC. In contrast to the responses reported in the RCC patients in the three phase I studies, no objective responses were seen in any of the patients. Therefore, further evaluation of the efficacy of the MTX-HSA drug in the phase IIb or phase III settings to estimate the true response rate cannot be advocated [13]. As ours was an uncontrolled trial, the true survival benefit of MTX-HSA could not be assessed. Since the drug is relatively safe, the efficacy of MTX-HSA may be further studied in different investigational settings and/or different patient groups, for example those with less-advanced stages of disease. In addition, MTX-HSA may prove of more value for patients with other malignancies than RCC, for example in urothelial cell carcinoma.

Metastatic RCC remains a challenging disease for which unfortunately no effective treatments are available after failure of first-line immunotherapy. Therefore, the search for new effective therapies to palliate the disease, to improve the quality of life, and preferably, to increase the long-term survival of these patients with a dismal prognosis continues.

Acknowledgements The trial was sponsored by Klinge Pharma GmbH, Fujisawa Group, München, Germany.

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