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# Phase I Clinical Trial with Carbetimer

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**Abstract**—*Carbetimer, a low molecular weight polymer derived from ethylene and maleic anhydride, belongs to a class of chemical compounds different from previously available anticancer agents. It has shown moderate antitumor activity against the Madison 109, Lewis lung, colon 26 and M5076 ovarian carcinomas. In the human tumor stem cell assay, antitumor activity was seen against carcinomas of the breast, ovary, lung, colon and kidney. A total of 26 patients with solid tumors were entered into this trial; carbetimer was given on 5 consecutive days as a 1–2-h intravenous infusion. The dose was escalated from 1.08 to 11 g/m²/day. The drug did not induce the usual side-effects of chemotherapy: leukopenia, thrombocytopenia, alopecia and mucositis were minimal or totally absent. Gastrointestinal toxicity was limited to mild to moderate nausea and vomiting; these were observed at all dose levels and required antiemetics in only two patients. The major side-effects of carbetimer consisted of hypercalcemia and neurotoxicity. Hypercalcemia was dose- and treatment duration-dependent. The precise mechanism of hypercalcemia is presently under investigation, but remains unclear. Neurotoxicity was observed only after prolonged therapy; two patients, who received cumulative doses higher than 250 g/m², developed a peripheral neuropathy with paresthesia, decrease in sensory perception and motor weakness. One patient recovered completely; the other patient improved slightly before developing fatal brain metastases. Two patients with malignant melanoma exhibited major antitumor response; both were previously treated; after excellent partial responses to carbetimer, both were operated on and one is presently disease-free 2 ½ years after completion of therapy with carbetimer. In conclusion, carbetimer is a new compound with an unusual pattern of side-effects and interesting antitumor activity against malignant melanoma. Its antitumor activity is presently being investigated in phase II trials.*

## INTRODUCTION

THE TREATMENT of several common adult tumor types remains unsatisfactory either because of insufficient activity of the currently available chemotherapeutic agents or because of excessive toxicity. Therefore, major efforts are being directed towards the discovery of novel anticancer agents. Carbetimer is a low molecular weight polymer derived from ethylene and maleic anhydride; interest in this new class of compounds has been ongoing since the nineteen-sixties [1, 2]. The pattern of preclinical antitumor activity of carbetimer is unusual [3]. The activity of carbetimer against P388-leukemia is quite limited; however, significant activity has been found against the Lewis lung, Madison 109 lung, colon 26 and M5076 ovarian carcinomas. In addition, carbetimer demonstrated antitumor activity in the human tumor cloning system [4]. In this assay, which has been considered as a potential screening system for new anticancer agents [5], carbetimer showed interesting activity against carcinomas of the breast, ovary, lung, colon and kidney.

Toxicological studies in mice, rats and dogs revealed that the major acute side-effects of carbetimer consisted, at high doses, of sedation, tremor, occasional convulsions and impaired respiration. In the dog, dose-related anemia, leukopenia and abnormalities of liver function tests were observed. In the mouse, the $LD_{10}$ with a single dose schedule was 4.4 g/m², while the $LD_{10}$ with the daily × 5 schedule was 4.3 g/m² [3].

The mechanism of action of carbetimer is still unclear. The fact that carbetimer has no direct cytotoxic activity against the Lewis lung carcinoma

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and good *in vivo* activity against this tumor indicates that the drug could act through immunomodulation or biological response modification. However, the activity of carbetimer in the stem cell assay suggests that the antitumor efficacy of the drug may be due to a direct antiproliferative effect. In addition, Ardalan and Paget have shown recently that carbetimer may affect the uridine, cytidine, uridine triphosphate and cytidine triphosphate pools [6]. Carbetimer may also decrease the uptake of uridine and cytidine; these results suggest that carbetimer may act on the uptake and/or phosphorylation of pyrimidines.

On these grounds, a comprehensive program to investigate carbetimer in patients was activated both in the United States and in Western Europe. We selected the daily × 5 schedule for our phase I trial.

## MATERIALS AND METHODS

*Patient selection*

A total of 26 patients with histologically confirmed solid malignancies no longer amenable to conventional therapy were entered into this study (Table 1). They all had a performance status (PS) ≤2 on the Eastern Cooperative Oncology Group (ECOG), except for three patients with PS 3, and a life expectancy of at least 6 weeks. Neither chemotherapy nor radiation therapy had been administered for at least 4 weeks before entry into this study (this was increased to 6 weeks for therapy with nitrosoureas or mitomycin). The patients had to have recovered from the toxic effects induced by previous therapy (with the exception of alopecia). All patients had normal renal (serum creatinine ≤1.5 mg/dl), hepatic (bilirubin ≤1.5 mg/dl) and hematologic (neutrophils >1500/μl, platelets >100,000/μl) functions. No patients had major intercurrent disease. All patients gave their informed consent before entry into the study.

*Table* 1. *Patient characteristics*

| | |
|---|---|
| No. of patients entered | 26 |
| Median age (range) | 60 (29–73) |
| Median PS (WHO) (range) | 1 (0–3) |
| Male/female | 14/12 |
| Prior radiotherapy | 1 |
| Prior chemotherapy | 6 |
| Prior chemotherapy + radiotherapy | 18 |
| No prior treatment | 1 |
| Tumor types | |
| Head and neck | 8 |
| Melanoma | 7 |
| Colorectal | 2 |
| Lung | 2 |
| Sarcoma | 2 |
| Other tumor types | 5 |

*Treatment*

Carbetimer was supplied by Searle & Company Ltd (High Wycombe, U.K.) in vials containing 1 g of carbetimer. The vials were reconstituted with 12.6 ml of sterile water for injection producing a 7.5% w/v isotonic solution. The reconstituted carbetimer solution was diluted with 5% dextrose for injection to give a total infusion volume of 500 ml. The treatment plan consisted of the intravenous infusion of carbetimer on 5 consecutive days every 4 weeks. The starting dose was 1.08 g/m$^2$/day; this selection was based on information from other phase I trials with carbetimer [7, 8]. In the absence of toxicity, three evaluable patients were entered per dose level and the dose was escalated according to a modified Fibonacci scale. Five to six patients were treated at toxic dose levels. Retreatment in a given patient was allowed if all eligibility criteria were still satisfied. Intrapatient dose escalation was allowed provided that two courses at the previous dose level were adequately tolerated; five patients were retreated at higher dosages. One patient was retreated at a lower dose because of a syncopal episode during his first course. For the other retreated patients, there was no dose escalation or reduction. Overall 64 courses were given: 12 patients received one course of therapy, seven, two courses, four, three courses, and one patient each received four, 10, and 12 courses, respectively.

*Follow-up studies*

Follow-up observation included a weekly history and physical examination; complete blood cell counts and chemistries were obtained weekly. In patients with measurable disease, tumor response was assessed according to the WHO criteria [9].

## RESULTS

Myelosuppression was infrequent in this trial; it was observed sporadically at all dose levels with no dose–effect relationship. Only five patients had leukopenia between 3000 and 4000 WBC/μl; none of these patients had neutrophils below 1500/μl. It is important to note that, to be eligible for the study, patients had to have neutrophils >1500/μl, independently of the leucocyte count; out of the five patients who had WBC between 3000 and 4000/μl, three entered the trial with WBC counts below 4000/μl before therapy with carbetimer. Thrombocytopenia was even less frequent with only two patients developing mild thrombocytopenia (81,000 and 71,000/μl). Both had rapidly progressive cancer and died from their disease before they had recovered normal platelet counts.

Non-hematologic side-effects of carbetimer were relatively limited (Table 2). Nausea and vomiting were mild to moderate in all cases; gastrointestinal

*Table 2. Non-hematological toxicities**

| Dose | No. of evaluable patients | No. of evaluable courses | Toxic patients | Nausea/ vomiting | Skin | Neuro-toxicity | Hepato-toxicity |
|---|---|---|---|---|---|---|---|
| 1080 | 5 | 7 | 2 | 1 | | | |
| 1620 | 4 | 6 | 1 | | | | 1 |
| 2430 | 3 | 6 | 1 | | | | 1 |
| 3645 | 3 | 5 | 0 | | | | |
| 5467 | 6 | 9 | 4 | 3 | 3 | | 3 |
| 6500 | 3 | 15 | 3 | 1 | 2 | 1 | 1 |
| 8200 | 8 | 13 | 5 | 4 (2) | | 1 | 4 |
| 11,000 | 2 | 2 | 2 | 1 | | | 1 |

*Number in parentheses: patients with grade III–IV toxicity; hypercalcemia is not included in this table.

toxicity was slightly more pronounced at higher dosage. Only two patients (treated at 8200 mg/m$^2$) required antiemetics. Mild phlebitis and/or irritation at the infusion site was observed in 3/6 patients treated at 5467 mg/m$^2$; the infusion duration was therefore increased from 1 to 2 h. Only 2/13 patients treated at doses between 6500 and 11,000 mg/m$^2$ over 2 h developed this problem. Alopecia was seen in one patient and mucositis in none. Abnormalities of liver function tests were observed in eight patients; these abnormalities were mild to moderate (maximum WHO grade II) and consisted of an increase of the SGOT, SGPT, lactate-dehydrogenase and/or alkaline phosphatase. In several patients, these abnormalities could also have been attributed to hepatic involvement by malignant disease. Liver function test abnormalities were never the reason for interrupting the treatment.

Hypercalcemia was the dose-limiting toxicity in this trial (Table 3). Overall, 11 out 26 patients developed hypercalcemia; seven of these had risk factors (four patients had head and neck tumors; two had bone metastases; one had renal cell cancer). There was no evidence of hyperparathyroidism in any patient and no patient was taking thiazide diuretics or vitamin D supplements. The day of maximum serum calcium was highly variable (median: 13, range 1–36); recovery occurred after 17–77 days after treatment (median day of recovery: 25). Three patients did not recover normal serum calcium levels after 22, 36 and 36 days of observation, respectively. There was no other significant alteration of the calcium metabolism (serum phosphorus, serum magnesium, total serum protein, serum albumin, alkaline phosphatase) (Table 4).

Two patients developed neurologic toxicity. Both patients were treated for a prolonged period of time: one patient received a total cumulative dose of 254.8 g/m$^2$ over 12 courses and the other 308.9 g/m$^2$ over 10 courses. In both cases, neurologic toxicity consisted of a peripheral neuropathy with decreased sensation and muscle strength and abolition of deep tendon reflexes. In the first patient, these symptoms gradually developed over a period of a few weeks just after the end of the treatment with carbetimer and resolved over a period of 3 months. In the second patient, the first obvious signs of peripheral neuropathy became apparent after the 9th course of carbetimer, and worsened during the 10th course and after treatment; the patient started to improve slightly 4 months after the

*Table 3. Effect of carbetimer on calcemia**

| Dose mg/m$^2$ | No. of evaluable patients | No. of evaluable courses | No. of courses with Ca > 10.5 | Median max. calcemia | Range |
|---|---|---|---|---|---|
| 1080 | 5 | 6 | 1 | 9.8 | 9.5–10.6 |
| 1620 | 3 | 4 | 0 | 9.9 | 9.5–10.4 |
| 2430 | 3 | 5 | 1 | 10.3 | 9.8–10.9 |
| 3645 | 3 | 5 | 3 | 10.7 | 10.1–12.7 |
| 5467 | 6 | 9 | 5 | 10.6 | 10.2–11.4 |
| 6500 | 3 | 15 | 1 | 10.1 | 8.7–10.6 |
| 8200 | 7 | 12 | 8 | 11.3 | 9.6–12.9 |
| 11,000 | 2 | 2 | 0 | 9.7 | 9.6–9.7 |

*Overall: 11 out of 26 patients developed hypercalcemia; seven had risk factors (four patients with head and neck tumors, two with bone metastases, one patient with renal cell cancer); calcium levels are expressed in mg/dl.

*Table 4. Effect of carbetimer on calcium metabolism*

| | Normal values | No. of evaluable patients | Median | Range |
|---|---|---|---|---|
| Maximum calcemia | 8.5–10.5 | 23 | 10.4 | 9.5–12.9 |
| Day of max. | — | 23 | 13 | 1–36 |
| Day of recovery | — | 11* | 25 | 17–77 |
| Phosphorus at max. Ca | 2.2–4.0 | 15 | 4.1 | 3.0–5.7 |
| Magnesium at max. Ca | 1.3–2.1 | 8 | 1.8 | 0.9–2.2 |
| Total protein at max. Ca | 6.0–7.5 | 23 | 6.9 | 5.5–8.4 |
| Percentage albumin at max. Ca | 40–65 | 16 | 52.7 | 31.2–63.9 |
| Alkaline phosphatase at max. Ca | <110 | 23 | 113 | 60–797 |

*Based on the 11 patients who developed hypercalcemia; three patients did not recover normal serum calcium levels at days 22, 36 and 36, respectively.

end of treatment with carbetimer. Unfortunately, he developed brain metastases and died despite radiation therapy on the brain. In both cases, the electrophysiological studies were consistent with a peripheral neuropathy. In the second patient, a spinal tab and a brain CT scan obtained 3 months before the development of brain metastases were within normal limits. A nerve biopsy was obtained and did not reveal any abnormality.

One patient treated at 8200 mg/m$^2$/day had a syncopal episode on the 2nd day of treatment. Treatment was interrupted and the patient recovered completely. He received nine further courses of carbetimer at a dose of 6500 mg/m$^2$/day without recurrence. The syncope was retrospectively attributed to a vaso-vagal attack.

At the end of the study, considering our own results and those from other phase I trials, it appeared that a dose between 5467 and 8200 mg/m$^2$/day could be recommended for phase II trials. A few patients were therefore treated at 6500 mg/m$^2$/day. In order to increase the dose intensity and in view of the lack of bone marrow toxicity, treatment was given every 3 weeks instead of every 4 weeks. These patients had side-effects entirely similar to those observed in other patients. Hypercalcemia was not a problem (Table 3).

*Antitumor responses*

Two patients exhibited a major antitumor response in this trial. Both had previously treated malignant melanoma. The first patient was a 62-year-old man with a malignant melanoma of the right foot previously treated with multiple surgical resections, extracorporeal circulation with dacarbazine, cisplatin and vindesine and radiotherapy. He had also received prior immunotherapy and a prior phase I drug (flavone acetic ester) for lung and skin metastases. The patient received 12 courses of carbetimer at doses ranging from 1080 to 8200 mg/m$^2$/day. A complete remission of the lung metastases was achieved after five courses; an excellent partial remission of the skin lesions was also obtained. After 12 months of therapy with carbetimer, the skin lesions had shrunk so that a surgical resection of the residual lesions was possible. The pathologic examination of these lesions was still positive for malignant melanoma. The patient remained disease-free for 9 months, after which he relapsed in the small intestine and the groin. The patient was again rendered disease-free by surgical resection. He is still in complete remission 2.5 years after the end of therapy with carbetimer.

The second patient was a 55-year-old man with malignant melanoma, metastatic to the lung and previously treated with mitozolomide, an experimental nitrosourea [10]. The patient received a first course of carbetimer at 8200 mg/m$^2$/day followed by nine courses at 6500 mg/m$^2$/day. An excellent partial remission of the lung metastases was achieved after eight courses of carbetimer (Fig. 1). Treatment was interrupted after 10 courses because of severe neurotoxicity. Three months after the interruption of the treatment, the lung lesions started to regrow and the patient was submitted to a thoracotomy. The resection of three residual lung metastases was possible; unfortunately, the mediastinum was still largely invaded and complete resection was not possible. Pathological examination of the resected lesions showed malignant melanoma. No further therapy was given for 4 months. The patient then developed brain metastases and received radiation therapy on the brain. After a temporary improvement, his neurological status worsened and the patient died 8 months after the end of the treatment with carbetimer.

No other antitumor response was observed among the five other patients with malignant melanoma and among the 19 patients with other tumor types.

## DISCUSSION

The purpose of this trial was to define the maximum tolerated dose for carbetimer after administration on 5 consecutive days every 4 weeks. The pattern of side-effects due to carbetimer was quite unusual for a chemotherapeutic agent since

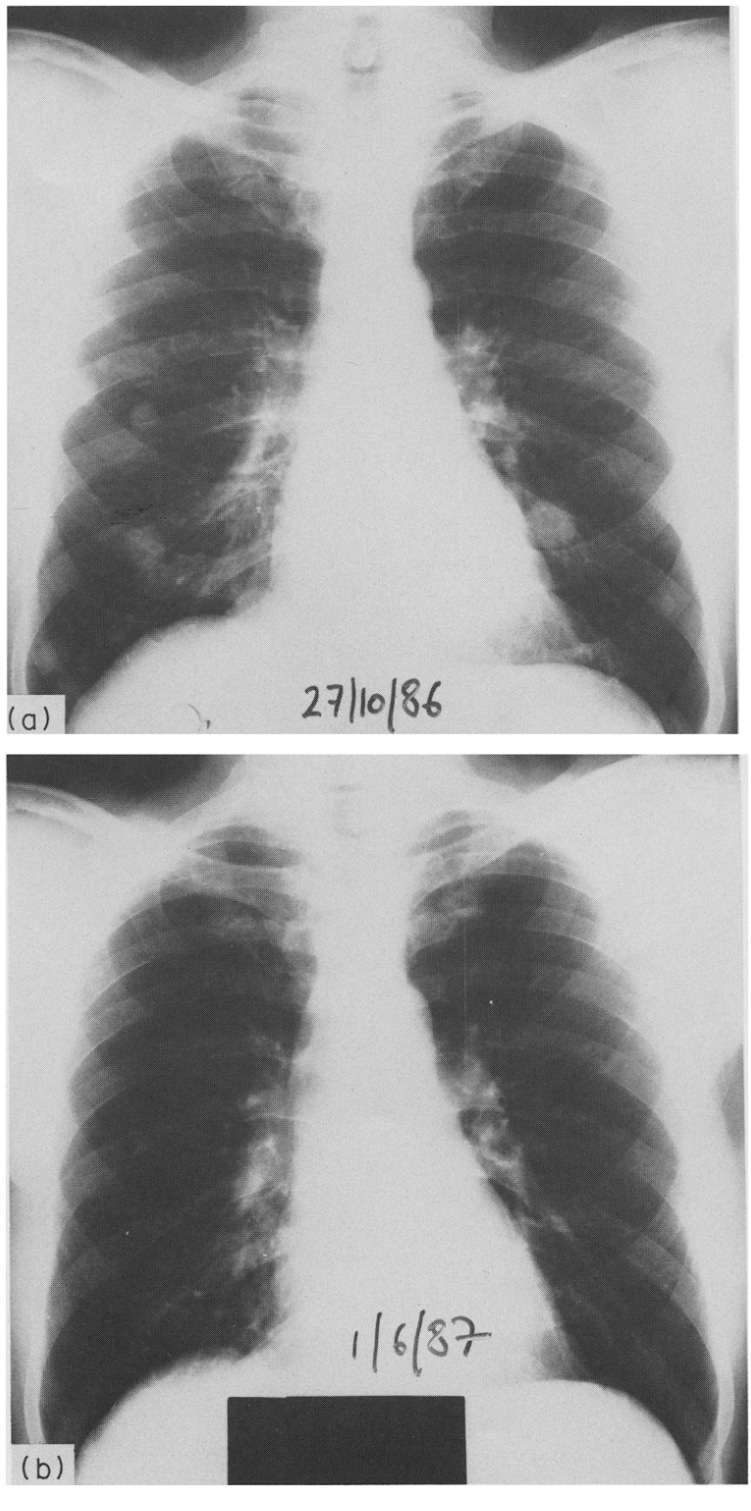


*Fig.* 1. *(a) Chest X-ray of patient* 23, *prior to carbetimer. (b) Chest X-ray of patient* 23, *after* 7 *months of treatment with carbetimer.*

the drug did not induce any significant myelosuppression, alopecia or mucositis; gastrointestinal toxicity was also minimal. The major side-effect observed in this study was hypercalcemia.

Hypercalcemia is an unusual side-effect for anticancer agents and, as a matter of fact, it is even not graded in the WHO criteria. This fact caused some difficulty defining precise criteria for severe hypercalcemia. Hypercalcemia had a tendency to be more frequent and more severe with an increasing dosage of carbetimer. However, we recognize that the dose could have been further increased in our trial. In other phase I trials with carbetimer, hypercalcemia was also observed [7, 8]. Hypercalcemia was noted in the two patients treated at 11,000 mg/m$^2$/day for 5 consecutive days in the trial conducted by Grunberg *et al.* [7]; similarly, 2/3 patients treated with 8500 mg/m$^2$ once every 4 weeks developed hypercalcemia in another trial conducted at the San Antonio Cancer Center [8]. The findings from these other phase I trials influenced our decision to close our own trial, rather than to attempt further dosage escalation.

We also recognize that hypercalcemia could have been due to other causes, at least in some of our patients. Out of the 11 patients who developed hypercalcemia, seven had risk factors for hypercalcemia (four patients with head and neck tumors, two patients with bone metastases and one patient with renal cell cancer). Although a neoplastic origin to the hypercalcemia is possible in these patients, other classical causes of hypercalcemia were excluded. Moreover, the overall incidence of hypercalcemia in this trial was much higher (42%) than the overall incidence of hypercalcemia in our four previous phase I trials (eight out of 124 patients: 6.5%). Therefore, we believe that hypercalcemia is truly a side-effect of carbetimer.

The mechanism of carbetimer-induced hypercalcemia remains unclear. The *in vitro* findings suggest that carbetimer binds directly to the bone matrix and displaces calcium from it [3, 11, 12]. However, this does not explain the post-carbetimer fall in ionized calcium that has been observed in a few patients investigated in that respect at this and other centers; this observation may suggest that carbetimer is chelating calcium. However, *in vitro*, carbetimer does not chelate calcium dissolved in water [3]. Our group is continuing to study the mechanism of carbetimer-induced hypercalcemia.

The occurrence of two cases of major peripheral neuropathy is disturbing. This was observed in the two patients who received prolonged therapy: both had received a cumulative dose above 250 g/m$^2$. The peripheral neuropathy developed by these two patients resembled clinically that induced by vinca alkaloids. However, histological examination of the nerve biopsy obtained in our second patient with neuropathy was normal and did not reveal the findings characteristic of vinca alkaloid-induced neuropathy [13]. The mechanism of this side-effect remains to be clarified. Carbetimer-induced neuropathy may prove to be a serious limitation to the use of this compound since it limits the duration of the treatment. If carbetimer proves to be an active drug, the development of analogs with reduced neurologic toxicity may be considered. Neurologic toxicity of carbetimer was not clearly foreseen by animal toxicology. In rats treated with intraperitoneal cumulative doses of 22–90 g/kg, vacuolization of the anterior horn cell of the spinal cord was observed. In dogs treated intravenously twice or weekly with a cumulative dose of 0.13–30 g/kg over 3 months, there was no pathologic neurotoxicity. The cumulative doses received by our patients in g/kg was 5.54 g/kg or higher.

When our results are compared with those from other phase I trials, they are very similar. Another trial with a daily administration for 5 consecutive days was conducted by Grunberg *et al.* [7]. As mentioned above, hypercalcemia was considered dose-limiting at 11,000 mg/m$^2$/day. The drug has also been given intermittently once every 28 days [14]; with this schedule, the maximum tolerated dose was 16,700 mg/m$^2$. The dose intensity in our schedule is therefore higher than in the intermittent schedule.

The antitumor activity observed in our trial is encouraging. Seven patients had malignant melanoma, and two exhibited a major antitumor response. Antitumor activity was noted after prolonged treatment with carbetimer and this must certainly be considered in the planning of phase II trials with the drug.

In conclusion, carbetimer is a new anticancer compound with a distinctly unusual pattern of toxicity. The drug is presently undergoing phase II testing using the daily × 5 schedule at a dose of 6.5 g/m$^2$/day.

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