A Prospective Open-Label Single-Arm Phase II Study of Chimeric Monoclonal Antibody cG250 in Advanced Renal Cell Carcinoma Patients

(monoclonal antibody / renal cell carcinoma / immunotherapy / G250)

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Abstract. cG250 is an IgG1 kappa light-chain chimeric monoclonal antibody that binds to a cell surface antigen found on 95% of clear-cell renal cancer. A multicentre phase II study was performed to evaluate the safety and efficacy of repeated doses of cG250.

Thirty-six patients with metastatic RCC were included. All patients were nephrectomized for the primary tumour. Twenty-one patients were pretreated (e.g. with IL-2, IFN-α). A weekly dose of 50 mg cG250 was given by iv infusion for 12 weeks. Patients with SD or tumour response (PR, CR) after 12 weeks of treatment could receive additional treatment for 8 more weeks.

None of the 36 enrolled patients had any cG250 grade III or IV toxicity. Only three patients had grade II toxicity possibly related to the study medication. ELISA testing gave no evidence for relevant amounts of HACA. Eleven patients presented with SD and ten were eligible for extension treatment. After the end of the study in the follow-up period, one patient demonstrated a CR in week 38 and another patient with SD showed a significant reduction of the overall tumour load in week 44. Six additional patients with progressive disease at study entry were stable for more than six months after the treatment start.

The weekly schedule of iv cG250 in patients with metastatic RCC was safe, very well tolerated and non-immunogenic in a 12-week treatment regimen. cG250 showed anti-tumour activity.

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Abbreviations: ADCC – antibody-dependent cellular cytotoxicity, AE – adverse events, CR – complete remission, HACA – human anti-chimeric antibodies, IFN-α – interferon α, IL-2 – interleukin 2, iv – intravenous, mAb – monoclonal antibody, PR – partial remission, RCC – renal cell carcinoma, SD – stable disease.

Renal cell carcinoma (RCC), which accounts for 3% of all adult malignancies, was estimated to cause 14 000 new cancer cases and 6 000 cancer deaths in Germany in 1999 and is the most lethal of the urologic cancers (Arbeitsgemeinschaft Bevölkerungsbezogener Krebsregister in Deutschland, 2002). Men are twice often afflicted as women, most often in the 5^{th} –7^{th} decade of life. Approximately a third of cases of RCC have metastatic disease at presentation and up to 50% of those resected for cure are expected to have relapse during the course of the disease (De Kernion and Berry, 1980; Golimbu et al., 1986; Dineen et al., 1988). To date radiation, chemo- and hormonal therapy have not demonstrated sufficient anti-tumour activity to prolong the survival with metastatic disease. Due to less than satisfactory response to these therapies, and to the indirect evidence that host immune mechanisms play a major role in the natural history of RCC, there is a continued exploration of immunotherapy in this malignancy since the eighties (McCune, 1983; Belldegrun et al., 1988; Bander, 1989; Graham, 1989; de Riese et al., 1991; Heicapell and Ackerman, 1991). The two principal cytokines available as non-investigational agents for RCC therapy are interferon-α (IFN-α) and interleukin-2 (IL-2). Estimate of complete (CR) or partial remission (PR) are generally in the 5% to 10% and 15% to 20% range, respectively, in larger series. Smaller series with higher response rates appear difficult to reproduce and probably related to case selection (Law et al., 1995; Negrier et al., 1998; Negrier et al., 2000; Atzpodien et al., 2001; Bukowski, 2001; Jonasch and Haluska, 2001; Malaguarnera et al., 2001). However, such immunotherapy protocols are limited by severe, mostly dose-dependent side effects and are tolerated by only a selected group of patients without significant co-morbidities (Jonasch and Haluska, 2001; Malaguarnera et al., 2001; Varga et al., 2001). In the last few years mAbs have become a well-tolerated treatment option in an increasing number of tumour cases (Weiner, 1999; Dillman, 2001).

The murine monoclonal antibody (mAb) G250, as originally developed, has an IgG1 isotype. The antibody recognizes an antigen present on more than 75% of renal

cancers. Murine mAb G250 detects a cell-surface antigen (MN or CA IX antigen) on renal cancer cells. In immunohistochemical assays on sections of fresh frozen tissues, mAb G250 reacts with 95% of renal cancers of the clear-cell type. Reactivity with renal cancers is homogeneous (greater than 75% reactive cells) in 75% of renal cancers. The reactivity of mAb G250 with normal human tissues is restricted to the gastric epithelium, the biliary ducts in the liver and astrocytes in brain and spinal cord (Oosterwijk et al., 1986; Oosterwijk et al., 1993; van Dijk et al., 1994; Oosterwijk and Debruyne, 1995; Oosterwijk et al., 1995; Grabmair et al., 2002; Stadick et al., 2002).

As expected, development of human anti-mouse immune response (HAMA) precludes repeated administration of the murine G250 in humans. To decrease and/or prevent the development of an immune response, a chimeric (mouse Fv with human Fc) antibody was constructed. Chimeric G250 mAb (cG250) is an IgG1 kappa light chain, chimeric version of an original murine monoclonal antibody, G250 IgG1 (mAb G250), first described by Oosterwijk et al. (1986). cG250 has been shown to be equivalent to murine mAb G250 in competitive binding assays and co-types with murine mAb G250 in binding reactivities on human cancer cell lines.

A phase I dose-escalation study with weekly administrations over six weeks per cycle has shown that the unconjugated (to ^{131}I) antibody is safe at dose levels of 5, 10, 25, and 50 mg/m^2. In this study, patients received up to 10 cycles (unpublished data). Regarding the preclinical results, cG250 can biolocalize efficiently in RCC. In addition, it has been shown that cG250 can induce NK cells to kill tumour cells *in vitro* by the ADCC mechanism. In animals the murine G250 was effective in delaying growth of established grafted renal tumours (van Dijk et al., 1994). The aim of the study was to show that cG250 has a good safety profile and induces possibly objective response in patients with advanced RCC.

Material and Methods

The study was designed as a phase II, non-randomized, open-label, single-arm, multicentre study. The statistical trial design was based on a sequential enrollment of two groups of patients with a maximum of 54 evaluable patients enrolled. After the first cohort of 32 patients the study was planned to continue with 22 additional patients if at least three objective responses had been observed. The study had to be stopped in the case of less than three or if five (or more) objective responses were already seen. The method of calculation was the Sequential Probability Ratio Test modified according to Wald and Wolfowitz (1948, 1950). The primary objectives were to evaluate the safety of cG250 and to achieve a minimum of 15% overall response rate (CR+PR) in patients with advanced RCC by treatment with 50 mg cG250 administered intravenously (iv) weekly for 12 weeks. The secondary objectives were to determine the immunogenicity of cG250 by measuring the human anti-chimeric antibody (HACA) levels. Furthermore, to specify the biological activity of patients' peripheral mononuclear blood cells caused by cG250 by evaluating the antibody-dependent cellular cytotoxicity (ADCC). All patients were selected by in- and/or exclusion criteria (Table 1) and received written patient information, and they had to give written informed consent prior to study entry. After re-evaluation in week 16 there was the possibility for extended treatment with additional 8-week cG250 therapy. For continuation patients needed to be objective responders or had to show stable disease after initial progression.

The patients' characteristics of the 32 evaluated patients are listed in Table 2. The high incidence of pretreated patients (56.3%) is remarkable.

Results

Thirty-two of 36 enrolled patients were evaluable, four discontinued within the first five weeks because of one protocol violation at inclusion and three with disease progression. Because of tumour progression three additional patients did not receive the planned 12 injections (7, 10, 11 injections). No dose reduction was necessary. The primary objective of more than 15% overall response was not reached. Eleven patients presented with stable disease (SD) in week 16 and were eligible for extended treatment. Ten patients received extended treatment and eight out of them still showed SD at week 24. A durable clinical benefit for at least six months or more, which is considered as clinically meaningful for this patient population, was achieved in eight patients (25%). Six patients achieved a stabilization of their disease lasting more than six months. In addition, one patient achieved a CR, and another patient experienced a 59% reduction of his target lesions and a minor response when considering all lesions. Both patients are free of progression for more than one year (16+ and 18+ months, respectively). The tumour regression in the CR patient occurred late, more than six months after the start of treatment with cG250. The mean time to progression for all patients was 27 weeks (range 4 to 70 weeks). As of February 2002, the date of study end, five patients are still free of progression (range 13+ to 18+ months) and 19 patients (59.4%) are alive. The median survival reached 15+ months. All 36 patients received at least one injection of cG250, and were assessed for safety. Thirty of 36 patients experienced a total of 160 adverse events (AE). Two thirds of the AE were mild to moderate. Ten patients (27.8%) had 33 grade 3 AE, and four patients (11.1%) had five grade 4 AE. Only in seven patients the AE were assessed as possibly and in one as probably related to cG250 medication. In one patient (nausea grade 1) the relationship to study drug was assessed as probable. This patient also experienced two episodes of fatigue grade 1, which was assessed as possibly related. The AE were observed four days after the 1st injection (nausea and fatigue) and at the day of the

Table 1. In- and exclusion criteria

Inclusion criteria
• Stage IV clear-cell renal carcinoma
• Nephrectomized for primary
• Indicated disease progression at study entry if present
• Bidimensionally measurable tumour with individual lesions under 5 cm and with at least one lesion > 1 cm in diameter
• Karnofsky performance status > 70%
• Life expectancy > 28 weeks
• Laboratory values obtained < 14 days to registration
• Negative HIV- and hepatitis test
• Negative pregnancy test for women of child-bearing potential
• Age > 18 years
• Ability to provide written confirmed consent
Exclusion criteria
• Known standard therapy for the patients disease that is potentially curative or definitely capable of expending life expectancy
• Any CNS metastases
• Patients with bone metastases only
• Any chemo-, immuno-, biologic or radiation therapy within 4 weeks prior the first dose of study agent
• Pretreatment with any antibody therapy
• Not fully recovered from effects of prior cancer therapy
• Concurrent use of systemic corticoids or immunosuppressive agents
• Cardiac disease with NYHA-classification III or IV
• Patients who are pregnant
• Any unrelated illness, eg active infection, or medical condition, which in the judgement of the investigator will significantly affect the patients' clinical status

Table 2. Patient characteristics

• Total number of patients	32
◦ Male	22
◦ Female	10
• Mean age (range)	63.8 years (43-76 years)
• Target lesions	95
◦ Lung	50 (52.6%)
◦ Liver	6 (6.3 %)
◦ Other locations (lymph node, adrenal gland, kidney, psoas muscle, spleen)	38 (40%)
◦ Missing documentation	1 (1.1%)
• Prior treatment	18 (56.25 %)
◦ Immunotherapy	
▪ IFN/vinblastin	7
▪ IL-2/IFN/5-FU	5
▪ IFN	3
◦ Dendritic-cell vaccination/IL-2	1
◦ Vaccination	1
◦ Radiation	1
• Documented disease progression at study entry	27 (84.4%)

9th application (fatigue). The severity of the AE was always rated as mild or moderate (grade 1 or 2). All of the possibly related AE resolved during the study period with the exception of the gastritis of one patient. Also the probably related AE (nausea grade 1) resolved during the observation period of the study (after one day). The AE which were assessed as possibly or probably related to the study drug were single observations and did not reoccur after consecutive administrations in five patients. Thus, it is very likely that these events were caused by the underlying disease. Neither allergic reactions nor the production of clinically significant HACAs were observed. There was no clinically significant development of the laboratory parameters. Urine analysis did not reveal any drug-related effects either.

Regarding the ADCC measurements, cG250 treatment had no effect on the proportion or activity of NK cells in patient peripheral blood. The level of cG250-mediated ADCC was dependent on the individual patient: 42% of the patients had moderate to high ADCC whereas 33% showed no ADCC at all. There was no clear correlation between the proportion of NK cells and the level of cG250-mediated ADCC. No correlation of levels of NK cell-related cytolytic activities and cG250 treatment results could be observed.

Discussion

Overall, the treatment with cG250 was very safe without any significant side effects. Six patients achieved a durable stabilization of their progressive disease for more than six months. In addition, one patient achieved a CR, and another patient experienced a minor response. Adverse events which were assessed as possibly or probably related to the study drug were observed only in seven out of 36 patients. Their severity was rated as mild or moderate. Neither allergic reactions nor a clinically significant production of HACAs were observed. The antitumour effect of antibodies can be mediated through different effector mechanisms. ADCC has been suggested in *in vitro* and *in vivo* studies as the main mechanism for cG250, but in our trial no clear correlation between the proportion of NK cells and the level of cG250-mediated ADCC could be shown. Molecular studies have shown significant polymorphism in the genes for differ-

ent Fc receptors and they may have important functional consequences. This could be the reason why no clear correlation between the proportion of NK cells and the level of cG250-mediated ADCC was observed (Vance et al., 1993). Further investigation into the mechanism of cG250 in RCC is currently ongoing. The good tolerability of this treatment with cG250 together with the clinical benefit in 25% of this hardly treatable group of metastatic RCC patients warrant further investigation. Especially patients with low tumour burden or patients with a high risk for recurrence of RCC after curative intent surgery may benefit from a cG250 treatment. Therefore, a randomized two-arm adjuvant study with cG250 versus observation is in preparation.

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