Effective Relief of Malignant Ascites in Patients with Advanced Ovarian Cancer by a Trifunctional Anti-EpCAM × Anti-CD3 Antibody: A Phase I/II Study

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Abstract

Purpose: Malignant ascites in ovarian carcinoma patients is associated with poor prognosis and reduced quality of life. The trifunctional antibody catumaxomab (anti-EpCAM × anti-CD3) enhances the antitumor activity by redirecting T cells and Fcγ receptor I/III – positive accessory cells to the tumor. This multicenter phase I/II dose-escalating study investigated tolerability and efficacy of i.p. catumaxomab application in ovarian cancer patients with malignant ascites containing epithelial cell adhesion molecule (EpCAM) – positive tumor cells.

Experimental Design: Twenty-three women with recurrent ascites due to pretreated refractory ovarian cancer were treated with four to five i.p. infusions of catumaxomab in doses of 5 to 200 μg within 9 to 13 days.

Results: The maximum tolerated dose was defined at 10, 20, 50, 200, and 200 μg for the first through fifth doses. Side effects included transient fever (83%), nausea (61%), and vomiting (57%), mostly CTCAE (Common Terminology Criteria for Adverse Events) grade 1 or 2. A total of 39 grade 3 and 2 grade 4 treatment-related adverse events (AE), 9 of them after the highest dose level (200 μg), were observed in 16 patients. Most AEs were reversible without sequelae. Treatment with catumaxomab resulted in significant and sustained reduction of ascites flow rate. A total of 22/23 patients did not require paracentesis between the last infusion and the end of study at day 37. Tumor cell monitoring revealed a reduction of EpCAM-positive malignant cells in ascites by up to 5 log.

Conclusion: I.p. immunotherapy with catumaxomab prevented the accumulation of ascites and efficiently eliminated tumor cells with an acceptable safety profile. This suggests that catumaxomab is a promising treatment option in ovarian cancer patients with malignant ascites.

Catumaxomab (anti-EpCAM × anti-CD3) is a trifunctional monoclonal antibody (MAb) with two different specificities binding simultaneously to the epithelial cell adhesion molecule (EpCAM) on tumor cells and the CD3-antigen on T cells. In addition, the unique Fc region composed of the two potent immunoglobulin (Ig) isotypes mouse IgG_{2a} and rat IgG_{2b} selectively binds to human FcγI and III-receptors on accessory cells (e.g., macrophages, dendritic cells, and natural killer cells), but not to the inhibitory FcγII receptor, expressed on B cells (1). These binding specificities of catumaxomab induce a simultaneous activation of different immune cell types at the tumor site, resulting in an effective destruction of tumor cells, including those resistant to apoptosis, through different mechanisms, i.e., perforin-mediated lysis, antibody-mediated phagocytosis, and cytokine release (1–3). In addition, results from two immunocompetent mouse models indicate a possible generation of a long-lasting humoral and cellular antitumor response (4).

Ovarian cancer is one of the most common causes of cancer death in women in the Western world (5). Because it can develop without symptoms for a long time, ~70% of patients have widespread disease with tumor dissemination into the peritoneal cavity at diagnosis. Malignant ascites emerges in 17% (FIGO [International Federation of Gynecology and Obstetrics] stage I/II) to 89% (FIGO stage III/IV) of patients (6). It is associated with a poor prognosis because the median survival is

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usually not much higher than 8 months (7). Malignant ascites can result when tumor cell deposits on the surface of the peritoneum cause mechanical obstruction, thereby preventing the adsorption of intraperitoneal fluid. Vascular endothelial growth factor, secreted by tumor cells, may also contribute to ascites formation by increasing vascular permeability (8).

Although in >90% of ovarian cancer patients EpCAM is overexpressed on tumor cells in the ascites fluid (9), the EpCAM antigen is also expressed on normal epithelial tissues (10). However, within the peritoneal cavity, EpCAM expression is tumor-specific because normal cells in the peritoneal compartment are of mesothelial origin and do not express EpCAM on their surface. Therefore, i.p. administration of catumaxomab offers the advantage of a targeted immunotherapy leading to a direct and specific attack on the ascites-causing tumor cells in the peritoneum. Furthermore, all effector cells essential for the antitumor activity of catumaxomab, like T cells and accessory immune cells, are located in the peritoneal cavity (11, 12). To target epithelial tumor cells within the peritoneal cavity, EpCAM is an attractive antigen in the otherwise mesenchymal peritoneal compartment.

The clinical management of malignant ascites is an unmet medical need because current treatment is dissatisfying. Commonly reported symptoms include abdominal bloating, anorexia, dyspnea, insomnia, fatigue, respiratory distress, lower extremity discomfort and edema, poor ambulatory capacity, and pain (13, 14). For most patients, the major therapeutic aim is the relief of ascites to improve their quality of life. Repeated ascites drainage provides temporary relief, but re-accumulation of fluid typically occurs within a short interval. Repeated paracentesis, as the consequence, removes large volumes of ascites fluid, resulting in protein loss and hypovolemia with subsequent circulatory problems including hypovolemic shock. Human albumin substitution is therefore often necessary. Repeated paracentesis can also increase the risk for bowel perforation and peritonitis.

There are no generally accepted evidence-based treatment guidelines for recurrent ascites worldwide (15). Current therapies include diuretics, frequent large-volume paracentesis, i.p. or systemic chemotherapy, and a variety of other experimental strategies such as radiolabeled antibodies and immunostimulants (16, 17). None of these approaches have been established as standard therapy for refractory malignant ascites because of limited efficacy or severe side effects (15, 18).

Based on these facts and promising findings with trifunctional antibodies (19, 20), this study was done in ovarian cancer patients with recurrent malignant ascites to investigate the tolerability and efficacy of i.p. catumaxomab.

Patients and Methods

The objectives of the reported phase I/II study were the investigation of the efficacy and tolerability, as well as the identification of the maximum tolerated dose (MTD), of increasing doses of 5 to 200 μg catumaxomab administered repeatedly for four to five doses at a constant-rate 6-h i.p. infusion to patients with ascites due to ovarian carcinoma.

Patients. Selection of patients was done according to the inclusion criteria shown in Table 1. Patients with other concurrent illnesses or unstable medical conditions were excluded. All patients gave written informed consent before participation in the trial.

The protocol and amendments were approved by independent ethics committees or institutional review boards. Most patients were hospitalized during the study. The study followed the Declaration of Helsinki and good clinical practice guidelines.

Administration and treatment plan. The trial was an open-label, sequential dose escalation study of i.p. application of catumaxomab in ovarian cancer with symptomatic ascites. Catumaxomab (removab) was manufactured by TRION Pharma GmbH, Munich, under good manufacturing practice conditions approved by the local authority. An i.p. catheter was placed for draining of ascites and catumaxomab administration. Six-hour i.p. infusions of catumaxomab were administered on days 0, 3, 6, and 9 for the first four dose groups and a fifth

Table 1. Inclusion and exclusion criteria for treatment with catumaxomab

Inclusion criteria	Exclusion criteria
Female patients of non–child-bearing potential	Acute or chronic infection
Body weight ≥45 kg	Symptoms or signs (including laboratory tests) of relevant concomitant hematologic, cardiovascular, pulmonary, hepatic, renal, pancreatic, or endocrine disease
Age ≥18 y	
Ovarian carcinoma	
Ascites that clinically required at least one peritoneal puncture	WBC <3 or >13 × 10^9/L Thrombocytes: <80 × 10^9/L Bilirubin more than twice the ULN
EpCAM+ tumor cells in ascites fluid	
Life expectancy of at least 8 wks	AST, ALT >3.5 times the ULN; if a patient has liver metastases, AST and ALT may be up to six times the ULN
One prior chemotherapy or not being suitable for chemotherapy or refusing chemotherapy	Abnormal coagulation
	Serum creatinine >2.0 mg%
Willing and able to provide informed consent	Evidence or suspicion of relevant psychiatric impairment including alcohol or recreational drug abuse
Willing to attend follow-up visits up to 1 month after application (with the provision for an open life-long follow-up)	Previous participation in the present trial
	Exposure to any investigational trial medication, cancer chemotherapy, or radiotherapy within the last 30 days before the first application
	Previous treatment with mouse Mab
	Known or suspected hypersensitivity to catumaxomab or similar antibodies
	Any further condition which, according to the investigator, resulted in an undue risk of the patient by participating in the present study.

Table 2. Actual doses of catumaxomab and number of patients receiving these doses

Dose group	Loading dose (μg)	Consecutive doses (μg)				Number of patients	
	Day 0	Day 3	Day 6	Day 9	Day 13	All	With all planned infusions
I	5	10	10	10		3	3
IIa	10	50	50	50		3	1
IIb	10	20	50	50		3	3
III	10	20	50	100		3	3
IV	10	20	50	200		4	3
V	10	20	50	200	200	7	4
Overall						23	17

infusion on day 13 for the fifth dose group. Intervals between the administrations could be prolonged in case of intolerability reactions provided that the time between the first and the last infusion did not exceed 23 days. The catheter was removed 24 h after starting the last infusion. Seven days after the last infusion, a follow-up safety evaluation was done. End of trial (EOT) examination, consisting of all safety and efficacy measurements, was done 28 days after the last infusion.

The patients were allocated to one of six dose-series groups until the MTD was achieved (Table 2). At least three patients had to be treated in each dose group. If none of the three patients experienced a dose-limiting toxicity (DLT) at a given dose, another three patients were treated at the next dose level. If one of three patients experienced a DLT, three additional patients, up to a total of six, were monitored at that dose level. A Dose Steering Board (DSB) was involved in data review and dose escalation decisions.

Intensity of adverse events (AE) was graded according to the National Cancer Institute Cancer Evaluation Program (CTEP)-Common Criteria (CTC) Version 2.0 dated April 30, 1999 (21).

The dosing schedule was selected based on pilot studies providing preliminary evidence about the effectiveness of i.p. doses between 5 and 200 μg in the treatment of peritoneal carcinomatosis due to different entities (19, 20). The number of patients in each dose group and the applied catumaxomab doses are shown in Table 2. In dose group IIa, one patient had a grade 3 serum bilirubin increase after the application of 50 μg. Therefore, the DSB decided to reduce this second dose to 20 μg, and the protocol was amended accordingly. Premedication consisted of 1,000 mg paracetamol given 30 min before the start of infusion. To improve the catumaxomab distribution within the peritoneal cavity, 500 mL of 0.9% NaCl solution was administered i.p. 30 min before the start of the catumaxomab infusion.

Table 3. Baseline characteristics of ovarian carcinoma patients receiving at least one i.p. infusion of catumaxomab

	Median (range)
Age (y)	61.7 (42-80)
Time since last diagnosis of ovarian carcinoma (months)	24 (1-106)
Number of past surgical procedures	2.0 (1-3)
Number of past medical ovarian carcinoma treatments	3.0 (1-8)
	Patients, *n* (%)
FIGO staging at primary diagnosis	
IIIb	2 (9)
IIIc	14 (61)
IV	7 (30)

Efficacy measurements. Reduction of ascites flow rate, tumor cell elimination from ascites fluid, and need of paracentesis from baseline to EOT were efficacy end points. The ascites flow rate was measured from 1 day before start of treatment until 1 day after last antibody application. The flow rate was calculated from the collected ascites volume (minus lavage volumes, if appropriate) and the time of collection.

Immunocytochemical analysis of ascites tumor cells. Ascites samples before therapy were evaluated to select patients with EpCAM-positive cells (>400/10^6 screened cells).

Additional ascites samples were collected before the second infusion on day 3, and 24 h after the last infusion (day 10 for dose groups I to IV; day 14 for dose group V) and shipped to the laboratory within 24 h. Samples before the third, fourth, or fifth infusion were optional. If there was no ascites flow, a NaCl lavage was done and processed as ascites samples.

Cells from ascites samples were prepared by density centrifugation and applied to slides (2.5×10^5 cells per slide). Slides were dried and stained with an anti-EpCAM antibody (Ho-3, isotype mouse IgG_{2a}, TRION Pharma, Munich), labeled with the Texas Red (TR) labeling kit (Molecular Probes). For evaluation, 10^6 cells were screened using the Micrometastasis Detection System (MDS; Applied Imaging). Ho-3 is the parental antibody of catumaxumab's EpCAM arm and ensures at screening that EpCAM+ tumor cells of the patient can be recognized by catumaxomab. To prevent competitive inhibition of the staining by catumaxomab residues in ascites samples during and after therapy, the follow-up samples were stained with the directly labeled Alexa Fluor 594 Texas Red anti-EpCAM antibody VU1D9 (TRION Research GmbH), which recognizes a different EpCAM epitope than Ho-3/catumaxomab.[13]

In selected patients, a double staining with anti–EpCAM-TR (VU1D9) and anti–CD45-FITC antibody was done to evaluate the tumor cell/leukocyte ratio and analyzed as mentioned above.

Immunologic parameters. Serum levels of interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) were determined as biological markers of immunopharmacologic effects.

As catumaxomab contains antigenic mouse protein, patients were analyzed for human antimouse antibody (HAMA) serum levels.

Statistical methods. Statistical analyses were done using SAS version 8.2. Continuous variables and changes from baseline were summarized using descriptive statistics. AEs were coded with MedDRA and grouped by treatment and infusion period. The volumes of ascites before and after catumaxomab treatment were compared by the Wilcoxon signed-rank test for related measurements.

Results

Patients. Out of 26 patients screened between November 2001 and May 2003, two patients died during screening, and one

[13] TRION Research GmbH, unpublished results.

Table 4. Incidence of drug-related TEAEs by SOC occurring in ≥30% of patients and by preferred term in >4% of patients

SOC	Drug-related TEAEs			
Preferred term	All		Grade 3	
	n (%)	*E*	*n* (%)	*E*
General disorders and administration site conditions	22 (96)	84	1 (4)	2
Fever	19 (83)	62	1 (4)	2
Pain	7 (30)	17	0 (—)	0
Fatigue	2 (9)	2	0 (—)	0
Gastrointestinal disorders	16 (70)	111	3 (13)	3
Nausea	14 (61)	29	0 (—)	0
Vomiting	13 (57)	43	1 (4)	1
Abdominal pain	9 (39)	21	0 (—)	0
Diarrhea	3 (13)	3	0 (—)	0
Constipation	2 (9)	4	0 (—)	0
Dyspepsia	2 (9)	4	0 (—)	0
Investigations	11 (48)	19	9 (39)	12
Blood alkaline phosphatase increased	4 (17)	4	4 (17)	4
Liver function test abnormal	4 (17)	5	2 (9)	2
γ-Glutamyltransferase increased	3 (13)	3	2 (9)	2
Aspartate aminotransferase increased	2 (9)	2	1 (4)	1
Hyperbilirubinemia	3 (13)	6	2 (9)	2
Metabolism and nutrition disorders	8 (35)	12	5 (22)	5
Hypercalcemia	2 (9)	4	1 (4)	1
Blood and lymphatic system disorders	7 (30)	12	6 (26)	6
Lymphocytopenia	6 (26)	10	6 (26)	10
Leukocytosis	2 (9)	2	0 (—)	0

NOTE: *n*, number of patients; *E*, number of AEs. Additional CTC Grade 3 TEAEs were observed as follows: hypertension (one patient), ileus (one patient), large intestinal obstruction (one patient), hyperuricemia (one patient), hypocalcemia (one patient), hypokalemia (one patient), hyponatremia (one patient), polyneuropathy (one patient), dyspnoe (one patient). A total of two CTC grade 4 toxicities were observed in two patients: one GGT increase, one hyperuricemia.

patient did not meet the inclusion criteria. Therefore, 23 patients were treated with catumaxomab. At the time of therapy, patients had advanced and heavily pretreated disease with a median of 3 prior treatments (1–8) and of 24 months from first diagnosis (Table 3). At primary diagnosis, most patients had FIGO stage IIIc (61%) and the others in stages IV (30%) and IIIb (9%). The most common prior medications were platinum compounds (22 patients), followed by taxanes (e.g., paclitaxel, $n = 20$) and pyrimidine analogues (e.g., fluorouracil, $n = 10$). Eleven patients received other antineoplastic agents (altretamin, topotecan). All patients had recurrent malignant ascites, i.e., requiring at least one prior paracentesis.

Safety. Catumaxomab doses ranged from 5 to 200 μg in five dose groups (Table 2). In dose group IIa, one patient experienced a grade 3 increase of serum bilirubin after the second application (50 μg). Although the definition of a DLT was not fulfilled, the study was amended and continued with a reduced second dose of 20 μg of catumaxomab. DLTs occurred in two out of seven patients of dose group V (patient 1, large bowel obstruction of grade 3 after 200 μg, and patient 2, GGT increase of grade 4 after 50 μg). Therefore, the DSB defined the MTD series as 10, 20, 50, 200, and 200 μg for 6-h constant-rate i.p. infusions of catumaxomab administered over 9 to 13 days, with a premedication of 1,000 mg paracetamol. In further clinical studies, a dose series of 10, 20, 50, and 150 μg i.p. are used as standard therapy, which is below the MTD.

All patients had treatment emerged AEs (TEAE) with fever, nausea, vomiting, abdominal pain, lymphopenia, and general pain being the most common, and all patients had at least one TEAE considered possibly related to treatment (Table 4). Sixteen patients experienced a total of 41 grade ≥3 possibly related TEAEs. Nine of these were observed after application of the highest catumaxomab dose (200 μg). A total of 6 out of 23 patients discontinued the trial early. Reasons included toxicity ($n = 3$), disease-related complications ($n = 2$), and catheter-related sepsis ($n = 1$). Most possibly related TEAEs were mild or moderate. Transient grade 3 decrease of lymphocytes related to catumaxomab infusion observed in six patients had returned to lower grades within 1 to 8 days. No association with an increased infection rate was observed. There was no apparent dose dependency for the frequency of possibly related TEAEs.

The most common TEAEs were from the system organ classes (SOC) "gastrointestinal disorders" and "general disorders and administration site disorders" (Table 4). These TEAEs usually occurred on the day of infusion or the day after and were fully reversible. There was no consistent correlation between the frequency of TEAEs and infusion period, dose, or serum cytokine values.

Fifteen patients experienced serious adverse events and those in six patients were considered possibly related to treatment: increase of liver enzymes, skin infection, catheter-related infection, extravasation, hemorrhagic erosive gastritis, large bowel obstruction, rash, and ileus. Three patients died during the study (19, 24, and 28 days after the last infusion). None was considered catumaxomab related but due to progression of underlying disease.

Abnormal liver parameters of grade ≥3 were observed in 10 patients, in one patient already at screening, most often after the second or third infusion. One patient experienced CTC grade 4 GGT elevation after the third (50 μg) infusion, which was reported as DLT. In seven of these patients, including the one with GGT grade 4, all values returned to normal (or grades 1 or 2) or baseline by EOT, whereas in the other two, the last available values at day 5 were still grade 3. The grade 4 and one grade 3 elevation of liver function parameters led to treatment discontinuation in two patients. Drug-related grade 4 hyperuricemia was observed in one patient of dose group V after the fourth and fifth infusion, which was not clinically relevant.

There were no significant changes in mean blood pressure during treatment. Relevant changes of systolic blood pressure were reported for 4 of 23 patients. Pulse rate and body temperature significantly increased in 15 and 21 patients, respectively, between 6 and 12 h after the start of particularly the first and second infusions. Cardial and physical examinations did not show any clinically relevant abnormalities/changes during the study period.

Efficacy. Mean and median ascites flow rates were comparable in all dose groups at baseline (range 46.2-446.9 mL/h) and decreased from a median of 105 mL/h at baseline to 23 mL/h 1 day after the fourth infusion, resulting in a median decrease by 52 mL/h.

In dose groups IIb, III, IV, and V, a significant decrease of ascites flow rate was observed after the third infusion ($P = 0.038$, Wilcoxon signed-rank test, Fig. 1A) compared with baseline. Only one patient needed a paracentesis during the study at the last individual visit.

The mean value of EpCAM+ tumor cells was reduced from 539,000 per 10^6 screened cells before treatment to 39 per 10^6 at the last individual measurement, resulting in a mean reduction of 99.9% (Fig. 1B). In 6 out of 23 patients, tumor cell elimination to levels below the detection limit was observed.

In selected patients, the evaluation of the EpCAM+ tumor cell/CD45$^+$ leukocyte ratio during therapy was possible. The data of one representative patient are shown in Fig. 2. Before therapy, only a minority of FITC-labeled CD45$^+$ leukocytes (*green*) was present in the malignant ascites cell population, whereas the majority of ascites cells were TR-labeled EpCAM+ tumor cell clusters (*red*). In the ascites sample from day 3 of treatment, after i.p. infusion of 10 μg of catumaxomab, this ratio was completely reversed (from 7:1 to 1:69), i.e., tumor cells decreased distinctly, whereas the portion of CD45$^+$ leukocytes increased. On day 6 after 20 μg administration of catumaxomab, mainly leukocytes were visible in the ascites sample, whereas tumor cells had almost disappeared.

Immunologic parameters. At baseline, 17 patients (74%) had normal serum levels of IL-6 and TNFα, and 6 (26%) had values above the upper limit of normal (ULN) for both cytokines. The proportion of patients with IL-6 values above ULN increased to about 80% on the days after infusion and decreased to almost baseline values before the next infusion. The proportion of patients with elevated TNFα values on the infusion days was about 60%.

At baseline, all patients were HAMA negative. One patient (dose group V) showed a weak HAMA positivity (533 ng/mL) 1 day after the last infusion (day 14). At EOT (day 37), HAMA values had exceeded the ULN with a mean of 1,043 ng/mL in all (14/15) but one tested patient.

Discussion

Trifunctional antibodies are being developed to enhance the immunologic response against tumors by binding cell types, which are essential for a complex immune reaction. Here, we present the results of a phase I/II study evaluating safety and efficacy of the trifunctional antibody catumaxomab administered i.p. to ovarian cancer patients with recurrent malignant ascites.

The described drug application schedule of four to five subsequently increasing doses of catumaxomab within 9 to 13 days was found to be safe and feasible for clinical practice. Severe AEs or organ failure requiring ICU treatment were not observed.

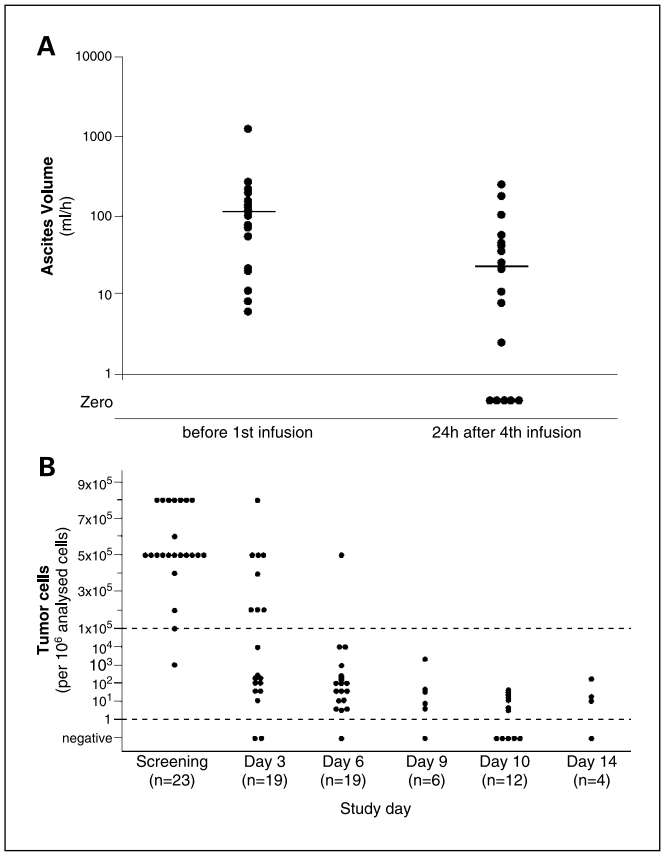

Fig. 1. Reduction of ascites flow and tumor cell number within the ascites fluid during i.p. therapy with catumaxomab. *A,* individual ascites flow rates in the 17 patients of dose groups IIb, III, IV, and V, before treatment and 24 h after the fourth antibody infusion. Lines over the dots, median values of 105 and 23 mL/h, respectively. *B,* EpCAM-positive tumor cells in ascites per 10^6 immunohistochemically analyzed cells in ascites. Days 3, 6, and 9, before second, third, and fourth infusion. Days 10 and 14, 1 d after fourth and fifth infusion.

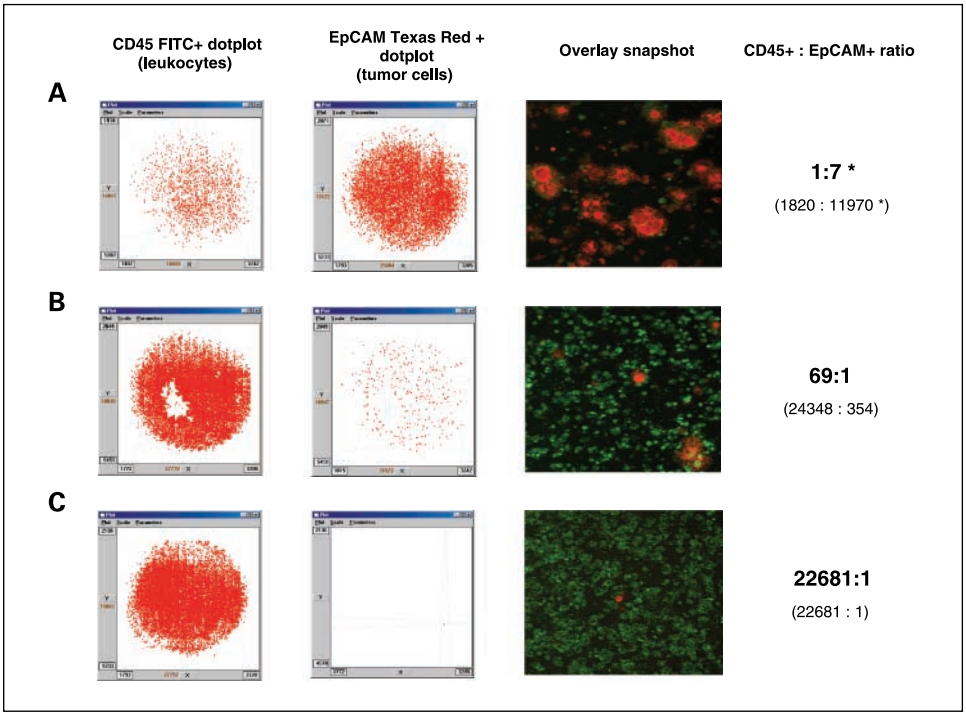

Fig. 2. Fluorescent double staining of ascites fluid cells: evaluation of EpCAM+ tumor cell/$CD45^+$ leukocyte ratio during catumaxomab therapy. *A,* malignant ascites harvested at screening puncture. *B,* at day 3 after 10 μg catumaxomab i.p. *C,* at day 6 after a total of 30 μg catumaxomab i.p. At day 11 after a total of 230 μg catumaxomab i.p., no spontaneous ascites flow was detectable anymore; a lavage was not done. n.a., not available. Dotplot analysis: every plot resembles a fluorescence-labeled cell that was detected and counted by the computerized image analysis system. *The computerized image analysis was not able to detect a correct number of single tumor cells because of the massive clustering of EpCAM+ cells, so the number of EpCAM+ tumor cells is relatively low compared with the overlay snapshot that shows the real situation with a huge amount of EpCAM+ tumor cell clusters and only small counts of $CD45^+$ leukocytes.

The most frequently observed AEs during catumaxomab therapy were fever, nausea, and vomiting. Those AEs may be attributed to a systemic treatment-associated cytokine release as a consequence of the complex immunoreaction against tumor cells caused by catumaxomab. Cytokine release–related symptoms are a well-known side effect of antibody therapies (22, 23) and are regarded as an indicator of efficacy. In the case of catumaxomab, the activation of Fc-receptor–bearing cells besides T cells leads to the additional secretion of cytokines as, e.g., IL-6 or TNFα, which could also be detected in peripheral blood after i.p. application. However, the third valence of trifunctional antibodies is essential because it leads to a substantial enhancement of antitumor efficacy.

As shown by Ruf and Lindhofer in a mouse tumor model, the additional binding to the activating Fcγ receptor types I and III was mandatory for effective tumor cell killing and the induction of a long-lasting antitumor immunity (4).

The release of cytokines may also contribute to the transiently elevated levels of liver enzymes which normalized mainly to baseline values at EOT. On the other hand, the increase in GGT, AP, and bilirubin may be caused by the penetration of catumaxomab into extraperitoneal tissues and binding to EpCAM, which is expressed, e.g., at the bile duct epithelium (24). Interestingly, in a phase I study with the trifunctional antibody ertumaxomab which binds to Her2 instead of EpCAM, the observed increase of liver enzymes was less pronounced than in the present study despite systemic i.v. application (25). However, the exact mechanisms by which the antibodies are transferred to adjacent EpCAM-positive tissues outside the peritoneal cavity are currently unknown.

There was no clear relationship between dose level and severeness of adverse events. This is not unusual especially for immunomodulating agents for cancer treatment (26). However, as a measure of precaution, the dose level 10-20-50-200-200 μg was defined as MTD and a dose schedule of 10-20-50-150 μg was recommended for further clinical trials, which is distinctly below this MTD.

The observed transient lymphocyte decrease has been reported in other studies, suggesting that infused bispecific antibodies induce a passive interaction of lymphocytes with endothelial cells, followed by a generalized redistribution of lymphocytes into tissues (27). As in studies in bone marrow/stem cell transplantation, a restoration of peripheral lymphocytes after cytotoxic damage due to high-dose chemotherapy took 1 to 6 months (28, 29), the transient decrease of lymphocytes for 1 to maximally 8 days is most probably due to a shift of the lymphocytes into another compartment, where they are not detectable by conventional blood sampling measures.

The administration of catumaxomab led to a considerable reduction of ascites production (Fig. 1A). Only 1 of 23 patients required a paracentesis by EOT 28 days after the last infusion. This is distinctly longer as the median interval of 7 to 11 days reported in literature for recurrent malignant ascites (30–32). As repeated paracentesis increases the risk of infection and cachexia, treatment with catumaxomab seems to be an encouraging alternative to conventional therapies.

The therapy with catumaxomab was accompanied by a rapid and significant elimination of EpCAM+ tumor cells in all patients. The average number of tumor cells in the ascites fluid decreased from $540{,}000/10^6$ analyzed cells before treatment to $39/10^6$ following the last infusion, indicating an antitumor effect of catumaxomab (Fig. 1B). These results confirm previous preclinical (*in vitro*, animal model) and clinical (pilot studies) data with trifunctional antibodies (1, 2, 4, 19, 20, 33).

In the present study, it could be shown that immune cells in the malignant ascites are also influenced by catumaxomab. I.p. application of catumaxomab leads to a dramatic increase of the $CD45^+$ leukocytes in the peritoneal cavity resulting in a reversion of the tumor cell/leukocyte ratio in favor of the leukocytes (Fig. 2). This observation indicates that tumor cell

elimination is caused by the proposed mechanism of action of catumaxomab and not simply by serial drainages of ascites followed by dilution with infused catumaxomab/sodium chloride.

Because catumaxomab is a mouse/rat MAb, there is a potential for immunogenicity resulting in HAMA reactions. Other studies showed that HAMA appears 2 to 3 weeks after the first antibody application (34, 35). In response to catumaxomab, the majority of tested patients (14 of 15) developed moderate HAMA values at EOT. During the study, only one of the tested individuals from dose group V had developed a weak HAMA response after the last infusion on day 14. This patient's follow-up showed that neither tumor cell elimination nor prevention of ascites production was affected by the observed HAMA titer. Our data are supported by the observations of Marme et al. (36) who reported a stop of ascites production after repeated i.p. applications of a bispecific anti-EpCAM × anti-CD3 antibody even in the presence of HAMA. These results suggest that certain HAMA titers have to be reached to inhibit antitumor cytotoxicity. Interestingly, elevated HAMA levels after treatment of ovarian cancer patients with a bispecific F(ab)2 OC/TR antibody were associated with longer median survival (37), which may indicate a superior antitumor immune reactivity in HAMA-positive patients.

In conclusion, the i.p. application of catumaxomab induced effective tumor cell destruction in malignant ascites, substantially decreased ascites accumulation, and reduced the necessity for paracentesis. Thus, i.p. infusion of catumaxomab represents a targeted tumor therapy within the peritoneal cavity associated with a substantial improvement of symptoms related to malignant ascites. The toxicity was acceptable. Most of the AEs were signs of the proposed mechanism of action and therefore predictable. They were limited as the intensity remained mainly within mild to moderate, and they were manageable and reversible as all relevant AEs resolved within days to weeks.

The promising results of this study will be investigated in further clinical studies, which will elucidate the clinical potential of trifunctional antibodies in several tumor entities.

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