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Catumaxomab

Anti-Ep-CAM Monoclonal Antibody
Oncolytic

Rec INN

Removab®

Trifunctional bispecific hybrid mouse-rat monoclonal antibody against human Ep-CAM and human CD3 (anti-Ep-CAM x anti-CD3), which also binds to Fcγ receptors I and III via its intact Fc region

Trifunctional bispecific hybrid monoclonal antibody consisting of anti-human Ep-CAM mouse IgG_{2a} and anti-human CD3 rat IgG_{2b}, which also binds to Fcγ receptors I and III via its intact Fc region

Immunoglobulin G_{2a}, anti-(human antigen 17-1A) (mouse monoclonal Ho-3/TP-A-01/TPBs01 heavy chain), disulfide with mouse monoclonal Ho-3/TP-A-01/TPBs01 light chain, disulfide with immunoglobulin G_{2b} anti-(human CD3 [antigen]) (rat monoclonal 26/II/6-1.2/TPBs01 heavy chain), disulfide with rat monoclonal 26/II/6-1.2/TPBs01 light chain

CAS: 509077-98-9

CAS: 810670-61-2

EN: 402346

Abstract

Catumaxomab (Removab®) is a trifunctional antibody that simultaneously activates T cells and accessory immune cells to destroy target tumor cells possessing the surface antigen epithelial cell adhesion molecule (Ep-CAM). The agent has been submitted for approval with the European authorities for the treatment of malignant ascites caused by Ep-CAM-positive metastatic epithelial-derived tumors, and is in phase II trials for the treatment of ovarian and gastric cancers.

Background

The human Ep-CAM (CD326) protein, also referred to as EpCAM, 17-1A, HEA125, MK-1, GA733-2, EGP-2, EGP34, KSA, TROP-1, ESA and KS1/4, among other terms, was the first human tumor-associated antigen (TAA) identified using monoclonal antibodies (mAbs) (1, 2). This highly immunogenic TAA is a type I transmembrane glycoprotein (39-42 kDa) that is comprised of a large extracellular domain with two epidermal growth factor (EGF)-like domains, a single transmembrane domain and a short cytoplasmic tail, and that acts as an epithelial cell adhesion and activating molecule (2-4). The protein is overexpressed on a wide variety of human tumors, including stomach, colon, prostate, ovarian, breast and lung cancers (2, 5-8), and recent experimental findings have demonstrated that its inhibition is associated with a decrease in the proliferation, migration and invasion of cancer cells (9, 10). Ep-CAM is therefore now considered an attractive target for the immunotherapy of cancer.

The development of mAbs specifically targeting a particular antigen expressed on tumor cells represented an important advance in cancer immunotherapy. Efforts to improve their immunological effector functions led to the development of bispecific antibodies that target both a tumor-specific antigen and T cells, and trifunctional bispecific antibodies which additionally target accessory cells (macrophages, dendritic cells, natural killer [NK] cells) were subsequently designed with enhanced antitumor activity. The trifunctional antibody is able to recruit and activate both T cells and accessory cells at the tumor site via two distinct, specific binding arms (tumor-specific antigen and CD3) and an intact Fc region that selectively binds to Fcγ receptor-positive accessory cells. The subsequent mutual costimulation of immune cells mediated by costimulatory receptors and cytokines results in a coordinated antitumor response involving T cell-mediated lysis, cytotoxicity by cytokines secreted by T cells, and phagocytosis and antibody-dependent cell cytotoxicity via activation of accessory cells (11-18).

Catumaxomab is a hybrid of a mouse IgG_{2a} heavy and light chain derived from the monoclonal antibody HO-3 and a rat IgG_{2b} heavy and light chain derived from the monoclonal antibody 26/II/6-1.2 (19). The IgG_{2a} arm recognizes human Ep-CAM (CD326), while the IgG_{2b} arm binds and activates T cells via the CD3 receptor (13, 17, 19) (Fig. 1). Catumaxomab (Removab®) was submitted for approval with the European Medicines Agency (EMEA) in late 2007 for the intraperitoneal treatment of malignant ascites (20). Besides malignant ascites, catumaxomab is also in phase II trials in patients with various advanced-stage tumors, including ovarian and gastric cancers, where it may provide a more effective and tolerable alternative to current chemo/radiotherapies.

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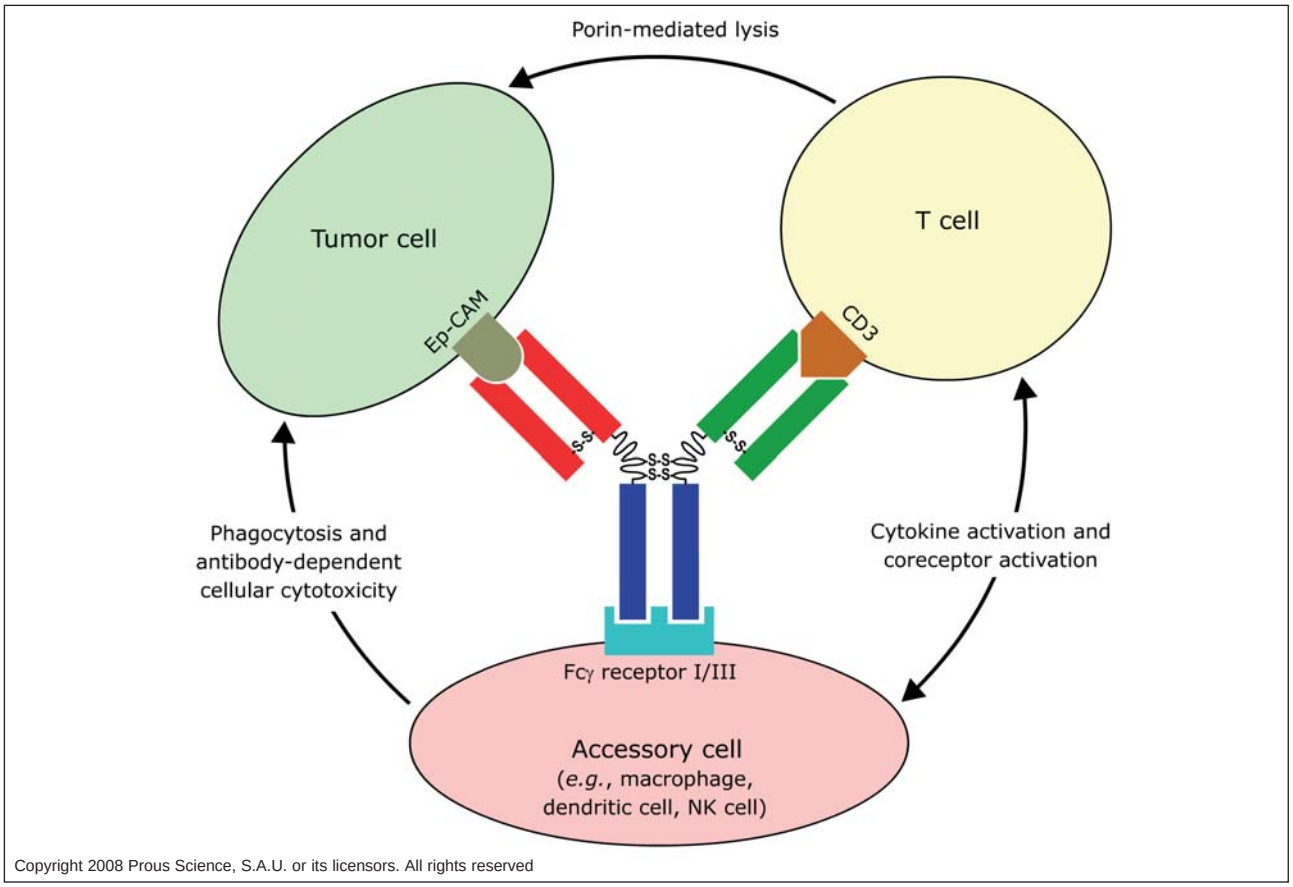

Fig. 1. The proposed tricell complex and cell interactions mediated by catumaxomab. The costimulatory receptors implicated in the activation of the tricell complex are CD40/CD40L, B7.1-2/CD28 and LFA-3/CD2. Elevated cytokines include IL-1, IL-2, IL-6, IL-12, TNF-α, interferon gamma and DC-CK1.

Preclinical Pharmacology

Binding studies using the extracellular domain of human Ep-CAM expressed in yeast showed that catumaxomab has high binding affinity and a low dissociation constant (K_D = 5.6 x 10^{-10} M), which was comparable to the parent of its Ep-CAM-binding arm HO-3. When peripheral blood mononuclear cells (PBMCs) from healthy donors were cocultivated with Ep-CAM-positive HCT-8 human colon cancer cells and catumaxomab for 3 days, catumaxomab mediated tumor cell killing at concentrations of 0.1 ng/ml and above, and was 1,000-fold more effective than HO-3 (19).

The Ep-CAM-positive human larynx carcinoma cell line BHY was cocultured with PBMCs from healthy volunteers in the presence or absence of catumaxomab. In the presence of catumaxomab tumor cells lysed, the secretion of interferon gamma by $CD3^+$ T cells increased and the $CD83^+$ dendritic cell population increased. Pretreatment of the PBMCs with catumaxomab (opsonization) followed by washing to remove excess antibody and released cytokines was equally effective at causing BHY cell lysis, stimulating interferon gamma secretion and activating dendritic cells. When isolated $CD8^+$ T cells were opsonized with catumaxomab and incubated with Ep-CAM-positive BHY cells, the levels of interferon gamma and granzyme B (a marker of cytotoxic T lymphocyte activation) increased, demonstrating that at least part of the tumor lytic activity stimulated by catumaxomab was due to cytotoxic T cell activity. In the chick chorioallantoic membrane (CAM) assay, BHY cells were incubated in the presence or absence of catumaxomab-opsonized PBMCs. Opsonized PBMCs caused significant lysis of the tumor cells at 24 h, which remained unchanged over the subsequent 24 h. When PBMCs alone were incubated with catumaxomab, the levels of TNF-α, interferon gamma and IL-2 all increased over the first 24 h and remained steady thereafter; no cytokines were released in the absence of catumaxomab. There was a particular emphasis on opsonization of PBMCs in these experiments because patients treated by direct i.v. injection with immunomodulating agents are at risk of adverse events caused by cytokine release syndrome, and opsonization potentially reduces that risk (21).

Tumor tissue and PBMCs were obtained from 22 patients with head and neck squamous cell carcinoma (HNSCC) and coincubated with or without catumaxomab in the CAM model. In comparison with PBMCs alone,

catumaxomab caused a 2-fold drop in the viability of the tumor cells. In addition, interferon gamma levels increased 10-fold and $CD8^+$ T cells and $CD83^+$ dendritic cells increased 5-fold, indicating activation of PBMCs by catumaxomab. Similar results were obtained when catumaxomab-opsonized PBMCs were substituted for catumaxomab + PBMCs in this model, and also using the tumor cell lines BHY and colon carcinoma HCT-8 (22).

Using the same model of autologous HNSCC with primary tissue samples from 36 patients (4 were Ep-CAM-negative), incubation of tumor tissue with autologous PBMCs in the absence of catumaxomab resulted in no change in the tumor cell viability after 48 h. In the presence of either cisplatin (positive control) or catumaxomab, the viable tumor cell count fell by approximately 50% over the first 24 h and remained steady over the next 24 h. In two of the samples with Ep-CAM-negative tumors, the viable tumor count was reduced by < 20% on treatment with catumaxomab (23).

An *in vitro* proliferation assay was used to assess the activity of catumaxomab in pleural fluid samples obtained from patients with Ep-CAM-positive malignant pleural effusion before they received catumaxomab therapy. Relative to cultures without catumaxomab, the levels of the cytokines IL-2, IL-6, interferon gamma and TNF-α were higher after 24 h of incubation with 100 ng/ml catumaxomab, indicative of T cell activation with a Th1 cytokine profile. After 72 h of incubation with catumaxomab, $CD4^+$ and $CD8^+$ T cell counts were increased, as were $CD11c^+$ monocyte counts, relative to the cultures without catumaxomab (24).

Safety

A phase I dose-escalating study investigated the safety and tolerability of a single i.v. infusion of catumaxomab (2-7.5 μg) administered with or without dexamethasone (40 or 10 mg i.v.) premedication in patients with non-small cell lung cancer (NSCLC). All patients were treated with antihistamines 30 min before the study medication. Six of 15 evaluable patients experienced treatment-related adverse events of grade 3 or higher, with a tendency towards a greater incidence of adverse events at higher drug doses. One patient receiving catumaxomab (2 μg) without dexamethasone experienced syncope during the drug infusion, resulting in discontinuation of the infusion, and there were no further treatments without dexamethasone premedication. Patients receiving 5 μg catumaxomab + 10 mg dexamethasone (n=5) or 7.5 μg catumaxomab + 40 mg dexamethasone (n=2) experienced grade 3 or grade 4 elevation of liver parameters, including elevated alanine aminotransferase, aspartate aminotransferase and γ-glutamyltransferase. Elevated liver enzymes decreased to grade 2 within a week and reached baseline levels within 2 weeks of the infusion. The most frequent grade 1/2 toxicities were pyrexia, nausea, dizziness, headache and tachycardia, which were all related to cytokine release. The maximum tolerated dose (MTD) was defined as 40 mg dexamethasone followed by 5 μg catumaxomab. There were no human anti-mouse or anti-rat antibody reactions detected at days 7 and 28 after the infusion, except in 1 patient who had preexisting anti-rat antibodies. Most patients received additional tumor therapies after catumaxomab treatment, although survival rates did appear to indicate clinical efficacy for catumaxomab, with 4 of 4 stage IIIB patients still alive 2 years after catumaxomab treatment (25-27).

A multicenter, uncontrolled, dose-escalating phase I/II study investigated the toxicity and MTD of catumaxomab in patients with recurrent Ep-CAM-positive malignant pleural effusion. Thirteen of 24 patients received all three planned doses of catumaxomab (5-200 μg) administered by intrapleural infusion. The reasons for early termination of treatment were deteriorating performance (3), exanthema (2), dyspnea (2) and others (2). Thirty-two serious adverse events were seen in 18 patients, of which death due to pleural empyema (1), pneumonia (1), erythema (1) and increase in liver enzymes (1) were considered possibly drug-related. The most frequent adverse events were symptoms of cytokine release syndrome, and most patients had elevated human anti-mouse or anti-rat antibodies at the end of treatment. The MTD was defined as 20, 50 and 100 μg for doses 1, 2 and 3 (28).

The safety of catumaxomab in patients with peritoneal carcinomatosis due to Ep-CAM-positive gastrointestinal cancer was explored in a phase I dose-escalating trial. Patients received three or four i.p. applications of escalating doses of catumaxomab (10-200 μg). In an interim analysis of 17 evaluable patients, the MTD was defined as 10, 20, 50 and 200 μg for the first through the fourth infusions, respectively. The most frequent adverse events > grade 2 were nausea/vomiting (14), abdominal pain (12), fever (6), exanthema (4) and elevation of liver enzymes (4), but all events could be controlled by conventional medication (29, 30).

In another multicenter, open-label, dose-escalating phase I/II study, 23 women with recurrent ascites due to Ep-CAM-positive ovarian cancer were treated with catumaxomab (5-200 μg) administered by i.p. infusion on days 0, 3, 6 and 9, and on day 13 in those receiving a fifth dose. Patients were premedicated with 1 g paracetamol 30 min before the start of the infusion. Two dose-limiting toxicities were seen in 7 patients treated with the maximum dosing schedule (10, 20, 50, 200 and 200 μg for doses 1 through 5), and this schedule was defined as the MTD. Three patients died during the study, which was not considered to be related to study treatment, and 6 patients discontinued the trial early due to toxicity, disease-related complications and catheter sepsis. Grade 3/4 adverse events consisted of elevated liver enzymes (seen in 10 patients mostly after the third or fourth infusion), skin infection, catheter-related infection, extravasation, hemorrhagic erosive gastritis, large bowel obstruction, rash and ileus. There was a transient decrease in peripheral lymphocytes in 6 patients, which returned to normal by day 8 after the cessation of treatment. Most of the adverse events were reversible. All patients experienced grade 1/2 adverse events, the most common of

which were transient fever (83%), nausea (61%) and vomiting (57%). These are all symptoms of cytokine release, and consistent with this observation, increased levels of IL-6 and TNF-α could be detected in the peripheral blood of the patients after each administration. Fourteen of fifteen tested patients had elevated human anti-mouse antibodies at the end of treatment (day 37) (31-33).

Clinical Studies

A pilot trial explored *ex vivo* opsonization of PBMCs with catumaxomab as a way of controlling the intravascular release of cytokines while maintaining its anticancer activity. PBMCs were collected from 4 patients with recurrent HNSCC, incubated *ex vivo* with catumaxomab, washed to clear the cells of released cytokines and excess catumaxomab, and then reinjected into the patients at escalating doses of 1 x 10^4, 1 x 10^5, 1 x 10^6 and 1 x 10^7 $CD3^+$ cells/kg body weight at 2-week intervals. *Ex vivo* treatment of the PBMCs with catumaxomab caused the release of the cytokines IL-2, interferon gamma and TNF-α, which were removed by the washing process. *In vitro* assays on the opsonized blood samples showed that the proportion of $CD4^+/CD8^+$ cells expressing the activation markers CD25 and CD69 increased, and the number of cells expressing CD83 increased, indicative of dendritic cell maturation. When the opsonized/washed PBMCs were incubated with Ep-CAM-positive BHY cells, interferon gamma and granzyme B were released, indicating biological activity of the material. Two patients received all four dose escalations, 1 of whom was in complete remission and still alive after 27 months; the other had stable disease for 4.5 months and survived 8.2 months after the initiation of treatment. A third patient had an emergency tracheotomy and skipped the second dose escalation but received the third and fourth doses, and this patient survived for 3.7 months. A fourth patient died of aspiration pneumonia during treatment. Neither the death nor the tracheotomy was considered to be related to the treatment. Treatment-related grade 3 elevated liver enzymes were observed in 2 patients at the highest dose. As these events were not seen at the penultimate dose escalation (1 x 10^6 cells/kg body weight), this was defined as the MTD (34, 35).

Nine patients with peritoneal carcinomatosis from various solid tumors (6 gastric, 2 ovarian and 1 adenocarcinoma of unknown primary origin) received two to five doses of either catumaxomab 5-80 μg or ertumaxomab (an anti-HER2/NEU x anti-CD3 trifunctional antibody in development by Trion Pharma for the treatment of metastatic breast cancer) 10-100 μg by i.p. infusion. After 4 weeks, patients received catumaxomab + PBMCs + irradiated tumor cells to boost the immune reaction. Treatment was well tolerated and there were no severe side effects. Five patients had an increased $CD4^+/CD8^+$ T cell count 1 week after the boost, indicating specific antitumor activity. Five patients had a clinical response, with a mean time to progression of 4.6 months (36).

Another pilot study assessed the safety and efficacy of catumaxomab in 8 treatment-experienced patients with peritoneal carcinomatosis and symptomatic ascites due to a variety of solid tumors (2 ovarian, 3 breast, 1 bronchial, 1 gastric and 1 adenocarcinoma of unknown primary origin). Seven patients received escalating i.p. doses of either catumaxomab alone or catumaxomab in combination with ertumaxomab over a period of up to 23 days to reach a total of 145-940 μg of antibody per patient. There were no severe adverse events or treatment discontinuations. Adverse events included fever, abdominal pain and skin reactions. Serum levels of IL-6 rose sharply after the first application and less so on subsequent applications, but serum levels of TNF-α rose progressively higher after each application, with the highest levels after the last application. During the treatment period, ascites production disappeared and the mean paracentesis-free interval was 38 weeks, which was associated with relief of the clinical symptoms of ascites. Flow cytometry of the ascites tissue demonstrated elimination of the tumor cells (> 5 log reduction) in 4 of 4 patients analyzed, and reverse transcriptase-PCR analysis showed the elimination of Ep-CAM transcripts in 5 of 6 patients analyzed. The elimination of tumor cells in ascites correlated with improvements in the clinical symptoms (37).

Analysis of efficacy in the above-mentioned multicenter phase I/II study showed a reduction in pleural fluid in 8 of 13 patients, and a reduction in tumor cells of up to 5 log in 10 of 13 patients (28).

Efficacy analysis in the trial in patients with peritoneal carcinomatosis due to Ep-CAM-positive gastrointestinal cancer showed decreased tumor cells in the ascites fluid at the end of antibody treatment in 7 of 8 patients. Patients were free to receive further cancer therapies after catumaxomab treatment and 11 of 17 patients were still alive at 10 months and 7 of 17 patients were still alive at 15 months following catumaxomab treatment (29, 30). In a subsequent analysis, the median survival time for 22 patients who had received catumaxomab was 12.2 months after the initial diagnosis of peritoneal carcinomatosis, which was significantly greater than the 9.7 months for patients receiving conventional treatment in a matched-pair analysis (38).

Efficacy endpoints in the phase I/II study in women with recurrent ascites due to Ep-CAM-positive ovarian carcinoma were reduction of ascites flow rate, the need for paracentesis during the study period and tumor cell elimination from the ascites. The median ascites flow rate in 17 patients was reduced from 105 ml/h at baseline to 23 ml/h 1 day after the fourth infusion, and only 1 patient required paracentesis during the 37-day study period. The number of Ep-CAM-positive malignant cells in the ascites fluid was reduced by a mean of 99.9% and was below the limit of detection in 6 patients. Peripheral blood analysis showed that i.p. administration of catumaxomab reduced the number of circulating tumor cells, detectable in 8 of 13 blood samples before therapy and in 4 of 11 samples after therapy (31-33, 39, 40).

A European, randomized, open-label phase II/III trial examined the paracentesis-sparing effect of catumaxomab in Ep-CAM-positive cancer patients with malignant ascites who could no longer be treated with chemotherapy (Fresenius Biotech study number: IP-REM-AC-01). Half of the cases were caused by ovarian cancer (n=129) and the remainder were due to gastrointestinal (25%), breast (5%), pancreatic (3.5%) and unknown/other (13%) primary cancers. Patients (n=258) were randomized 2:1 to i.p. catumaxomab following paracentesis on days 0, 3, 7 and 10 at ascending doses of 10, 20, 50 and 150 μg (n=170), or to paracentesis alone (n=88). One hundred and fifty-seven patients received at least one dose of catumaxomab and 131 patients received all four doses. The primary endpoint –the median puncture-free survival (*i.e.*, the time until the first therapeutic paracentesis or death)– was 46 days in the catumaxomab group and 11 days in the control group. Secondary endpoints also showed significant improvements: 1) the time to the first therapeutic puncture (excluding patients who died before the next puncture) was 77 days in the catumaxomab group compared to 13 days in the control group; 2) the median time to disease progression was 111 days in the catumaxomab group *versus* 35 days in the control group; 3) the median overall survival was 86 days in patients receiving all four doses of catumaxomab *versus* 68 days for patients in the control group (survival advantage of 18 days); and 4) both physician and patient questionnaires showed improvements in the ascites symptoms in the catumaxomab treatment arm. Stratification of the patients by the primary origin of the cancer showed that those with ovarian cancer had the best improvement, with a median puncture-free survival time of 52 days in the catumaxomab arm (n=88) *versus* 11 days in the control arm (n=44); the time to the first therapeutic puncture was 71 days *versus* 11 days, the time to the next therapeutic puncture was 26 days in the catumaxomab group *versus* 13 days in the control group, the median time to disease progression was 111 days *versus* 35 days and the median survival advantage was 29 days in those receiving per-protocol catumaxomab treatment. Those with gastric cancer showed similar improvement, with a puncture-free survival time of 44 days *versus* 15 days and the time to the first therapeutic puncture of 118 days *versus* 15 days. The median survival advantage for the gastric cancer patients receiving per-protocol catumaxomab was 27 days. All other patients had a median puncture-free survival time of 30 days (catumaxomab) *versus* 9 days (control) and a median time to first therapeutic puncture of 69 days *versus* 15 days. Overall and progression-free survival were longer in the ovarian cancer patients treated with catumaxomab. Catumaxomab was safe and no deaths were attributed to the active treatment. Laboratory tests showed transient changes in the blood cell count and liver function, most of which were mild to moderate and rarely considered clinically significant. The most frequent adverse effects were abdominal pain and those related to cytokine release syndrome, including fever, nausea and vomiting. Pharmacodynamic analysis showed a reduction in the number of tumor cells in the ascites fluid in the catumaxomab group. This was accompanied by an increase in the number of $CD45^+$ T cells (a general marker of T cells) and the ratio of $CD45^+$ T cells to Ep-CAM^+ tumor cells increased from 6:1 at baseline to 10,000:1 after the first infusion of catumaxomab. Intraperitoneal expression levels of CD69 (a marker of T cell activation) increased, as did the secretion of IL-2 and interferon gamma. Serum IL-6 levels also increased after each infusion, consistent with the most frequent adverse events being related to cytokine release and indicating systemic effects of catumaxomab. *In vitro* analysis of samples take from the ovarian cancer patients and from controls confirmed the *in vivo* findings, and additionally showed that catumaxomab treatment mediated the upregulation of IL-2, interferon gamma and proliferation of $CD4^+$ and $CD8^+$ T cells and of $CD11c^+$ accessory cells. These findings are consistent with the proposed mode of action of catumaxomab (41-46).

In a phase II study designed to evaluate two different doses of catumaxomab (AGO Ovarian Cancer Study Group study number AGO-OVAR 2.10), 45 women with platinum-refractory epithelial ovarian cancer were randomized to either 10, 10, 10 and 10 μg or 10, 20, 50 and 100 μg of catumaxomab administered by i.p infusion on days 0, 3, 7 and 10, respectively. There was a modest dose effect, with 6 patients in the high-dose arm showing clinical response compared to 3 in the low-dose arm. After a median follow up of 5 months, the overall survival time was 182 days for the high-dose and 114 days for the low-dose arm. Catumaxomab was safe and toxicity was acceptable when administered at the high dose (47-49).

An ongoing open-label phase II study is assessing i.p. catumaxomab in ovarian cancer patients with recurrent symptomatic malignant ascites (Fresenius Biotech study number IP-REM-AC-02-US) (50), two open-label phase II trials are assessing catumaxomab in women with epithelial ovarian cancer who experience a complete response to chemotherapy (Fresenius Biotech study number IP-CAT-OC-01) (51) or as adjuvant treatment after tumor resection (Fresenius Biotech study number IP-CAT-OC-02) (52), and a randomized, open-label, uncontrolled study in patients with gastric adenocarcinoma after curative resection is examining the safety and efficacy of catumaxomab administered subsequent to neoadjuvant chemotherapy (Fresenius Biotech study number IP-CAT-GC-03) (53).

Sources

Fresenius Biotech GmbH (DE); TRION Pharma GmbH (DE).

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