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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 07-115803- -00006-0000

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Señor

**SUPERINTENDENTE DE INDUSTRIA Y COMERCIO
DIVISIÓN DE NUEVAS CREACIONES**

E. S. D.

REF: Expediente No.:
Patente de Invención:

07-115.803

**"ELIMINACIÓN DE POBLACIÓN
CELULAR HETEROGÉNEA O
MEZCLADA EN TUMORES"
IMMUNOGEN, INC.**

Titular:

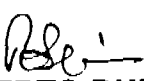
**Publicada en la Gaceta de la Propiedad Industrial No. 594 de
31 de Julio de 2008**

SOLICITUD DE EXAMEN DE PATENTABILIDAD

HUMBERTO RUBIO CAMACHO, mayor de edad, domiciliado en Bogotá, D.C., identificado con la cédula de ciudadanía número 17.192.062 de Bogotá, abogado en ejercicio, portador de la Tarjeta Profesional No. 50.007, expedida por el Consejo Superior de la Judicatura, actuando en carácter de **APODERADO** de la sociedad **IMMUNOGEN, INC.**, respetuosamente solicito a esa Dependencia:

- 1.- De conformidad con lo establecido en el Artículo 44 de la Decisión 486 de la Comisión de la Comunidad Andina y estando dentro del término legal de seis (6) meses contados desde la fecha de publicación en la Gaceta No. 594 de 31 de Julio de 2008, de la solicitud de la patente de la referencia, solicito se sirvan proceder a efectuar el examen de patentabilidad de la Invención.
- 2.- Para que la presente petición sea viable, estoy anexando el siguiente documento:
 - 2.1. Recibo de pago No. 09-29 de 2 de Enero de 2009; de acuerdo con la Resolución No. 55819 de 26 de diciembre de 2.008.
- 3.- Por lo anterior solicito se continúe con el trámite correspondiente.

Atentamente,


HUMBERTO RUBIO CAMACHO
C.C. 17.192.062 de Bogotá
T.P. 50.007 Consejo Superior
de la Judicatura.
COD. 4134

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**PARA USO EXCLUSIVO DE
HUMBERTO RUBIO & CIA. LTDA.**

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

NIT : 800176089-2

RECIBO OFICIAL DE CAJA : 09 - 29
FECHA : ENERO 2 DE 2009

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



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***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	No. PAGO	FECHA PAGO	Vr. PAGO
HUMBERTO RUBIO & CIA ASOC	CONSIGNACION	BANCO DE BOGOTA	062754387	92984710	02/01/2009	3,069,000.00

***** CONCEPTO *****

CANT.	RENTISTICO	CONCEPTO	TOTAL CONCEPTO
1	50005-01-01 SOLICITUDES	112 PTO CAP-I EXAMEN DE PATENTIBILIDAD	452,000.00
TOTAL :			\$ 452,000.00

SON: CUATROCIENTOS CINCUENTA Y DOS MIL PESOS

RESPONSABLE : _____

RECIBO DE CAJA APLICADO AL EXPEDIENTE No. _____

DIVISIÓN DE NUEVAS CREACIONES

RADICACIÓN EXPEDIENTE No. 07-115803

EL EXTRACTO DE LA PRESENTE SOLICITUD DE **PATENTE DE INVENCION**

FUE PUBLICADO EN LA GACETA DE PROPIEDAD INDUSTRIAL No **594** DE

FECHA **31 DE JULIO DE 2008** EL TÉRMINO HÁBIL SEÑALADO POR LA LEY

EXPIRA EL **28 DE OCTUBRE DE 2008**

NOTA: Dentro del plazo de los seis (6) meses siguientes a partir de la fecha de publicación en la gaceta de Propiedad Industrial, deberá solicitar y cancelar el examen de patentabilidad so pena de que la solicitud caiga en abandono, de acuerdo con lo establecido en el Artículo 44 de la Decisión 486/2000.

SE PRESENTARON OPOSICIONES POR PARTE DE TERCEROS.

SI _____

NO X

Eradication of large colon tumor xenografts by targeted delivery of maytansinoids

(immunoconjugate/colon cancer xenografts)

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Communicated by Stuart Schlossman, Dana-Farber Cancer Institute, Boston, MA, May 22, 1996 (received for review March 29, 1996)

ABSTRACT The maytansinoid drug DM1 is 100- to 1000-fold more cytotoxic than anticancer drugs that are currently in clinical use. The immunoconjugate C242-DM1 was prepared by conjugating DM1 to the monoclonal antibody C242, which recognizes a mucin-type glycoprotein expressed to various extents by human colorectal cancers. C242-DM1 was found to be highly cytotoxic toward cultured colon cancer cells in an antigen-specific manner and showed remarkable antitumor efficacy *in vivo*. C242-DM1 cured mice bearing subcutaneous COLO 205 human colon tumor xenografts (tumor size at time of treatment 65–130 mm³), at doses that showed very little toxicity and were well below the maximum tolerated dose. C242-DM1 could even effect complete regressions or cures in animals with large (260- to 500-mm³) COLO 205 tumor xenografts. Further, C242-DM1 induced complete regressions of subcutaneous LoVo and HT-29 colon tumor xenografts that express the target antigen in a heterogeneous manner. C242-DM1 represents a new generation of immunoconjugates that may yet fulfill the promise of effective cancer therapy through antibody targeting of cytotoxic agents.

Colorectal cancer is one of the most common malignancies and is among the leading causes of death from cancer. Surgical resection is the primary treatment modality for these tumors, but about half of all patients will die of disseminated disease (1). Because of the high incidence and poor prognosis of patients with metastatic disease, successful treatment of colorectal cancer requires effective systemic therapy in addition to surgery, either as adjuvant treatment to surgery or for primary treatment of those 25% of all patients for whom surgery alone cannot achieve a complete response (2). Unfortunately, the conventional systemic treatment options for colon cancer, including radiation therapy, chemotherapy, and immunotherapy, have limited efficacy (3, 4). To date, 5-fluorouracil (5-FU) has served as the standard cytostatic drug for adjuvant therapy after surgery. However, the overall response rate to 5-FU is less than 25%, and the treatment has not significantly improved patient survival (1–3). Although the improved regimen of 5-FU plus levamisole in the adjuvant setting has proven to be more effective in patients with stage II and III colorectal cancers, the estimated reduction in the mortality rate is still less than 30% (2, 5). Thus, there is an urgent clinical need for new agents with greater efficacy.

Conventional chemotherapeutic agents are limited in their therapeutic effectiveness by severe side effects due to their poor selectivity for tumors. The development of monoclonal antibodies against specific tumor antigens made it possible to think of enhancing the selectivity of anticancer drugs by a targeted delivery approach. However, several such reported

attempts using monoclonal antibodies and the anticancer drugs doxorubicin (6), methotrexate (7), and *Vinca* alkaloids (8), have been largely unsuccessful. These antibody-drug conjugates were only moderately potent and usually less cytotoxic than the corresponding unconjugated drugs. In fact, antigen-specific cytotoxicity toward cultured tumor cells was rarely demonstrated (6–8). *In vivo* therapeutic effects with these conjugates in tumor xenograft animal models were, in general, observed only when the treatments were commenced before the tumors were well established (8) or when exceedingly large doses (up to 90 mg/kg, drug equivalent dose) were used (6). It is, therefore, not surprising that in human clinical trials, no significant antitumor effects were observed with these agents (9, 10). Indeed, the peak circulating serum concentrations of conjugates were only in the same range as their *in vitro* IC₅₀ values and, thus, capable of eliminating at best only about 50% of tumor cells.

These observations have led us (11, 12) and others (13, 14) to conclude that the previous attempts at delivering therapeutic doses of cytotoxic drugs via monoclonal antibodies have met with little success in clinical trials because of inappropriate choices of drug. We concluded that immunoconjugates must be composed of drugs possessing much higher potency than the clinically used anticancer agents if therapeutic levels of conjugate at the tumor sites are to be achieved in patients. We have recently described antibody conjugates with CC-1065 analogs and with maytansinoids that are 100- to 1000-fold more cytotoxic than the chemotherapeutic agents doxorubicin, methotrexate, and *Vinca* alkaloids (11, 12). Herein, we report the results of preclinical efficacy tests with C242-DM1, a maytansinoid drug (DM1) linked to the monoclonal antibody C242 directed against human colorectal cancer.

MATERIALS AND METHODS

Preparation of C242-DM1 Conjugate. Ansamitocin P-3 (compound 1) provided by Takeda (Osaka) was converted to the disulfide-containing maytansinoid DM1 (compound 2) (Fig. 1) as described (15). The C242 antibody, a murine IgG1 (16), was provided by Pharmacia. C242-DM1 (compound 3) was prepared as described (12). The conjugate was purified by gel filtration through a column of Sephacryl S300 and the peak corresponding to monomeric conjugate (>80% overall yield) was collected. The final conjugate contained on the average four DM1 molecules linked per antibody molecule.

Specific Affinity of C242-DM1. The specific binding affinity of C242-DM1 conjugate and C242 antibody to CanAg-positive COLO 205 cell membranes was determined by a binding assay as described (17). Samples of C242-DM1 or C242 at various concentrations (10⁻¹² to 10⁻⁹ M) were incubated for 18 h at

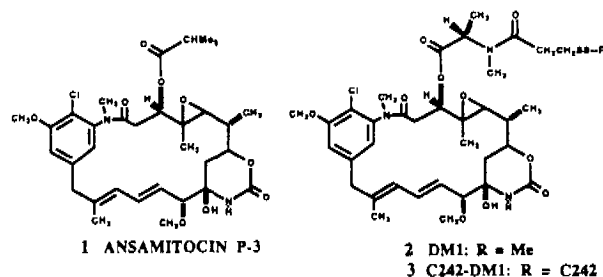


FIG. 1. Structural representation of Ansamitocin P-3 (compound 1), DM1 (compound 2), and C242-DM1 (compound 3).

ambient temperature with COLO 205 cell membranes immobilized in 96-well plates. The membranes were then washed and the amount of bound conjugate or antibody was determined using a Eu^{3+} -labeled anti-mouse IgG. Results are plotted as concentration of conjugate or antibody versus relative fluorescence.

In Vitro Cytotoxicity of C242-DM1 Conjugate. The cytotoxicity of C242-DM1 was measured on antigen-positive human colon carcinoma cell lines COLO 205 [American Type Culture Collection (ATCC) CCL 222], LoVo (ATCC CCL 229), and HT-29 (ATCC HTB 38) and on the antigen-negative human melanoma cell line A-375 (ATCC CRL 1619) in a clonogenic assay. Cells were plated in 96-well tissue culture plates with each plate containing a fixed number of cells (ranging from 3 to 10,000 cells per well) in 0.2 ml of DMEM containing 20% fetal calf serum. Immunoconjugate at varying concentrations (4×10^{-11} to 4×10^{-8} M) was added and the cells were maintained in a humidified atmosphere at 37°C and 6% CO_2 for 18–21 days. In some experiments, the cells were incubated with C242-DM1 for 24 h and then washed, and the medium was replaced with fresh medium without drug. Colonies were then counted and the plating efficiency was determined. Surviving fractions of cells were then calculated as the ratio of the plating efficiency of the treated sample and the plating efficiency of the control.

Immunohistochemical Studies. Tumor tissues excised from either humans or mice were frozen in O.C.T. embedding medium (Miles), sectioned, and treated with biotinylated-C242 antibody. The bound antibody was detected using the avidin-biotin immunoperoxidase technique as described (18).

In Vivo Tumor Growth Assays. Female CB-17 SCID mice, 6–7 weeks of age, were obtained from Massachusetts General Hospital. The human colon cancer cell lines COLO 205, LoVo, and HT-29 were maintained as adherent cultures in DMEM containing 10% fetal bovine serum at 37°C in a humidified atmosphere of 6% CO_2 /94% air. Each mouse was inoculated subcutaneously at the right flank with tumor cells (2×10^6 to 1×10^7 cells in different experiments) in 0.1 ml of medium. Treatments were started on days 7–9 after tumor inoculation, when the tumor sizes reached from 65 to 500 mm^3 , depending on the experiment. The therapeutic agents were administered intravenously to groups of 7–10 mice. Tumor size was measured weekly in two dimensions using a caliper, and the volume was expressed in mm^3 using the formula: $V = 0.5a \times b^2$, where a and b are the long and short diameters of the tumor, respectively.

Measurement of Concentration of C242-DM1 in Serum. A group of eight CD1 mice obtained from Charles River Breeding Laboratories were injected with C242-DM1 at a dose of 300 μg per kg per day² for five consecutive days. Blood (0.1 ml) was withdrawn from the retroorbital sinus once per day from each mouse, either at 1 h (four mice) or at 24 h (four mice) after injection of the conjugate. C242-DM1 was determined by an ELISA using a murine monoclonal IgG2a anti-DM1 antibody

(developed at ImmunoGen) to capture the C242-DM1. The amount of bound conjugate was then quantified by detection of the C242 antibody using IgG1-specific goat anti-mouse IgG-alkaline phosphatase/*p*-nitrophenyl phosphate as described (17).

Immunostaining of Cells. Cells grown on coverslips were fixed with 2% paraformaldehyde, permeabilized in methanol at -20°C , and stained with C242 antibody for fluorescence microscopy as described (19). A similar protocol was used for flow cytometry (Becton-Dickinson FACScan), except that cells were trypsinized, stained live without fixation, and then fixed with 1% paraformaldehyde in phosphate-buffered saline (PBS).

Magnetic Bead Depletion. Cells were harvested with trypsin, counted, incubated with C242 antibody, and washed. Cells were then mixed with magnetic beads (Dynabeads M-450, goat anti-mouse IgG coated, Dynal, Oslo) at a beads/cells ratio of 5:1 and incubated for 30 min with rocking at 4°C . Beads plus adhering cells were magnetically removed, and an equal number of fresh beads were added for a second cycle. The remaining cells were analyzed by flow cytometry.

RESULTS

Evaluation of C242-DM1 for Specificity, Cytotoxicity, and Selectivity. The delivery agent of C242-DM1, the C242 antibody, recognizes a sialidase-sensitive carbohydrate epitope on the CanAg antigen, a mucin-type glycoprotein expressed to various degrees by all human colorectal cancers (20–22). C242 has only minimal cross-reactivity with normal tissues (21, 22). C242-DM1 was prepared in a manner similar to that described for other maytansinoid conjugates (12) (Fig. 1). The conjugate contains, on the average, four covalently linked DM1 molecules per antibody molecule. In a binding assay, C242-DM1 binds as well as unconjugated C242 to the CanAg antigen expressed on COLO 205 cell membranes (Fig. 2A), indicating

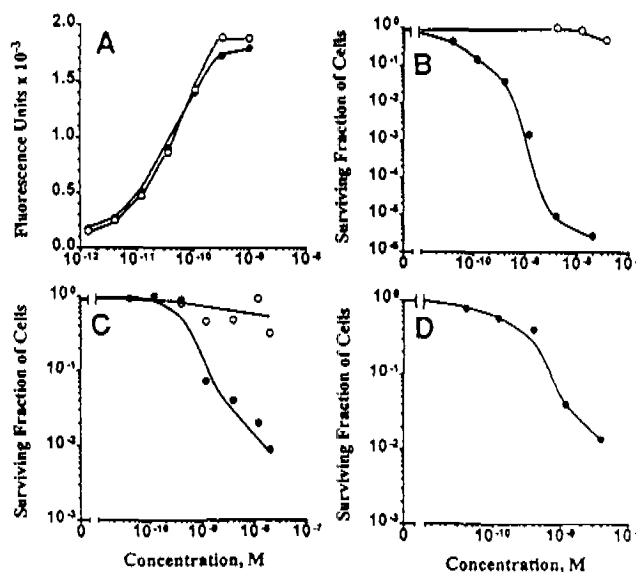


FIG. 2. Evaluation of binding and cytotoxicity of C242-DM1. (A) Binding affinity of C242-DM1. The specific affinity of conjugated C242 (●) for the CanAg antigen expressed on COLO 205 cell membranes is compared with that of C242 (○). (B) *In vitro* cytotoxicity and selectivity of C242-DM1. The *in vitro* cytotoxicity of C242-DM1 for antigen-positive human colon carcinoma COLO 205 cells (●) and antigen-negative human melanoma A-375 cells (○) was measured in a clonogenic assay. (C) *In vitro* cytotoxicity and specificity of C242-DM1 for antigen-positive colon carcinoma COLO 205 cells in the absence (●) or presence (○) of 1×10^{-6} M C242 antibody. Cells were incubated with immunoconjugate for 24 h in this experiment. (D) *In vitro* cytotoxicity of C242-DM1 for the human colon cancer cell line LoVo, which expresses the CanAg antigen heterogeneously.

¹All concentrations used *in vitro* refer to conjugated DM1.

²All doses used *in vivo* refer to conjugated DM1. A DM1 dose of 1 μg corresponds to 54 μg of C242-DM1 conjugate.

that the conjugation of DM1 does not diminish the binding avidity of C242. The cytotoxic potency and selectivity of C242-DM1 was assayed with the antigen-positive COLO 205 cell line and the antigen-negative A-375 melanoma cell line (Fig. 2B); both cell lines were equally sensitive to free DM1 ($IC_{50} = 4 \times 10^{-11}$ M). C242-DM1 was found to kill COLO 205 cells with an IC_{50} value of 3.2×10^{-11} M (23.5 pg/ml), and treatment of cells with a concentration of 4.5×10^{-9} M (3.3 ng/ml) left a surviving fraction of less than 1×10^{-5} (>99.999% of cells killed, detection limit of the assay). In contrast, C242-DM1 was 1100-fold less cytotoxic for the antigen-negative A-375 cells ($IC_{50} = 3.6 \times 10^{-8}$ M; 26.5 ng/ml), demonstrating that cell killing was selective for the antigen-positive colon cell line (Fig. 2B). COLO 205 cells were killed even after a 24-h exposure to C242-DM1, with an IC_{50} value of 6×10^{-10} M (Fig. 2C). Furthermore, a large excess of free C242 antibody greatly diminished the cytotoxicity of the conjugate toward the target cells (Fig. 2C), further demonstrating that the cytotoxic effect was dependent on specific binding through the antibody component of the conjugate.

The COLO 205 cell line cultured *in vitro* expresses the target antigen homogeneously on all cells (22). We also evaluated the cytotoxic potency of C242-DM1 against two colon tumor cell lines, LoVo and HT-29, which express the CanAg antigen heterogeneously on only 20–30% of their cells when grown *in vitro*, as judged by indirect immunofluorescence analysis of C242 binding using flow cytometry (data not shown). In spite of this low expression, treatment of these cells with C242-DM1 could eliminate 99% of the cells at a concentration of 4×10^{-9} M (shown in Fig. 2D for the LoVo cell line).

Immunohistochemical Analysis of Tumor Xenografts and Human Colon Tumor Samples. The three human colon tumor cell lines, COLO 205, LoVo and HT-29, were grown subcutaneously in SCID mice to test the *in vivo* therapeutic efficacy of C242-DM1. The particular cell lines were chosen because their antigen expression, when grown *in vivo*, was in the range of that seen by immunohistochemical examination of human colon tumor specimens from 20 patients. COLO 205 tumor xenografts excised from mice on day 7 after tumor inoculation exhibited, on immunohistochemical analysis, uniform staining of the CanAg antigen (Fig. 3A) in a manner similar to that of the section of a human colon tumor biopsy representative of 6/20 specimens shown in Fig. 3B. Tumor xenografts established with LoVo cells expressed the antigen heterogeneously at all time points. The staining pattern of a section taken on day 7 after tumor inoculation was classified as moderately heterogeneous (Fig. 3C) and resembled the staining pattern of the typical (10/20 specimens) human colon tumor biopsy shown in Fig. 3D. The third human colon tumor xenograft model established with HT-29 cells showed very heterogeneous staining for antigen, with many cells being antigen-negative (Fig. 3E), again in a fashion similar to that seen in some biopsies (4/20) of human colon tumors (Fig. 3F).

Antitumor Efficacy of C242-DM1. In the first therapy experiment (Fig. 4A), animals bearing COLO 205 tumors were treated with five daily injections of C242-DM1 at a dose of 300 μ g per kg per day, with an equivalent dose of the isotype-matched conjugate N901-DM1 that does not bind to COLO 205 cells, or with a mixture of corresponding amounts of C242 antibody (16 mg per kg per day) and unconjugated DM1 (300 μ g per kg per day). Treatment with C242-DM1 completely

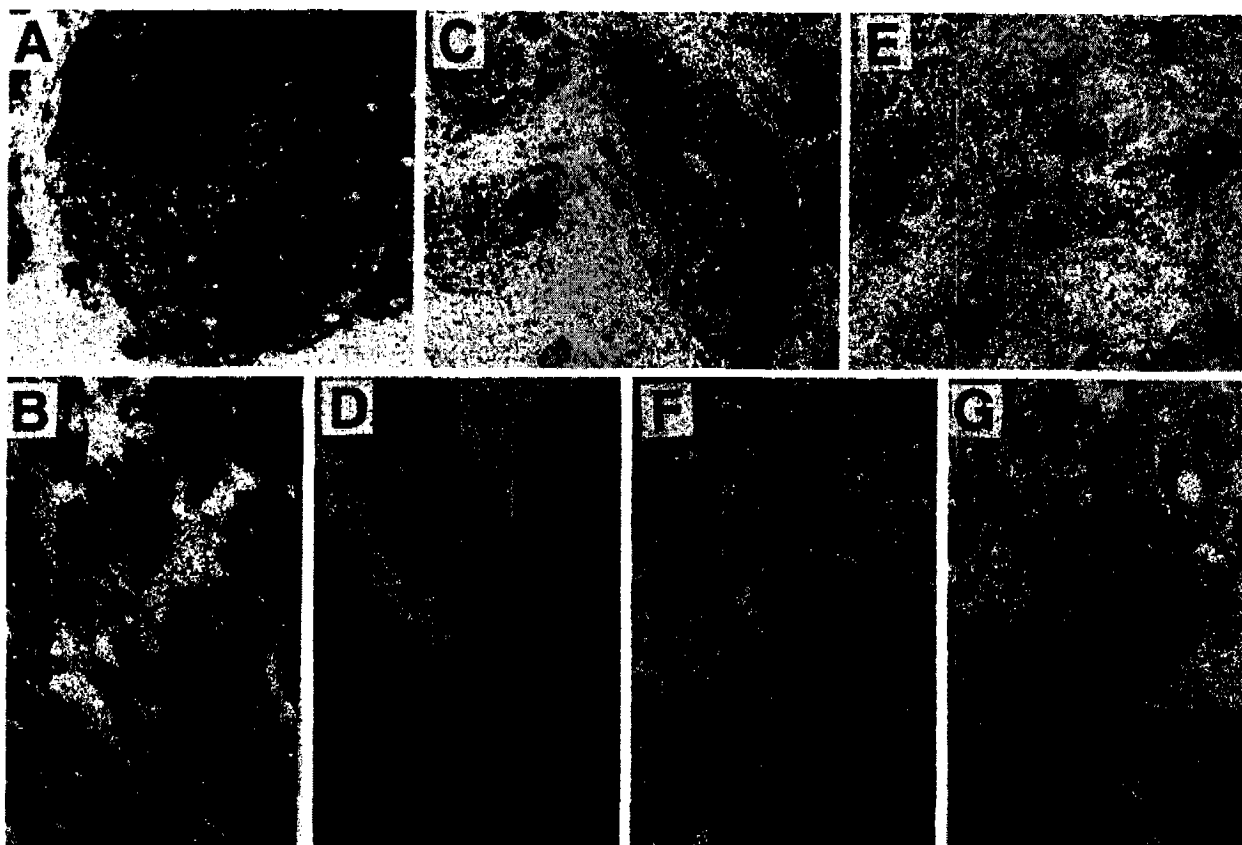


FIG. 3. Immunohistochemical analysis of tumor xenografts excised on day 7 after tumor inoculation and comparison with human colon tumor biopsies. (A) A COLO 205 xenograft. (B) A human colon tumor biopsy with homogeneous expression of antigen. (C) A LoVo xenograft. (D) A human colon tumor biopsy with moderately heterogeneous expression of antigen. (E) An HT-29 xenograft. (F) A human colon tumor biopsy with very heterogeneous expression of antigen. (G) A relapsed LoVo xenograft removed on day 91 from a mouse that received one course of C242-DM1 treatment.

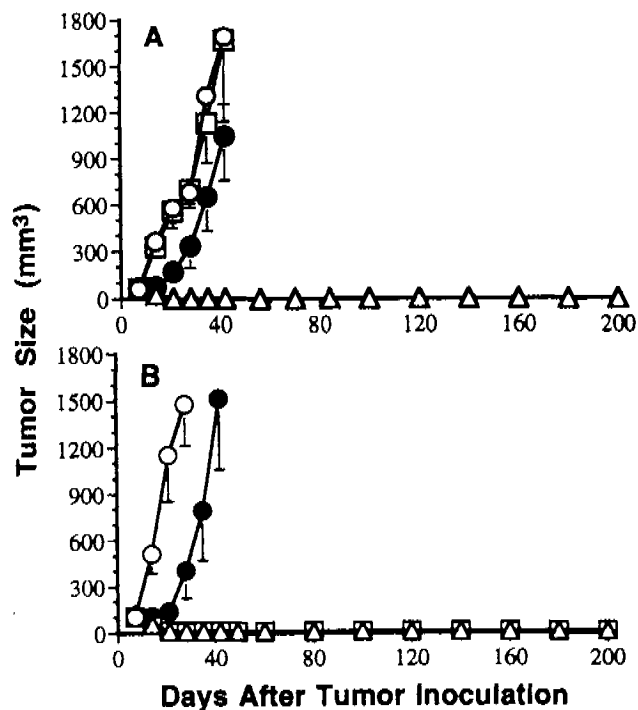


FIG. 4. Antitumor activity of C242-DM1 conjugate in SCID mice bearing COLO 205 human colon tumor xenografts. Each mouse was inoculated with 2×10^6 COLO 205 cells. The treatments were given from day 7 to day 11 after tumor inoculation (average tumor size = 65–100 mm³). (A) Antigen-specific antitumor activity of C242-DM1. The antitumor activity of C242-DM1 (300 µg per kg per day for 5 days) (Δ) was compared with that of PBS (0.2 ml per mouse per day for 5 days) ($\circ$), a mixture of C242 (16 mg per kg per day for 5 days) plus free DM1 (300 µg per kg per day for 5 days) ($\square$) or a nonbinding conjugate, N901-DM1 (300 µg per kg per day for 5 days) ($\bullet$). (B) Dose dependence of antitumor activity of C242-DM1. Tumor-bearing animals were treated with PBS (0.2 ml per mouse per day for 5 days) ($\circ$), C242-DM1 (150 µg per kg per day for 5 days) ($\bullet$), C242-DM1 (225 µg per kg per day for 5 days) (Δ), or C242-DM1 (300 µg per kg per day for 5 days) ($\square$).

eliminated any measurable tumors within 2 weeks of the initiation of therapy, and all eight animals were tumor-free for 200 days (duration of the experiment). Furthermore, toxic side effects were minimal at this dose as judged by the absence of body weight loss. The dose of C242-DM1 used in this experiment was below its maximum tolerated dose (MTD), which was defined for these experiments as the highest dose that could be administered to tumor-bearing mice without causing drug-related deaths (MTD = 380 µg per kg per day for five consecutive days). In contrast, very little antitumor activity was observed in animals treated with nontargeted conjugate or with the mixture of antibody and free DM1 (Fig. 4A). Thus, the DM1 moiety is a potent therapeutic agent against colon cancers *in vivo* when targeted to the tumors as a conjugate with the C242 antibody and shows high antitumor efficacy at doses that cause little toxicity.

The circulating serum concentrations of C242-DM1 were determined in CD1 mice by ELISA. One hour after each injection (five daily injections of 300 µg per kg per day), the concentration of C242-DM1 was about 1.8 µM, equivalent to DM1 at 1.3 µg/ml. After 24 h, the serum concentration was about 0.26 µM, which is still 58-fold higher than the concentration required to kill >99.999% cells *in vitro*.

Next, the dose-response effect of C242-DM1 in the COLO 205 xenograft model was evaluated. Animals were treated with C242-DM1 at doses ranging from 150 to 300 µg per kg per day for 5 days (Fig. 4B). C242-DM1 eliminated tumors in all animals at a daily dose as low as 225 µg per kg per day when

given for 5 consecutive days, which is 59% of the MTD. Even at the lowest dose tested (150 µg per kg per day for 5 days), a significant delay in tumor growth was observed.

These results encouraged us to evaluate the therapeutic efficacy of C242-DM1 in mice bearing larger (average size, 260 mm³) subcutaneous COLO 205 xenografts (Fig. 5A). Animals received two courses of 5-day treatment with C242-DM1 or, for comparison, treatment with 5-FU, the standard chemotherapeutic drug used for the treatment of colorectal cancer. C242-DM1 again cured all animals rendering them tumor-free for greater than 200 days (duration of the experiment). This therapeutic effect on large tumors is especially remarkable in view of the finding that 5-FU at its MTD (15 mg per kg per day for 5 days) only slightly (by about 5 days) delayed the tumor growth. We extended this study to even larger tumors. A group of animals bearing the largest COLO 205 tumor xenografts tested (average size 500 mm³) was treated with one course of C242-DM1 at a dose of 300 µg per kg per day for 5 days (Fig. 5B). Complete tumor regressions were achieved in all animals. In six out of eight animals, the complete response lasted 7 weeks. In the remaining two animals, no signs of tumor could be detected when the experiment was terminated on day 120 after tumor inoculation (representing more than 17 tumor size doubling times *in vivo*).

The COLO 205 cell line, both cultured *in vitro* and grown as tumor xenografts, expresses the target antigen homogeneously on all cells (Fig. 3A). We then evaluated the antitumor activity of C242-DM1 against established colon tumor xenografts from the LoVo and HT-29 cell lines that express the CanAg antigen heterogeneously on only 20–30% of their cells when grown *in*

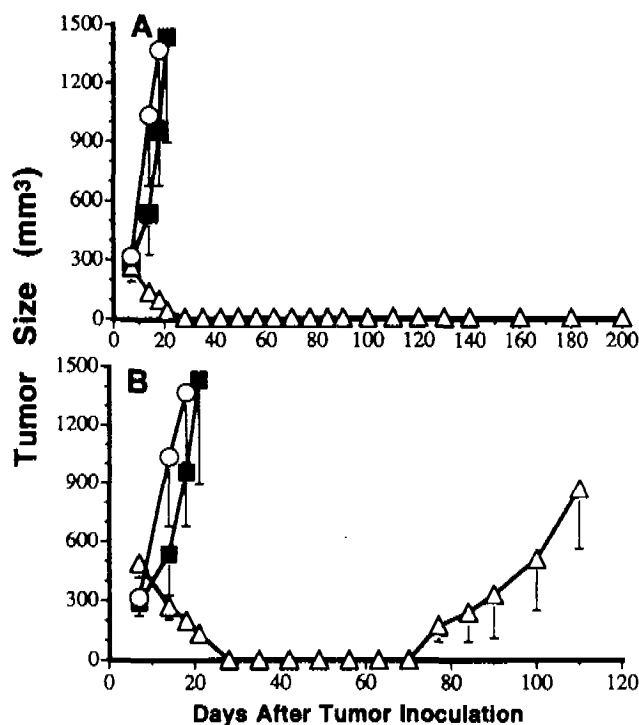


FIG. 5. Antitumor activity of C242-DM1 against large COLO 205 tumors. Each mouse was inoculated with 5×10^6 COLO 205 cells and treatments were started on day 7 after tumor inoculation. (A) Efficacy in treatment of large COLO 205 xenografts (mean tumor size = 260 mm³). Tumor-bearing animals were treated with PBS (0.2 ml per mouse per day for 5 days) ($\circ$), 5-FU (15 mg per kg per day for 5 days) ($\bullet$), or two courses of C242-DM1 (300 µg per kg per day for 10 days; days 7–11 and days 14–18) (Δ). (B) Efficacy in treatment of very large COLO 205 xenografts (mean tumor size = 500 mm³). Tumor-bearing animals were treated with PBS (0.2 ml per mouse per day for 5 days) ($\circ$), 5-FU (15 mg per kg per day for 5 days) ($\bullet$), or one course of C242-DM1 (300 µg per kg per day for 5 days, days 7–11) (Δ).

vitro or, as shown in Fig. 3 (C and E), *in vivo*. Animals bearing LoVo tumor xenografts were treated with either one or two courses of C242-DM1 (300 μ g per kg per day for 5 days). Two additional groups of tumor-bearing animals were treated with either a mixture of C242 antibody (16 mg per kg per day for 5 days) and unconjugated DM1 (300 μ g per kg per day for 5 days) or with 5-FU at its MTD (15 mg per kg per day for 5 days). Remarkably, complete tumor regressions lasting 5 weeks were observed in all animals treated with one course of C242-DM1 (Fig. 6A). The LoVo tumors from mice that were treated with C242-DM1 and relapsed after the period of complete regressions were evaluated for antigen expression. A section of the tumor excised on day 91 exhibited similar heterogeneous staining with C242 (Fig. 3G) as was seen prior to therapy (day 7, Fig. 3C). The period of complete regression could be prolonged to 9 weeks by a second course of treatment with C242-DM1 initiated 21 days after the start of the first course (Fig. 6A). No indication of toxic side effects as assessed by body weight loss was observed for either treatment protocol. In contrast, tumors in animals that were treated with the mixture of antibody and DM1, or with 5-FU, grew rapidly to large sizes (Fig. 6A). Similar effects were obtained in the tumor model with HT-29, classified as expressing the CanAg antigen very heterogeneously (see Fig. 3E). C242-DM1 (375 μ g per kg per day for 5 days) induced complete tumor regressions lasting 4 weeks (Fig. 6B). These results demonstrate that C242-DM1 is an effective therapeutic agent against colorectal cancer xe-

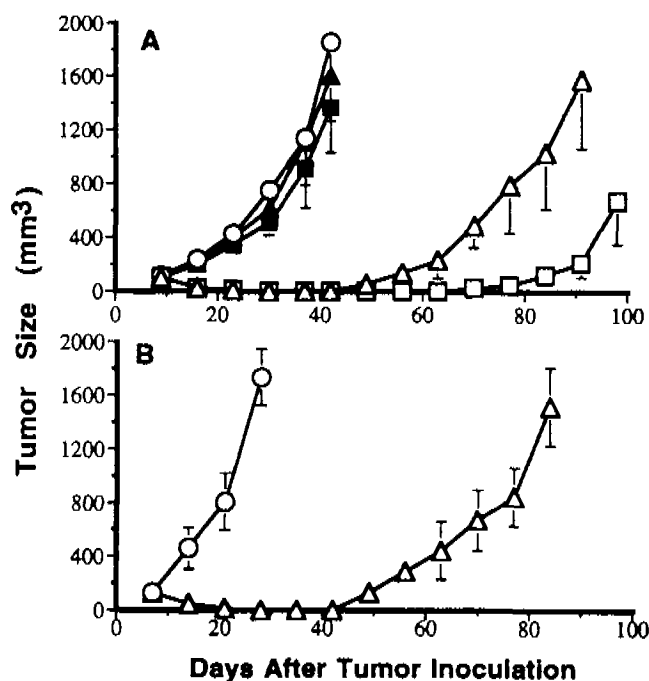


FIG. 6. Antitumor activity of C242-DM1 in SCID mice bearing human colon tumor xenografts that express the antigen heterogeneously. (A) Antitumor activity of C242-DM1 against LoVo tumor xenografts (mean tumor size = 103 mm³). Each mouse was inoculated with 5×10^6 LoVo cells, and treatments were started on day 9 after tumor inoculation. Tumor-bearing mice were treated with PBS (0.2 ml per mouse per day for 5 days) (○), 5-FU (15 mg per kg per day for 5 days) (●), a mixture of C242 (16 mg per kg per day for 5 days) plus DM1 (300 μ g per kg per day for 5 days) (▲), one course of C242-DM1 (300 μ g per kg per day for 5 days) (△), or two courses of C242-DM1 (300 μ g per kg per day for 10 days, days 9–13 and days 30–34) (□). (B) Antitumor activity of C242-DM1 against HT-29 tumor xenografts (mean tumor size = 130 mm³). Each mouse was inoculated with 1×10^7 HT-29 cells and treatments were given from days 7 to 11 after tumor inoculation. The tumor-bearing mice were treated with PBS (0.2 ml per mouse per day for 5 days) (○) or C242-DM1 (375 μ g per kg per day for 5 days) (△).

nografts including those that express the antigen heterogeneously and causes little toxicity even after two courses of treatment.

Relationship Between CanAg Expression and Colony Formation in Colon Cancer Cell Lines. The rather unexpected *in vivo* results that C242-DM1 induced complete regressions of tumors in which 70–80% of the cells do not express detectable amounts of the CanAg antigen led us to evaluate whether it was possible *in vitro* to generate homogeneous antigen-positive and antigen-negative sublines from cell lines that express the antigen heterogeneously. Repeated attempts to subclone the LoVo and HT-29 cell lines to select sublines that were completely antigen-negative (by staining with C242) were unsuccessful; no CanAg-negative subclones grew in a total of 210 subclones. In fact, most subclones expressed more antigen (about 60% positive cells) than the parental cell lines (20–30% positive cells). In another attempt, an HT-29 cell culture was first depleted of antigen-positive cells using magnetic beads coated with the C242 antibody and the remaining cells, which had a very low level of CanAg expression (<1%, by flow cytometry analysis of C242 binding), were subcloned. Initial screening of subconfluent cultures by immunofluorescence showed that many clones expressed very low levels of CanAg (about 70% of 79 clones had <10% antigen-positive cells). However, as the colonies were expanded, CanAg expression on some sublines again increased to levels greater than in the parental line or else the colonies died out (only 34 sublines could be expanded sufficiently for flow cytometry analysis). These results suggest that there is a direct relationship *in vitro* between the ability to form colonies and CanAg expression and may also provide an explanation for the experimental observations in the LoVo and HT-29 tumor xenograft models whereby the elimination of antigen-positive cells *in vivo* by C242-DM1 may cause the collapse of the entire tumor in animals.

DISCUSSION

C242-DM1 represents a new generation of immunoconjugates that may yet realize the potential of effective cancer therapy through antibody targeting of cytotoxic agents. The conjugate is highly cytotoxic *in vitro* in an antigen-dependent and tumor-cell-selective manner and produced long-term cures of mice bearing human colon tumor COLO 205 xenografts at doses that caused little toxicity. Cures were even obtained at doses that were well below the MTD of the conjugate. Importantly, the circulating serum concentrations of C242-DM1 (1.8 μ M at 1 h after injection of 300 μ g/kg) were about 380-fold higher than that required to kill greater than 99.999% of target cells *in vitro*. C242-DM1 was capable of curing mice bearing very large COLO 205 tumors, even those that were 500 mm³ at the start of treatment. In contrast, 5-FU, the standard chemotherapeutic drug used for the treatment of colorectal cancer, showed very little therapeutic benefit against the same tumors.

In many human colorectal tumor biopsies, it was demonstrated that CanAg, the target antigen for C242, is expressed in a heterogeneous manner. C242-DM1 showed remarkable antitumor activity in tumor models derived from the LoVo and HT-29 colon cancer cell lines that express the antigen on only 20–30% of the cells. The immunoconjugate induced complete regressions of such tumors lasting 4–5 weeks. Tumors harvested from regrowing LoVo xenografts after this period of complete regression exhibited heterogeneous staining similar to that seen prior to treatment (see Fig. 3 C and G), suggesting that the phenotype of the regrowing tumor xenografts was unchanged. Indeed, the period of complete regression could be prolonged by a second course of treatment that was well tolerated by the mice, suggesting that using multiple cycles of this immunoconjugate for treatment of colorectal cancer may be a feasible clinical regimen, with the potential for totally eradicating even those tumors that show heterogeneous expression for the CanAg antigen. The similarity of antigen

expression and tumor morphology between the xenografts and the human tumor biopsies suggests to us that antitumor activity of C242-DM1 exhibited in these models may well predict potent antitumor activity in the clinical situation.

The *in vitro* experiments suggest that CanAg antigen-negative cells do not grow (for long) in the absence of antigen-positive cells, which could provide a mechanism by which C242-DM1 could induce complete regressions of tumors that express the CanAg antigen heterogeneously. Secretion of cytokines essential for the survival of CanAg-negative cells by the CanAg-positive cells could explain these results, although initial experiments indicated that conditioned medium did not support growth of CanAg-negative colonies derived from the HT-29 or LoVo cell lines. Other possible mechanisms may be (i) all cell lineages go through a period of transient CanAg expression during which they are susceptible to the action of C242-DM1; (ii) a bystander effect, that is, antigen-positive cells may concentrate sufficient C242-DM1 at the tumor site to cause killing of neighboring antigen-negative cells either directly or by release of DM1 from the conjugate concentrated inside antigen-positive tumor cells, followed by diffusion of DM1 into neighboring cells via gap junctions; and (iii) recruitment of host immune mechanisms upon killing of CanAg-positive cells, including activated macrophages and natural killer cells (23). Also, it cannot be ruled out that CanAg-negative cells actually express antigen at low density, below the sensitivity of detection by flow cytometry or immunofluorescence microscopy but sufficient to mediate cell death using this potent immunoconjugate.

C242-DM1 exhibits a degree of potency and selectivity *in vitro* and *in vivo* that is superior to that of other immunoconjugates against colorectal cancer described thus far (6–8, 24–26). C242 has been linked to other cytotoxic agents, such as ricin A chain (24) or *Pseudomonas* exotoxin (25, 26). The ricin-A-chain conjugates showed very limited efficacy in the COLO 205 model (inducing a tumor growth delay of 16 days) and no significant efficacy in tumor models that express the CanAg antigen heterogeneously (24). The *Pseudomonas* exotoxin conjugates also showed little efficacy in the mouse models (25, 26). In one case where a “cure” was reported, the follow-up period was short (only 4 weeks), and the dose used was toxic and left only one survivor in the group (24). Of the other antibody-drug conjugates developed against colorectal cancer (6–8), cures were reported only in the case of BR96-Dox (6), a conjugate of the antibody BR96 with doxorubicin. However, 100% cures were only achieved in mice with BR96-Dox at its MTD (a doxorubicin dose of 20 mg per kg per day for 3 days). C242-DM1 cured all tumor-bearing mice at doses that are well below its MTD and that are 53-fold lower than those of BR96-Dox. This is an important result since the clinical MTDs of toxin- or drug-conjugates in humans are, in general, up to 10-fold less than those determined in animal studies, resulting in the inability of achieving therapeutically effective concentrations of conjugates in patients in the clinical studies to date (9, 10). Thus, from the preclinical data, C242-DM1 stands out as a promising new candidate for clinical evaluation against colorectal cancer. C242-DM1 made with a humanized antibody (27) will allow the treatment of patients with several courses of targeted chemotherapy with the potential for substantial clinical benefit.

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Cantuzumab Mertansine, a Maytansinoid Immunoconjugate Directed to the CanAg Antigen: A Phase I, Pharmacokinetic, and Biologic Correlative Study

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Purpose: To determine the maximum tolerated dose and pharmacokinetics of cantuzumab mertansine, an immunoconjugate of the potent maytansine derivative (DM1) and the humanized monoclonal antibody (huC242) directed to CanAg, intravenously (IV) once every 3 weeks and to seek evidence of antitumor activity.

Patients and Methods: Patients with CanAg-expressing solid malignancies were treated with escalating doses of cantuzumab mertansine administered IV every 3 weeks. The pharmacokinetic parameters of cantuzumab mertansine, the presence of plasma-shed CanAg, and the development of both human antihuman and human anti-DM1 conjugate antibodies also were characterized.

Results: Thirty-seven patients received 110 courses of cantuzumab mertansine at doses ranging from 22 to 295 mg/m². Acute, transient, and reversible elevations of hepatic transaminases were the principal toxic effects. Nausea, vomiting, fatigue, and diarrhea were common but rarely severe at the highest dose levels. Dose, peak concen-

tration, and area under the concentration-time curve correlated with the severity of transaminase elevation. The mean ($\pm$ SD) clearance and terminal elimination half-life values for cantuzumab mertansine averaged 39.5 ($\pm$ 13.1) mL/h/m² and 41.1 ($\pm$ 16.1) hours, respectively. Strong expression (3+) of CanAg was documented in 68% of patients. Two patients with chemotherapy-refractory colorectal carcinoma had minor regressions, and four patients had persistently stable disease for more than six courses.

Conclusion: The recommended dose for cantuzumab mertansine is 235 mg/m² IV every 3 weeks. The absence of severe hematologic toxic effects, preliminary evidence of cantuzumab mertansine tumor localization, and encouraging biologic activity in chemotherapy-refractory patients warrant further broad clinical development of this immunoconjugate in CanAg-expressing tumors.

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CANTUZUMAB MERTANSINE (SB-408075, huC242-DM1) is a tumor-activated immunoconjugate derived from the conjugation of approximately four molecules of the potent maytansinoid antimicrotubule agent DM1 (N²-deacetyl-N²-(3-mercaptopropyl)-maytansine C₃₅H₄₈ClN₅O₁₀S) to the humanized monoclonal antibody huC242 (Fig 1). HuC242 binds specifically to the extracellular domain of a tumor-associated carbohydrate epitope of CanAg (a novel glycoform of MUC1), which is strongly expressed in most pancreatic, biliary, and colorectal cancers as well as in a large proportion of non-small-cell lung (40%), gastric (55%), uterine (45%), and bladder (40%) cancers.¹⁻³ In contrast, only minimal immunostaining of normal tissues has been reported.^{1,2} Once cantuzumab mertansine is bound to the external domain of CanAg, the complex is internalized, and the DM1 molecules are released intracellularly by cleavage of the DM1-huC242 disulfide linkage.

DM1 is a derivative of the natural microbial fermentation product and extraordinarily potent antimicrotubule agent ansamitocin P-3.⁴ Maytansine was evaluated by the National Cancer Institute in phase I and II studies in the 1970s.⁵⁻⁹ Although complete and partial regressions of advanced non-Hodgkin's lymphoma, melanoma, thymoma, acute lymphocytic leukemia, and ovarian and breast carcinomas were noted in these early clinical trials, severe toxic effects, particularly nausea, vomiting, diarrhea, elevations of hepatic function tests and, less commonly, weakness and lethargy precluded further development of maytansine in favor of other antimicrotubule agents.⁵⁻⁹

On the basis of the extremely potent cytotoxic properties and clinical activity in limited early studies, maytansine was believed to be an ideal cytotoxic agent for conjugation to tumor-specific monoclonal antibodies. Chemical derivatization of maytansine resulted in the synthesis of a maytansine derivative DM1, which is three- to 10-fold more potent than maytansine, with an inhibitory concentration (IC₅₀) in the picomolar range and broad cytotoxic activity against a broad range of human tumors *in vivo*.⁴ The free sulfhydryl group of DM1 enabled stable disulfide linkage to huC242 that was modified to contain sulphydryl groups. Experimental studies demonstrated that the conjugation of DM1 to huC242 (cantuzumab mertansine) permits the selective distribution of DM1 molecules to specific antigen-bearing cells, resulting in enhancement of antitumor activity and reduction of normal tissue toxicity.^{4,10,11}

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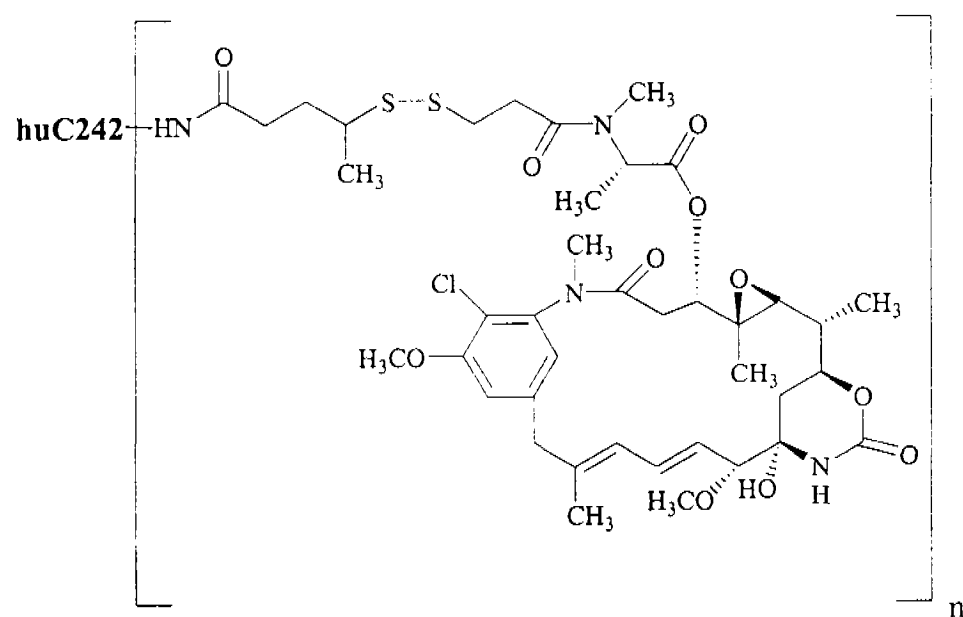


Fig 1. Schematic of immunoconjugate structure of DM1 ($n = 3$ to 4).

In addition to its extraordinary potency *in vitro*, cantuzumab mertansine demonstrated impressive antitumor activity against a broad range of CanAg-expressing, well-established human tumor xenografts.^{4,10} Treatment of mice bearing established human xenografts of Colo 205, HT-29, and LoVo colon cancer resulted in both cures and complete responses at relatively nontoxic cantuzumab mertansine doses of 48 mg/m^2 intravenously (IV) daily for 5 days.¹⁰ In contrast, tumor regressions were not observed in mice treated with maytansine, the "naked" huC242 antibody, a mixture of nonconjugated maytansine and huC242, fluorouracil (FU), or irinotecan as single agents at their respective maximum tolerated doses (MTDs). Furthermore, treatment of mice bearing established human lung cancer cell line H441 or pancreatic cell lines SW-1990, BxPC-3, and SU-8686 with even lower doses of cantuzumab mertansine (24 to 27 mg/m^2 daily for 5 days) resulted in complete responses.¹²

The toxicologic and pharmacologic profiles of cantuzumab mertansine were evaluated in both mice and cynomolgus monkeys—the latter animal model exhibiting comparable normal tissue expression of CanAg as in humans.¹ In the single-dose toxicologic studies, the lethal dose (LD) in 10% and 50% of mice (LD_{10} and LD_{50}) was 228 and 333 mg/m^2 , respectively.¹ Histomorphologically, gastrointestinal, hematopoietic, and neuronal tissues were the most sensitive tissues to the toxic effects of cantuzumab mertansine. Transient and reversible elevations of serum lipase, amylase, ALT, and AST also were observed in monkeys. In addition, pathologic studies revealed modest neuron axonal degeneration in monkeys treated with a single dose at 341 mg/m^2 , whereas mild axonal degeneration was noted in animals treated with lower doses of 58 to 228 mg/m^2 weekly, thereby demonstrating the potential for both peak and cumulative dose-related neurotoxicity. The plasma clearance of cantuzumab mertansine in mice was biphasic, with terminal half-life ($t_{1/2}$) values averaging approximately 48 hours.¹ In monkeys treated with a single IV dose of 228 mg/m^2 , pharmacokinetics were dose independent, with $t_{1/2}$ and volume of distribution averaging 40.3 hours and 59 mL/kg , respectively.¹

The rationale for the clinical development of cantuzumab mertansine included the potential for targeted delivery of the potent maytansinoid DM1 on the basis of the prevalence of CanAg expression on several common human tumors and the relative lack of expression on normal tissues, as well as the impressive activity of cantuzumab mertansine in a broad spectrum of CanAg-expressing experimental tumors at nontoxic doses. The principal objectives of this phase I and pharmacokinetic study were to (1) determine the MTD of cantuzumab mertansine administered as an IV infusion every 3 weeks, (2) determine the toxic effects of cantuzumab mertansine on this schedule, (3) characterize the pharmacokinetic behavior of cantuzumab mertansine, (4) seek preliminary evidence of anticancer activity in patients with advanced solid malignancies, and (5) characterize the expression of CanAg on tumor cells and shed CanAg expression and explore the relationships between these parameters and both the toxicity and pharmacokinetics.

PATIENTS AND METHODS

Patient Selection

Patients with histologically or cytologically confirmed solid malignancies refractory to standard therapy or for whom no standard therapy existed were eligible. Immunohistochemical assessment of CanAg expression was performed in all patients with available tissue. As CanAg is highly expressed in the majority of pancreatic and colorectal carcinomas, patients with these malignancies could be treated without prior confirmation of CanAg expression; however, patients with other malignancies required immunohistochemical confirmation of CanAg expression before enrollment. Eligibility also included an age ≥ 18 years; a life expectancy of at least 12 weeks; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; no prior chemotherapy within 4 weeks (6 weeks for mitomycin or a nitrosourea); adequate hematopoietic (hemoglobin $\geq 9 \text{ g/dL}$, absolute neutrophil count $[\text{ANC}] \geq 1,500/\mu\text{L}$, platelet count $\geq 100,000/\mu\text{L}$), hepatic (bilirubin $\leq 1.5 \text{ mg/dL}$, AST, ALT, and alkaline phosphatase less than or equal to three times the upper limit of normal, or less than or equal to five times institutional upper limit of normal if the elevation was from hepatic metastases), and renal (serum creatinine $\leq 1.5 \text{ mg/dL}$) functions; measurable or evaluable disease; no evidence of brain metastases; and no coexisting medical problem of sufficient severity to limit compliance with the study. Patients gave written

informed consent before treatment for all clinical and research aspects of the study according to federal and institutional guidelines.

Dosage and Drug Administration

IV cantuzumab mertansine was administered at a rate of 1 mg/min for 30 minutes and then increased to 3 mg/min if hypersensitivity phenomena were not observed. Treatment courses were repeated every 3 weeks. An accelerated dose-escalation method was used to guide dose escalation in cohorts of new patients, and toxicity was graded according to the National Cancer Institute's (NCI) Common Toxicity Criteria, version 2. Three patients were treated with cantuzumab mertansine at the starting dose of 22 mg/m², which represented one tenth of the LD₅₀ in mice. The dose was doubled in each new cohort of patient(s) until moderate toxicity (grade $\geq$ 2) was observed, at which time the cohort size was a minimum of three patients and a more conservative dose-escalation scheme was invoked. If one of three patients experienced dose-limiting toxicity (DLT), the cohort was expanded to six patients. The MTD was defined as the highest dose at which fewer than two of six patients experienced treatment-related DLT during the first course of therapy. DLT was defined as any grade 3 or 4 nonhematologic toxicity (including grade $\geq$ 3 nausea or vomiting despite optimal antiemetics), grade 4 thrombocytopenia, or grade 4 neutropenia (ANC $<$ 500/ μ L) lasting more than 4 days or accompanied by fever. In patients with hepatic transaminase elevations before treatment because of liver metastases, DLT was defined as an elevation of at least two grade levels above pretreatment.

Cantuzumab mertansine was supplied in 20-mL single-use vials by ImmunoGen, Inc. (Cambridge, MA). Each vial contained protein at a concentration of 0.7 mg/mL in a buffered solution (acceptable pH, 6.5 $\pm$ 0.5) consisting of monobasic potassium phosphate (0.57 mg/mL), monobasic sodium phosphate monohydrate (0.20 mg/mL), dibasic sodium phosphate (0.555 mg/mL), and sodium chloride (8.16 mg/mL) in purified water.

Pretreatment and Follow-Up Studies

A complete medical history, physical examination, concurrent medication profile, assessment of performance status, and routine laboratory studies were performed pretreatment and weekly during treatment. Routine laboratory studies included a complete blood count (CBC), differential WBC count, prothrombin and partial thromboplastin times, electrolytes, blood urea nitrogen, serum creatinine, uric acid, glucose, alkaline phosphatase, lactate dehydrogenase, ALT, AST, total bilirubin, calcium, total protein, albumin, cholesterol, triglycerides, amylase and lipase, and urinalysis. Pretreatment studies also included an ECG, relevant radiologic studies for the evaluation of all measurable and evaluable sites of disease, and an assessment of appropriate tumor markers. Radiologic studies for disease status were repeated after every other course. Patients were able to continue treatment if they did not develop progressive disease or experience intolerable toxicity. A complete response was scored if there was disappearance of all measurable and evaluable disease for at least two measurements performed at least 4 weeks apart without worsening of disease-related symptomatology or declining performance status. A partial response required at least a 50% reduction in the sum of the product of the bidimensional measurements of all lesions documented by at least two measurements separated by at least 4 weeks. Any concurrent increase in the size of any lesion by 25% or more or the appearance of any new lesion was considered disease progression. In course 1, patients were observed for 3 hours following treatment. ECGs were performed 1, 3, 6, and 24 hours posttreatment. A complete physical and neurologic exam was performed 24 hours following cantuzumab mertansine dosing along with a CBC and chemistry profile including lipase and amylase. Laboratory assessments also were performed on days 5, 8, 15, and 22 of course 1 and weekly for subsequent courses.

Plasma Pharmacokinetic Sampling and Assay

Blood samples were collected into heparinized tubes before the first infusion and at the end of infusion. Samples also were collected at 15 minutes and 3, 8, 24, 48, and 96 hours after the end of the infusion and on days 8, 15, and 22. Such intensive sampling was performed on the first and fifth courses, and peak and trough blood samples were obtained immediately before every course. The blood samples were centrifuged at 3,000 rpm for 10

minutes immediately after collection, and the plasma was transferred to separate tubes and frozen to -20°C until assayed.

Determination of Intact Cantuzumab Mertansine (huC242-DM1 Conjugate) in Plasma

The intact cantuzumab mertansine immunoconjugate was measured using a dual antibody enzyme-linked immunosorbent assay (ELISA) method using two specific murine IgG₁ monoclonal antibodies developed at ImmunoGen, Inc. One antibody, anti-huC242, is specific for huC242 (an anti-idiotypic), and the second antibody is specific for the DM1 moiety.

Briefly, ELISA plates were coated with anti-huC242 antibody (KM142) to capture cantuzumab mertansine from the test samples. Standard curves using cantuzumab mertansine concentrations ranging from 1.56 to 200 ng/mL diluted in 2% normal human plasma were run on each plate. The amount of cantuzumab mertansine that was captured in each well was detected using biotinylated antimaytansinoid antibody (ND137) followed by streptavidin-horseradish peroxidase (HRP) and 2,2'-azino-bis (3-ethylbenzethiazoline-6-sulfonic acid) (ABTS) to develop the signal absorbance, which was recorded at 405 nm. For each sample dilution, the mean of the triplicates was calculated, and the concentration of the intact conjugate was determined by comparison with the standard curve. The lower limit of detection (LLD) for this assay was 31.25 ng/mL in plasma.

Determination of Total huC242 Antibody (huC242-DM1 and Free huC242) in Plasma

The total huC242 antibody, including both unconjugated huC242 and huC242-DM1 containing varying amounts of DM1, was measured in patient plasma samples using an ELISA method, which measured the competition of binding of biotinylated huC242 antibody to anti-huC242 antibody by the standard huC242 antibody and the test samples. Briefly, anti-huC242 antibody was captured using goat antimouse IgG(Fc)-coated ELISA plates. Test samples and standard curve solutions were mixed with an equal volume of 20 ng/mL biotinylated-huC242 antibody, and then the mixtures were added to the coated ELISA plates. The standard curves were made using concentrations of huC242 antibody ranging from 3.9 to 500 ng/mL. The plates were developed using streptavidin-HRP and ABTS, and the absorbance was recorded at 405 nm. For each sample dilution, the mean of triplicates was calculated and the concentration of total huC242 antibody was determined by comparison with the standard curve. The LLD for this assay was approximately 1,560 ng/mL in plasma.

Detection of Human Anti-huC242 Antibodies and Anti-DM1 Antibodies

Human anti-huC242 antibodies (HAHA) and anti-DM1 antibodies (HADA) were detected in the plasma of patients with ELISA methods that use the multivalency of the Ig molecules. For detecting HAHA, ELISA plates were coated with huC242 antibody to capture any anti-huC242-specific human antibodies from the plasma. Bound human anti-huC242 antibody was then detected by its capture of biotinylated huC242, followed with streptavidin-HRP and ABTS reagent to develop the signal, and absorbance was recorded at 405 nm. Values were compared with a standard curve (4,000 ng/mL to 7.81 ng/mL in 2% human serum) of anti-huC242 (a murine IgG₁ monoclonal antibody), which also served as a positive control. All wells were run in triplicate. The LLD for this assay was 781 ng/mL.

For detection of HADA, ELISA plates were coated with bovine serum albumin conjugated with DM1 (BSA-DM1) to capture any specific human anti-DM1 antiserum. Biotinylated antigen (generated by the reaction of the thiol of DM1 with a maleimide-biotin derivative) was then added to detect any bound human anti-DM1 antibody by capture of the biotinylated DM1, followed by streptavidin-HRP and ABTS to develop the signal, and absorbance was recorded at 405 nm. Values were compared with a standard curve (4,000 ng/mL to 1.95 ng/mL in 2% human serum) of antimaytansinoid antibody (ND137; a murine IgG₁ monoclonal antibody), which served as a positive control. All wells were run in triplicate. The LLD for this assay was 390 ng/mL.

Determination of Nonprotein-Bound Maytansinoid in Plasma

Non-protein-bound maytansinoid ("free" maytansinoid) was detected using a competition ELISA method to detect maytansinoids in a protein-free extract of patient plasma samples. Protein-free extracts were made by precipitation of plasma proteins (including, therefore, cantuzumab mertansine) by the addition of an equal volume of chilled (-20°C) acetone to ice-cold aqueous samples. After 30 minutes' incubation on ice, precipitated protein was removed by centrifugation. Fifty microliters of appropriately diluted extracts, control samples, and a standard curve (50 pM to 400 pM maytansine) were mixed with 50 μL of biotinylated antimaytansinoid antibody, and then 50 μL of this mixture was transferred to ELISA plates coated with BSA-DM1. Streptavidin-HRP and the 3,3',5,5'-tetramethylbenzidine-substrate system were used for development of the assay, reading the absorbance at 630 nm. Samples were assayed in duplicate. The LLD was approximately 1.2 nmol/L in plasma.

Detection of Shed Antigen (Shed CanAg)

Shed CanAg was detected in plasma using an ELISA that took advantage of the multivalency of the antigen. Briefly, Immulon-2 plates were coated with the murine C242 antibody, which was used to capture CanAg from test samples. A standard curve was made from CanAg prepared as described previously³ and calibrated against a commercial antigen standard (CanAg Diagnostics, Gothenburg, Sweden). Bound antigen was detected by its capture of biotinylated murine C242 antibody, and the assay was developed with streptavidin-HRP and TMB reagent, recording the absorbance at 630 nm. The LLD was 10 units/mL.

Pharmacokinetic and Pharmacodynamic Analyses

Individual plasma concentration data sets for cantuzumab mertansine were analyzed by noncompartmental methods (WinNonLin Professional 3.1, Pharsight, Inc., Mountain View, CA). Peak concentrations (C_{max}) were determined by inspection of plasma concentration-time data. Elimination rate constants were estimated by linear regression of the last three data points on the terminal log-linear portion of the concentration-time curves, and $t_{1/2}$ was calculated by dividing 0.693 by the elimination rate constants. The area under the concentration-time curve (AUC) was calculated using the linear trapezoidal rule up to the last measurable data point (for AUC_{0-t}), then extrapolated to infinity ($\text{AUC}_{0-\infty}$). The systemic clearance (CL) was determined by dividing the dose (in mg m^{-2}) by the $\text{AUC}_{0-\infty}$. The apparent volume of distribution at steady state (Vd_{ss}) was determined using the following formula: $\text{Vd}_{ss} = (\text{dose} \times \text{AUC}/\text{AUC}^2) - (\text{dose} \times \text{duration of infusion}) / (2 \times \text{AUC})$, where AUC is the area under the moment curve extrapolated to infinity. Dose proportionality was assessed using a one-way analysis of variance (ANOVA) using Scheffe's multiple comparison test of the effect of cantuzumab mertansine dose on clearance.

The relationships between cantuzumab mertansine pharmacokinetic parameters reflecting drug exposure (eg, AUC and C_{max}) and indices reflecting hepatic toxicity (AST, ALT, bilirubin, and alkaline phosphatase) and myelosuppression (ANC and platelets) in the first course were explored. Relevant parameters of myelosuppression that were evaluated included ANC and platelet nadir values, and the percentage decrements in the ANC and platelet counts were calculated as follows: $100 \times [(\text{pretreatment counts} - \text{nadir counts}) / \text{pretreatment counts}]$. For hepatic toxicity, both categorical toxicity grade and percentage elevation in hepatic function test ($100 \times [\text{peak value} - \text{pretreatment value}] / \text{pretreatment value}$) were evaluated. Pharmacodynamic relationships were fit to both linear and sigmoidal maximal effect (E_{max}) models of drug action (ie, percentage change in toxicity parameter = $E_{\text{max}} \times \text{AUC}_{50\gamma} / \text{AUC}_{50\gamma} + \text{AUC}_{50\gamma}$), where E_{max} was fixed at 100% and $\text{AUC}_{50\gamma}$ is the AUC at which the effect is 50% of E_{max} . The exponent γ is a constant that describes the sigmoidicity of the curve. The sigmoidal E_{max} model was fit to the data by nonlinear least squares regression. The coefficient of determination (R^2) and the SEs for the estimated parameters were used as measures of goodness of fit for the pharmacodynamics model. Parameter values were expressed as means and SD values. Differences in mean $\text{AUC}_{0-\infty}$ values between patients who did or did not experience severe hematologic toxicity were compared using the Student's t test (two-sided). A multivariate regression analysis was used to examine the association between pharmacokinetic parameters and parameters of hepatic toxicity. Separate

regression models were estimated for either percentage elevation in AST or percentage elevation in ALT, with independent variables consisting of dose (mg m^{-2}), clearance, and volume. Statistical significance was declared if the P value on the corresponding coefficient was less than 0.05.

Immunohistochemical Staining of Tumor Tissues

One patient with readily accessible tumor tissue for biopsy consented to a biopsy procedure 24 hours after treatment. The tissue was coated with Tissue-Tek OCT embedding compound (Sakura Finetech USA, Torrance, CA), snap frozen, and sectioned on a cryostat, and then 6- μm sections were air dried and fixed in acetone for 2 minutes at room temperature. Tissues were blocked with Peroxidase Blocking Reagent and Biotin Blocking System (Dako, Carpinteria, CA) to reduce nonspecific endogenous antigen sites. The antibodies, murine C242, antihuman IgG, and anti-DM1, were biotinylated with FluoReporter Mini-Biotin-Protein Labeling Kit (Molecular Probes, Eugene, OR). Tissues were incubated with the antibodies for 30 minutes, and peroxidase-conjugated streptavidin (LSAB2; Dako, CA) was used to detect the presence of antibody binding on the tissue sections. The sections were developed in Liquid DAB (3,3'-diaminobenzidine) Substrate-Chromogen System (Dako, CA) and counterstained with hematoxylin (Sigma Co., St. Louis, MO).

The distribution of the epitope of CanAg recognized by the murine C242 antibody also was determined on sections cut from formalin-fixed, paraffin-embedded tumor biopsies from all patients where available and examined by immunohistochemical staining using the avidin-biotin immunoperoxidase technique. A control murine monoclonal IgG₁ antibody was obtained from Coulter Immunology (Hialeah, FL).

RESULTS

General

Thirty-seven patients, whose pertinent demographic characteristics are displayed in Table 1, received a total of 110 courses of cantuzumab mertansine at doses ranging from 22 to 295 mg m^{-2} . The total number of new patients treated and the number of courses at each dose level, as well as the overall dose-escalation scheme, are depicted in Table 2. The median number of courses administered per patient was two (range, one to 10). Doses were increased in two patients, whereas the dose was reduced because of DLT in three patients.

After no or negligible drug-related effects were noted in the first three patients treated at the 22- mg m^{-2} dose level, the dose of cantuzumab mertansine was doubled twice, to 44 and 88 mg m^{-2} , respectively. The first patient treated at the 88- mg m^{-2} dose level

Table 1. Patient Characteristics

Characteristic	No. of Patients
No. of patients	37
Median no. of courses/patient (range)	2 (1-10)
Median age (range)	54 (19-70)
Performance status	
0	19
1	13
2	5
Males/females	21/16
Previous therapy	
Chemotherapy	37
Radiation therapy	19
Median no. of prior chemotherapy regimens (range)	3 (1-9)
Tumor types	
Colorectal	32
Pancreas	4
Lung (non-small-cell)	1

Table 2. Dose-Escalation Scheme

Cantuzumab Mertansine Dose Level (mg/m ²)	No. of Patients			No. of Courses	Patients With DLT	
	New	Modified to Level*	Total		First Course	All Courses
22	3	0	3	5	0/3	0/5
44	1	0	1	1	0/1	0/1
88	3	0	3	11	0/3	0/11
132	3	1	4	12	0/3	0/12
176	8	3	11	28	0/8	0/28
235	16	1	17	44	1/16	2/44
295	3	0	3	9	2/3	2/9
TOTAL	37			110		

*Patients whose doses were escalated to the next higher dose level in the absence of DLT, or whose dose was reduced to the next lowest dose because of DLT.

experienced grade 2 fatigue, precluding further 100% dose-escalation increments and single-patient cohorts. Thereafter, no DLT was observed in three patients treated with cantuzumab mertansine at the 132- and 176-mg/m² dose levels. At the next highest dose level, 235 mg/m², one of the three initial patients experienced dose-limiting elevations in hepatic transaminases, resulting in three additional patients entered at this dose level, none of whom had DLT. Two of the three patients treated at 295 mg/m² experienced DLT, including a grade 3 AST and grade 3 fatigue in course 1. At this juncture, 10 additional patients were subsequently treated with the 235 mg/m² dose. At the 235-mg/m² dose level, one of these additional patients had DLT (grade 3 elevation in hepatic transaminase) during course 1, for a total of two of 16 patients with DLT. Because there was a preponderance of clinically significant episodes of hepatic transaminitis in patients who had extensive hepatic metastases, five additional patients with significant hepatic metastases were treated with cantuzumab mertansine at 176 mg/m², which resulted in notable but asymptomatic grade 3 elevation of lipase in one of these individuals but no other DLT during the first course. On the basis of these results, the recommended dose of cantuzumab mertansine for phase II studies is 235 mg/m² IV every 3 weeks.

Toxicity

Hepatic toxicity. The principal toxic effects of cantuzumab mertansine treatment were reversible elevations of hepatic transaminases and occasionally alkaline phosphatase and bilirubin.

The onset of transaminitis was typically within 48 hours following treatment, with median peak values observed on day 5 (range, days 2 to 8) and recovery toward pretreatment values by day 8. Elevations in alkaline phosphatase values occurred somewhat later, with median peak values noted by day 8 and recovery toward pretreatment values by day 15. Most drug-related hepatic toxic effects completely resolved by the time of scheduled retreatment, and no treatment delays were required for persistent elevations, except in those cases in which elevations were clearly attributable to progressive disease. In addition, although elevations in hepatic parameters recurred in subsequent courses in patients who experienced hepatotoxicity in course 1, the magnitude of hepatotoxicity did not worsen significantly with cumulative treatment.

The distributions of grades of related hepatic toxic effects as a function of dose are shown in Table 3. Clinically significant hepatic function test elevations attributable to cantuzumab mertansine were not observed at doses below 176 mg/m². Of eight patients treated at the 176-mg/m² dose level, one patient, who had grade 2 elevation in serum alkaline phosphatase values before treatment because of underlying malignancy, experienced further elevations to grade 3 concomitant with progressive growth in hepatic metastases that, therefore, was not considered a DLT. At the next higher dose level, 235 mg/m², 13 of 16 patients had elevations of liver function tests from pretreatment values by one or two grades, and two of 16 patients ultimately experienced DLT.

Table 3. Hepatic Toxicity Related to Cantuzumab Mertansine

Dose Level (mg/m ²)	No. of Patients (courses)	No. of Patients Experiencing Toxicity During First Course (all courses)											
		AST			ALT			Alkaline Phosphatase			Bilirubin		
		1	2	3	1	2	3	1	2	3	1	2	3
22	3	0	0 (1)	0	0	0	0	1	0 (1)	0	1 (1)	0	0
44	1	0	0	0	0	0	0	0	0	0	0	0	0
88	3	1 (2)	0	0	0 (2)	0	0	1 (2)	0	0	0	0	0
132	3	2 (2)	0	0	0 (1)	0	0	2 (2)	0	0	0	0	0
176	8	4 (4)	3 (3)	0	4 (4)	1 (2)	0	0 (2)	2 (2)	1* (1)	1 (1)	1 (1)	0
235	16	2 (4)	5 (5)	6† (8)	5 (6)	6 (8)	2‡ (3)	7 (9)	4 (6)	2* (4)	1 (3)	0	3§ (4)
295	3	1 (1)	0	2† (2)	1 (1)	2 (2)	0 (0)	2 (2)	1 (1)	0	0	1 (1)	0

*These patients had grade 2 elevations in alkaline phosphatase prior to treatment.

†Four patients had grade 2 elevations prior to treatment.

‡One patient had a grade 1 elevation prior to treatment.

§One patient had a grade 2 elevation prior to treatment.

Table 4. Hematologic Toxic Effects

Dose Level (mg/m ²)	No. of Patients (courses)	No. of First Courses (all courses) With Toxicity							
		Neutropenia					Thrombocytopenia		
		Grade 1-2	Grade 3	Grade 4	Grade 4 > 4 days	Grade 4 With Fever	Grade 1-2	Grade 3	Grade 4
22	3 (5)	0	0	0	0	0	0	0	0
44	1 (1)	0	0	0	0	0	0	0	0
88	3 (11)	0 (1)	0	0	0	0	2 (2)	0	0
132	3 (12)	0	0	0	0	0	0	0	0
176	8 (28)	1 (4)	0	0	0	0	0	0	0
235	16 (44)	1 (3)	0 (1)	1 (1)	0	0	0	1 (1)	0
295	3 (9)	2 (2)	0	0	0	0	0	0	0

Severe dose-limiting toxicity occurred in two of three patients treated at 295 mg/m². One patient, who experienced a three-grade increase in AST (grade 0 to 3), associated with grade 2 nausea and vomiting in course 1, received no further treatment; the second patient who had a two-grade increase in both AST (grade 1 to 3) and ALT (grade 0 to 2) had grade 3 fatigue, and the dose for this patient was reduced to 235 mg/m² for two subsequent courses, with the patient experiencing mild transaminitis (grade 1).

Possible determinants of hepatotoxicity were explored. Neither the presence of any single elevated hepatic function (AST, ALT, alkaline phosphatase, bilirubin) nor the combinations thereof at pretreatment predicted drug-related elevations in hepatic functions ($P > .05$ for elevations in AST, ALT, alkaline phosphatase, and bilirubin). Although hepatotoxicity appeared to be related to the extent of hepatic metastases as assessed by computed tomography (CT) scanning, the limited numbers of patients with hepatotoxicity at any one dose level and difficulties measuring the total liver involvement on the basis of CT therefore precluded the formulation of rigid quantitative scoring of liver disease for toxicity prediction.

Hematologic toxicity. The distribution of NCI grades of neutropenia and thrombocytopenia are displayed in Table 4. Effects on neutrophils and platelets were rarely clinically significant (grade 3 or 4) and never dose limiting. In fact, the most severe hematologic event consisted of grade 3 thrombocytopenia (nadir platelet count 40,000/ μ L) and uncomplicated, brief (< 5 days) grade 4 neutropenia in the first course of a patient treated with cantuzumab mertansine at the 235-mg/m² dose level. Neither dose reduction nor treatment delay was required because of severe or unresolved hematologic toxicity.

Neurotoxicity. Cumulative and dose-related peripheral sensory neuropathy was experienced by 16 patients (43%). Neurosensory symptoms were characterized by painful and nonpainful paraesthesias involving the feet and hands that persisted following discontinuation of treatment. Eight of the 17 patients (47%) treated at the 235-mg/m² dose level and two of three patients (67%) at the 295-mg/m² dose level experienced these symptoms. Only one of eight patients treated at this dose level had received prior oxaliplatin therapy. Two patients, both treated at 235 mg/m², experienced severe (grade 3) peripheral neuropathy after one and four courses, respectively. Interestingly, the patient who experienced an accelerated onset of severe peripheral neuropathy after the first course of cantuzumab mertansine received prior oxaliplatin and was the same patient who experienced the aforementioned grade 4 neutropenia and grade 3 thrombocytopenia during course 1.

Miscellaneous Toxic Effects

Nausea and vomiting, diarrhea, arthralgias, fever, peripheral neuropathy, and fatigue were common. The distributions of the NCI grades of these toxic effects as a function of dose are depicted in Table 5. Twenty-five patients (68%) and 12 patients (32%) experienced nausea and vomiting, respectively, at some time during treatment. Nausea and vomiting were generally mild or moderate (grade 1 or 2); however, one patient at the 235-mg/m² dose level experienced grade 3 vomiting at a later course of cantuzumab mertansine. Nausea and vomiting were usually managed and/or prevented with prochlorperazine or a serotonin (5HT₃) receptor antagonist. Thirteen patients (35%) experienced diarrhea at some time during treatment. The diarrhea was generally mild to moderate; however, one patient

Table 5. Nonhematologic Toxicity (excluding hepatic)

Dose Level (mg/m ²)	No. of Patients (courses)	No. of Patients Experiencing Toxicity During First Course (all courses)											
		Nausea/Vomiting			Diarrhea			Peripheral Neuropathy			Myalgia		
		1	2	3	1	2	3	1	2	3	1	2	3
22	3	0	0	0 (1)	0	0	0	0	0	0	0	0	0
44	1	0	0	0	0	0	0	0	0	0	0	0	0
88	3	0	1 (1)	0	0	0	0	1	0	0	0	0	0
132	3	1 (2)	0 (1)	0	1 (2)	0 (1)	0	0 (2)	0	0	0	0	0
176	8	3 (4)	0 (2)	0	2 (3)	1 (2)	0 (1)	1 (3)	0 (1)	0	1 (2)	0	0
235	16	7 (7)	2 (4)	0 (1)	4 (4)	1 (3)	0	0	0 (6)	1 (2)	4 (4)	0 (1)	0 (1)
295	3	2 (2)	1 (1)	0	0	0	0	1 (2)	0	0	2 (2)	0	0

Table 6. Noncompartmental Pharmacokinetic Parameters of Intact Cantuzumab Mertansine

Dose Level (mg/m ²)	No. of Patients	C _{max} (μg/mL)	T _{1/2} (h)	AUC _{0-∞} (mg/mL·hr)	CL mL/h/m ²	V _{dss} (mL/m ²)
22	3	33.3 (15.7)	18.9 (4.6)	0.94 (0.78)	35.3 (29.1)	814 (381)
44	1*	30.3				
88	3	104.0 (47.8)	48.5 (9.4)	2.47 (0.24)	35.9 (3.6)	1,333 (512)
132	3	158.5 (29.4)	32.4 (16.9)	3.97 (.45)	33.5 (3.6)	1,109 (219)
176	8	166.6 (34.6)	33.3 (8.3)	4.04 (0.76)	44.8 (7.9)	1,570 (308)
235	16	240.8 (87.9)	48.4 (18.0)	6.83 (3.56)	41.0 (16.3)	1,728 (733)
295	3	477.2 (56.0)	43.4 (11.0)	9.79 (1.52)	30.6 (5.0)	1,234 (111.0)
Mean (±SD)	—	—	41.1 (16.1)	—	39.5 (13.1)	1,497 (584)

NOTE: All values represent means (±SD).

Abbreviations: AUC_{0-∞}, area under the concentration-time curve extrapolated to infinity; C_{max}, peak plasma concentration observed; CL, clearance; T_{1/2}, half-life of elimination; V_{dss}, volume of distribution at steady state.

*Too few samples collected to estimate parameters.

experienced grade 3 diarrhea within 24 hours of the sixth course of cantuzumab mertansine; the diarrhea was not believed to be related to the investigational agent.

Three patients experienced hypersensitivity reactions. One patient experienced mild flushing, diaphoresis, dyspnea, transient bradycardia, and nausea within 15 minutes of the second course of cantuzumab mertansine at the 22-mg/m² dose level. The symptoms resolved with temporary interruption of the infusion and IV dexamethasone and antihistamine administration. The infusion was restarted within 1 hour, and the patient had no further recurrence of symptoms on this course. Two additional patients treated at 235 mg/m² experienced a similar, although more modest, reaction during the first course. Again, the patients received antihistamine and corticosteroids and were successfully retreated without further recurrence of symptoms during this or subsequent courses.

Three patients who had no clinical manifestations of pancreatitis experienced isolated grade 3 elevations of serum lipase. These events occurred at the 22-mg/m² (course 2), 132-mg/m² (course 3), and 176-mg/m² (course 1) dose levels.

Other mild to moderate (grade 1 or 2) nonhematologic toxic effects that were possibly related to cantuzumab mertansine included anorexia in 22% of patients, constipation in 3%, and intestinal ileus in 3%. These effects were noted across the entire cantuzumab mertansine dose range, and definite temporal relationships could not be discerned for any of these toxic effects. The underlying malignant processes might have contributed to these events.

Pharmacokinetics and Pharmacodynamics

Thirty-six patients had plasma sampling performed in the first course for pharmacokinetic studies, and 33 patients had complete sampling at all time points. All data sets were analyzed by noncompartmental methods. The mean pharmacokinetic parameters of the intact immunoconjugate cantuzumab mertansine at each dose level are listed in Table 6. The dose proportionality was confirmed by the demonstration of cantuzumab mertansine clearance being constant over the dose range administered ($P = .588$, one-way ANOVA). The mean (±SD) V_{dss} for cantuzumab mertansine approximated the intravascular plasma volume, averaging $1,497 \pm 584$ mL/m², whereas the mean plasma clearance was 39.5 ± 13.1 mL/h/m² and the elimination t_{1/2} was long, at 41.1 ± 16.1 hours. Neither cantuzumab mertansine clearance nor V_{dss} increased with

increasing with BSA ($R^2 = 0.011$, $P = .56$, and $R^2 = 0.015$, $P = .49$, respectively), thereby indicating that alternative dosing methods including flat dosing could be used in future phase II studies.

Five patients had complete pharmacokinetic sampling performed in both courses 1 and 5. A representative patient's plasma concentration data fit for both course 1 and 5 at 235 mg/m² is shown in Fig 2. In these individuals, the clearance of cantuzumab mertansine did not change following repetitive treatments; clearance values (±SD) averaged $37.9 (\pm 7.18)$ and $38.2 (\pm 14.1)$ for course 1 and 5, respectively ($P > .05$).

The total concentration of huC242 antibody also was measured in the plasma. The ELISA detects total huC242 irrespective of whether the antibody molecules are unconjugated or conjugated to DM1 molecules. The pharmacokinetic parameters for the total antibody are depicted in Table 7. The modest differences between peak concentrations of the total antibody versus immunoconjugate, illustrated in Fig 3, are attributable to the different ELISAs used. The clearance of total antibody was markedly slower than the immunoconjugate, with a mean clearance value of 11.0 ± 8.7 mL/h/m² and a prolonged mean t_{1/2} of 230.4 ± 97.5 hours. As the clearance of the total antibody was markedly slower than that of the immunoconjugate, the concentrations of unconjugated antibody exceeded those of the immunoconjugate at later time points postinfusion. Representative concentration-time profiles for both the immunoconjugate and total antibody, including peak and trough values, in courses 1 through 5 are depicted in Fig 3.

Free maytansinoid (DM1 non-protein bound) plasma concentrations were measured in two patients at the highest dose level

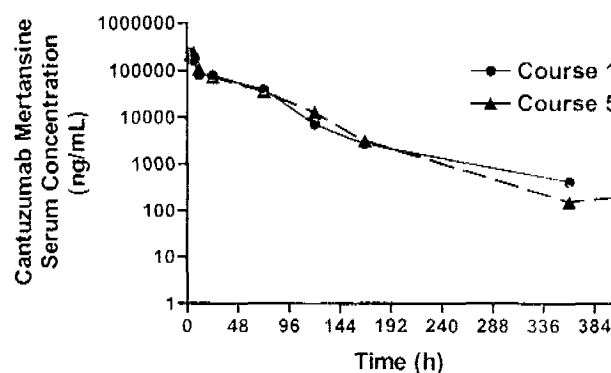


Fig 2. A representative patient's plasma cantuzumab mertansine concentration versus time curve for courses 1 and 5 at the 235-mg/m² dose level.

Table 7. Noncompartmental Pharmacokinetic Parameters of Total Antibody

Dose level (mg/m ²)	No. of Patients	C _{max} (μg/mL)	T _{1/2} (h)	AUC _{0-∞} (mg·h/mL)	CL mL/h/m ²	V _{dss} (mL/m ²)
22	3	13.2 (2.4)	189.4 (50.4)	2.22 (0.47)	10.2 (2.0)	2,796.8 (203.4)
44	1*	17.1				
88	3†	77.0	307.3	18.2	5.7	2,615.4
132	3	104.4 (27.0)	312.4 (16.3)	31.8 (5.8)	4.3 (0.9)	2,163.7 (506.3)
176	8	92.7 (12.5)	215.9 (65.9)	22.0 (10.5)	9.3 (3.3)	2,884.6 (695.2)
235	16	135.3 (67.4)	216.7 (127.7)	26.0 (18.2)	14.0 (12.1)	3,427.0 (1,506.6)
295	3	124.0 (19.7)	244.9 (55.2)	26.5 (3.0)	11.2 (1.2)	3,657.5 (616.8)
Mean (SD)	—	—	230.4 (97.5)	—	11.0 (8.7)	3,104.9 (1,180.0)

*Too few samples collected to estimate parameters.

†Not enough data from one patient; values are the average of data from only two patients.

of 295 mg/m² using an ELISA of a protein-free extract of blood. DM1 was detected in the plasma up to 48 hours following treatment but decreased below the lower limit of detection (1.2 nmol/L) at 96 hours posttreatment. The free maytansinoid concentrations in the plasma of the two patients were 52 and 56 nmol/L immediately following cantuzumab mertansine infusion and declined to 4.6 and 5.5 nmol/L by 48 hours. At all time points, the free maytansinoid represented less than 1% of the total circulating maytansinoid (huC242-conjugated immunoconjugate plus free maytansinoid).

The relationships between the pharmacokinetic parameters that reflect intact cantuzumab mertansine exposure (C_{max} and AUC) and the principal toxic effects encountered in this study also were explored. Scatterplots of the percentage increase in AST and ALT values as functions of C_{max} and AUC_{0-∞} are depicted in Fig 4A-D; all relationships were roughly linear (R² = 0.137 to 0.312), with the strongest being those relating transaminitis to the magnitude of C_{max}. The mean cantuzumab mertansine AUC_{0-∞} values at the 235- and 295-mg/m² dose levels were greater, albeit not significantly so, for patients who experienced severe hepatic toxicity (grade 3 or 4 transaminitis) compared with patients without toxicity of this magnitude (eg, for ALT, 75.74 ± 31.28 mg/mL × hr v 55.80 ± 23.52 mg/mL × hr; *P* = .12).

Although a rough relationship of C_{max} and AUC_{0-∞} to elevation in AST and ALT was observed using univariate analysis, a multivariate analysis of the relationship of independent variates of dose, AUC_{0-∞}, C_{max}, cantuzumab mertansine clearance, and V_{dss} indicated that none of the variables was statistically significant (*P* > .05).

In contrast to transaminitis, elevations in alkaline phosphatase or bilirubin could not be related to either C_{max} or AUC values (data not shown). With regard to hematologic effects, no pharmacodynamic relationships were apparent, and the few patients who experienced grade 3 or 4 neutropenia did not have lower clearance rates for cantuzumab mertansine compared with other patients.

Circulating Shed CanAg

There was marked interindividual variability in pretreatment levels of shed CanAg levels in plasma. Thirty-three of 37 patients (89%) had quantifiable plasma levels of shed CanAg before treatment; the median value was 184 units (range, from 10 [lower limit of detection] to 31,240 units; Fig 5). Following the first dose of cantuzumab mertansine, the shed CanAg levels decreased to undetectable levels in 25 of 30 patients (83%) who had complete pre- and posttreatment data sets (Fig 5). An analysis of all paired data sets indicated that this effect was highly significant (*P* < .0001, two-tailed paired *t* test). In eight patients who had

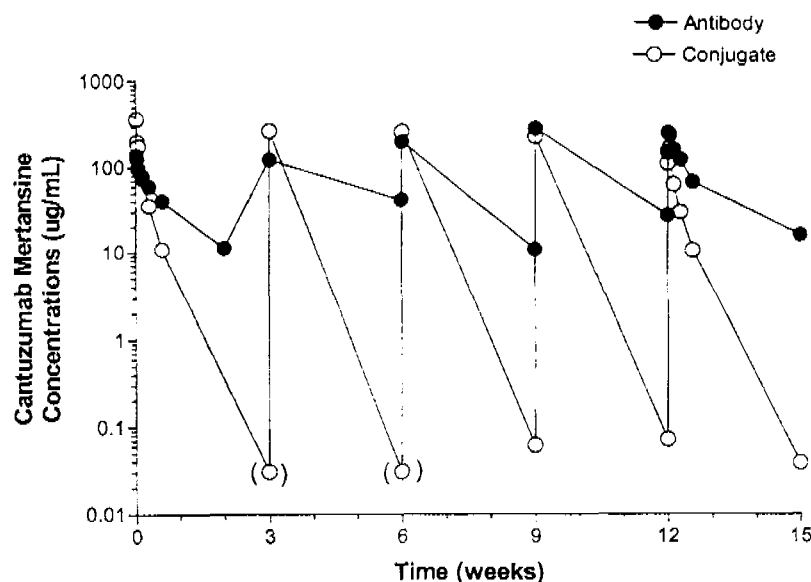


Fig 3. Representative patient's plasma concentration versus time curve for courses 1 and 5 for the intact immunoconjugate (open circles) and total antibody (closed circles), and peak and trough values for courses 2, 3, and 4. Symbols in parentheses represent data below the level of detection for assay.

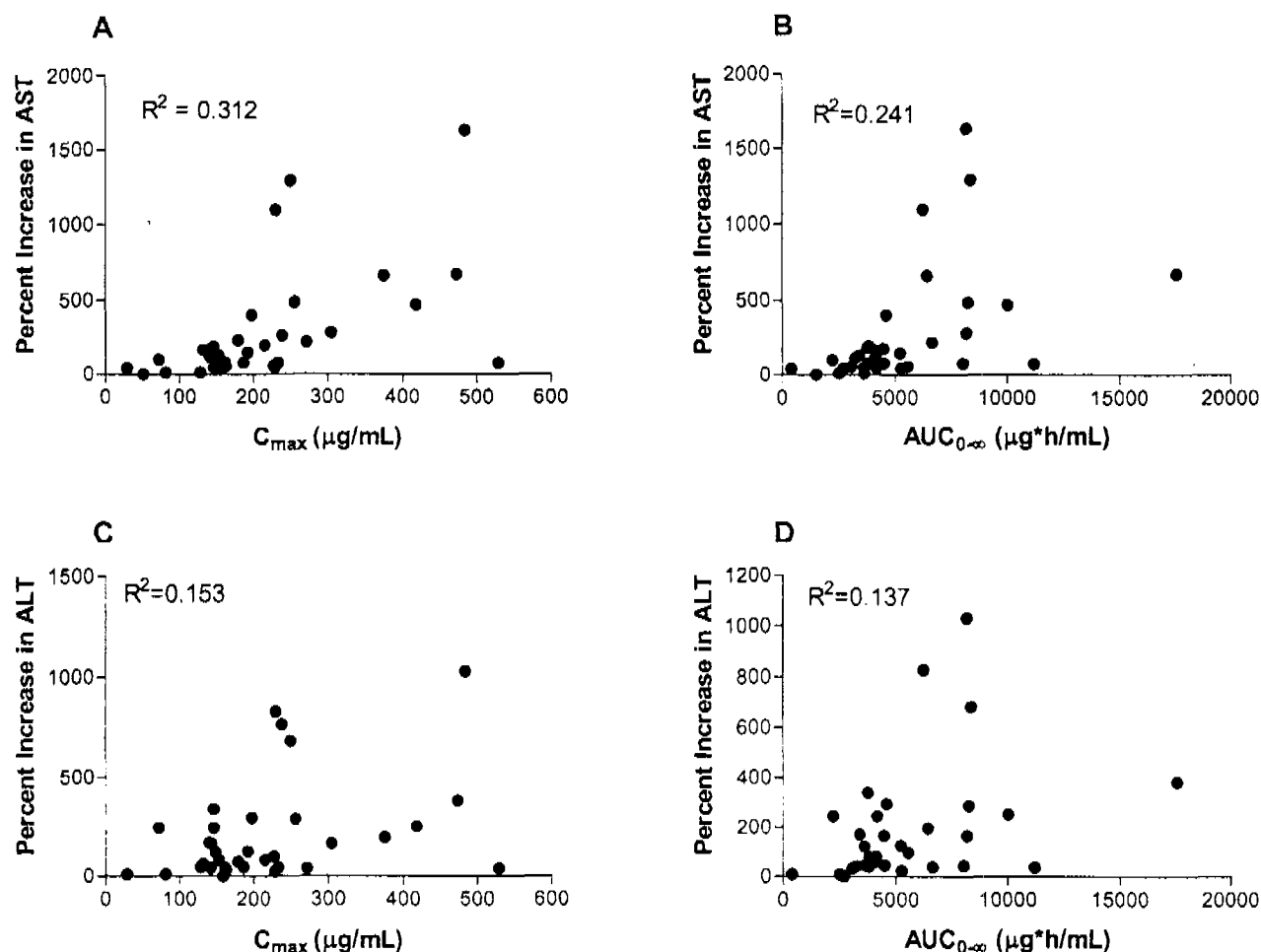


Fig 4. Scatterplots depicting the relationships between percentage increase in AST values during the first course of cantuzumab mertansine and C_{max} (A) and $AUC_{0-\infty}$ (B) and the percentage increase in ALT values during the first course and C_{max} (C) and $AUC_{0-\infty}$ (D).

multiple measurements of shed CanAg performed in the peritreatment period, plasma clearance of shed CanAg was rapid, with reductions to undetectable levels noted by the first time point assayed (3-hour and 8-hour samples in five and three patients, respectively). In 21 patients (70%), shed CanAg levels decreased to undetectable levels that persisted until the start of course 2 (day 22). Shed CanAg decreased to undetectable levels in patients treated

across the entire cantuzumab mertansine dose range, indicating that CanAg clearance was not dose-dependent, at least not in the dose range examined in this study.

The relationships between the magnitude of pretreatment shed CanAg levels and both the clearance of cantuzumab mertansine and the principal clinical toxic effects encountered during the first course of treatment were examined. No relationships between the pretreatment shed CanAg values and the clearance of cantuzumab mertansine were evident (data not shown). Similarly, neither linear nor nonlinear models were satisfactory in describing the relationships between pretreatment shed CanAg values and the percentage increases in relevant hepatic function parameters (data not shown), although only a few patients (four) had high CanAg values ($> 1,500$ units; Fig 5).

Formation of Human Antihuman and Human Anti-DMI Antibodies

Neither HAAHA nor HADA was detected in any of the 37 patients entered onto the study, including the patients who experienced hypersensitivity reactions.

Immunohistochemistry for Tumor CanAg Expression

Thirty-five of the 37 patients entered had tumor specimens available for immunohistochemical determination of CanAg

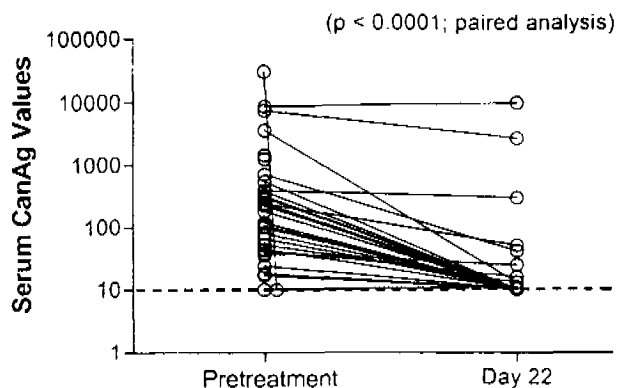


Fig 5. Scatterplots of individual circulating shed CanAg values pretreatment and following the first course (day 22) of cantuzumab mertansine. The patient with the highest CanAg value (31,240 units) sampled to 8 hours postinfusion only. Dashed line indicates lower limit of detection for assay.

Table 8. Tumor Immunohistochemistry for CanAg

Staining Distribution* and Intensity†	No. of Patients
Homogeneous	
3+	11
2+	4
1+	0
Heterogeneous	
3+	12
2+	2
1+	0
Focal	
3+	5
2+	0
1+	0
Total no. of evaluable specimens	34

*Distribution: homogeneous, > 75% of all cells stained; heterogeneous, 25-75% of all cells stained; focal, < 25% of all cells stained.

†Intensity scale: 0 = equal to background; 1 = weak; 2 = moderate; 3 = strong.

expression, and the results are shown in Table 8. A homogeneous distribution pattern of CanAg staining was observed in 15 patients (44%), whereas most patients had either heterogeneous (14 [40%] patients) or focal (five [14%] patients) staining. The 30 colorectal cancer patients exhibited a broad range of immunostaining distribution and intensity scores. Three patients with pancreatic carcinomas demonstrated homogeneous 3+, heterogeneous 2+, and focal 3+ immunostaining, and the single non-small-cell lung cancer patient had heterogeneous 3+ immunostaining. Relationships between CanAg immunostaining and the duration that patients remained on study were explored. Neither the distribution pattern nor the intensity of immunostaining was related to the number of cumulative courses.

One patient with accessible subcutaneous colon carcinoma metastases consented to a tumor biopsy 24 hours following cantuzumab mertansine infusion. Immunohistochemical staining of the tissue indicated 2+ homogeneous CanAg expression and

DM1 localization to the tumor using a murine monoclonal anti-DM1 antibody as depicted in Fig 6. This patient had evidence of a minor response of pulmonary metastases after two and four courses and received a total of six courses.

Antitumor Activity

No major objective responses were noted; however, two patients had minor responses. One patient, a 48-year-old female with metastatic colorectal carcinoma previously treated with FU and irinotecan, had a greater than 33% reduction in the size of a retroperitoneal lymph node following treatment with cantuzumab mertansine at 235 mg/m² and continued on treatment for a total of eight courses. The second patient, a 57-year-old female with metastatic colorectal carcinoma refractory to both FU and irinotecan, had a 36% reduction in the size of the lung metastases following cantuzumab mertansine treatment at the 235-mg/m² dose level and continued for a total of six courses. Four other patients had persistent stable disease exceeding 6 months. Seven patients experienced decrements of elevated carcinoembryonic antigen (CEA) values of greater than 30%, the most vivid of which was a 76% decrease after one course. Interestingly, five of 14 patients who had elevated CEA values pretreatment and were treated at the two highest dose levels, 235 and 295 mg/m², experienced decrements of at least 30% or greater. The possibility of cantuzumab mertansine interfering with the CEA radioimmunoassay was ruled out in comixing studies (data not shown).

DISCUSSION

Cantuzumab mertansine represents the first in a series of DM1-linked immunoconjugates currently in preclinical and early clinical development.^{10,11,13,14} Immunoconjugates represent novel delivery modalities for highly potent cytotoxic agents, particularly those that cannot feasibly be delivered via conventional systemic administration routes. The maytansinoid antimicrotubule agents exemplify such agents. Maytansine, although active at extremely low concentrations, exhibited too narrow a therapeutic index, with significant and sometimes unpredictable gastrointestinal toxic effects that precluded further clinical



Fig 6. Immunohistochemical detection for (A) CanAg expression (2+ homogeneous), (B) human IgG, and (C) DM1-huC242 (cantuzumab mertansine) in the tumor biopsy obtained 24 hours after the first infusion of cantuzumab mertansine.

development. Cantuzumab mertansine was selected for clinical development on the basis of the selective delivery of the potent antimicrotubule agent DM1, the impressive antitumor activity of this agent in CanAg-expressing human tumor cell lines and xenograft models at nontoxic doses, and the prevalence of CanAg expression in common human tumors.^{4,10}

The extrapolation of cantuzumab mertansine toxicology data from mice and monkeys to humans closely approximated the level at which clinically relevant toxicity was observed in this study. The starting dose for this phase I study, 22 mg/m², was equivalent to one tenth of the LD₅₀ in mice, which is a conventional algorithm for cytotoxic dose selection in phase I studies. In the current study, cantuzumab mertansine doses were escalated more than 10-fold before the MTD was reached and significant toxicity was noted. Also predicted from the preclinical toxicology studies, the principal toxic effects of cantuzumab mertansine were reversible elevations in hepatic transaminases and other nonhematologic gastrointestinal and neurosensory toxic effects. Although elevations of AST and ALT were common at the 235-mg/m² dose level, the increments were rarely severe. Furthermore, at dose levels of 176 mg/m² and below, the occurrence of hepatic toxicity was rare even in patients with significant preexisting hepatic metastases.

The etiology of the reversible transaminase elevations may be attributable to one or a combination of mechanisms. Hepatocytes do not express the CanAg antigen; therefore, a direct toxic injury secondary to cantuzumab mertansine binding to normal hepatocytes is unlikely. DM1, however, may possess intrinsic hepatotoxic properties. In the previous clinical phase I and II studies of maytansine, a pattern of transient, but also at times severe, hepatotoxicity was observed.⁵⁻⁹ Because cantuzumab mertansine is degraded into individual components, including the release, albeit in extremely low concentrations, of free maytansinoid into the systemic circulation, this may contribute, at least in part, to the transaminitis observed.

An alternative mechanism for the hepatic transaminase elevations may include cantuzumab mertansine localizing to hepatic metastases with a "bystander injury" to adjacent normal hepatocytes. The observation that patients with bulky hepatic metastases were at greatest risk for the development of this toxicity, even if they had normal or near normal transaminases at pretreatment, may support this bystander injury effect. Finally, hepatic transaminitis may represent a toxicity associated with the immunoconjugate "class" of antineoplastics. Reversible and sometimes severe hepatic transaminase elevations have been observed in clinical studies of other immunoconjugates, including gemtuzumab (calicheamicin-anti-CD33 immunoconjugate) and an antiB4-blocked ricin immunoconjugate, both of which target hematopoietic and lymphoid antigens not cross-reactive to hepatobiliary cells.¹⁵⁻¹⁷ Common to most therapeutic IgG immunoglobulins, despite widely different antigen targets, is their ultimate clearance mediated by the reticuloendothelial system. This pathway may result in the delivery of high concentrations of the immunoconjugate and the cytotoxic agent directly to hepatobiliary cells.

In addition to the hepatic toxic effects observed with cantuzumab mertansine, a constellation of nonhematologic manifestations that included nausea, vomiting, fever, and fatigue were observed at the two highest dose levels and precluded treatment

beyond the observed hepatic biochemical abnormalities. Nausea and vomiting, including delayed onset, occurred frequently in patients who experienced significant transaminase elevations, but it could be successfully managed, in most patients, with prochlorperazine and/or serotonin 5HT₃ receptor antagonists. The perturbations in hepatic function also may have contributed to fatigue reported in 65% of patients treated at the 235 mg/m² dose level and 100% of patients (including one grade 3 fatigue) treated at the 295-mg/m² dose level. Fever, although not severe, did occur in 35% and 33% of patients treated at the 235- and 295-mg/m² dose levels, respectively.

Hypersensitivity reactions were uncommon events with cantuzumab mertansine, and only three patients (8%) experienced hypersensitivity reactions. Furthermore, no patient's treatment was permanently discontinued because of a hypersensitivity reaction. Premedication with antihistamines and dexamethasone permitted retreatment with cantuzumab mertansine in all cases. These reactions may represent idiosyncratic reactions to the immunoconjugate because they occurred during the first course without prior sensitization to the agent. Moreover, the absence of detectable HAHA and HADA in all 37 patients, including the three patients with hypersensitivity reactions, and evidence of comparable clearance values of the immunoconjugate despite multiple courses of treatment, further supports the absence of clinically significant immunogenicity with cantuzumab mertansine. This is particularly noteworthy because huC242 is the first clinical evaluation of an antibody that was humanized via the variable domain resurfacing method.¹⁸

The degradation of cantuzumab mertansine occurs via cleavage of the disulfide linkage intracellularly and in the plasma, resulting in the progressive depletion of DM1 molecules eventually yielding the individual components of unconjugated antibody and "free" DM1. The free maytansinoid concentration, where measurable, was less than 1% of the total circulating maytansinoid and was readily cleared. There was a good correlation between the mean elimination half-life observed in the current study with that derived from preclinical animal studies. Although the plasma elimination half-life of 41.1 hours is longer than that of many conventional cytotoxic agents, it is considerably shorter than that reported for "naked" antibodies such as trastuzumab, rituximab, and the huC242 antibody product of cantuzumab mertansine.^{19,20} Because of the marked differences in clearances between the conjugated and unconjugated antibodies, the plasma concentration of unconjugated antibody eventually exceeds the immunoconjugate. Although there is a theoretical concern that cantuzumab mertansine's antitumor effect may be abrogated from the competition between the conjugated and unconjugated antibody for antigen sites, this does not occur in preclinical models examining this issue.²¹ Furthermore, abrogation would only occur when CanAg antigen-binding sites were near or totally saturated—an implausible situation, based on the density of CanAg expression on tumor cells.

The elevations of hepatic transaminases were a function of cantuzumab mertansine dose, peak plasma concentration (C_{max}), and to a lesser extent, $AUC_{0-\infty}$. One strategy to reduce the risk of peak dose- and peak plasma concentration-mediated hepatotoxicity while concurrently maximizing the total dose of cantuzumab mertansine delivered to CanAg-expressing tumor cells would in-

clude the evaluation of fractionated dosing schedules. With the elimination half-life of this agent (41.1 hours), frequent dosing even up to every other day would be predicted to be feasible. At present, phase I studies are examining cantuzumab mertansine administration either weekly or three times a week for 3 weeks every 28 days and will address whether this strategy is valid.

Because of the prevalence of CanAg expression in colorectal carcinomas, patients with this tumor type represented the vast majority of patients entered onto this study. Historically, colorectal carcinomas have demonstrated intrinsic chemotherapy resistance to the anticancer effects of antimicrotubule agents in general, and to maytansine in particular.^{9,22} Although no objective responses were observed with cantuzumab mertansine in this phase I study, two minor responses and persistent stable disease beyond 6 months in several patients was encouraging. Defining the optimal population to select for maximal therapeutic effect, however, remains a critical facet for the future clinical development of cantuzumab mertansine. Evidence from the current study demonstrates that a broad spectrum of CanAg expression exists between patients with similar histologic tumors, and also within an individual's tumor. Several lines of evidence indicate that the activity with this agent may be dependent on the distribution and intensity of CanAg expression within tumors. Mechanistically, the number of DM1 molecules delivered to an individual cell is dependent on the intensity of CanAg expression, and, plausibly, the optimal population for cantuzumab mertansine treatment will be those patients who express high

numbers of CanAg molecules on individual cells throughout their tumor (eg, homogeneous v heterogeneous or focal). The latter is supported by preclinical studies that indicate that mice bearing tumors with high CanAg expression were the most sensitive to cantuzumab mertansine treatment.^{4,10} Furthermore, clinical experience with trastuzumab indicates that clinical antitumor activity is dependent on target antigen expression within a tumor.²³ In the present study, only a minority of patients' tumors (32%) exhibited maximal CanAg expression (homogeneous 3+), which may have contributed to the absence of antitumor activity observed. Furthermore, on the basis of the aforementioned intrinsic resistance of colorectal carcinomas, studies should be initiated in CanAg-expressing tumors that also exhibit intrinsic sensitivity to antimicrotubule agents, such as non-small-cell lung, bladder, and uterine cancers. Identifying these favorable populations and tumor classes for cantuzumab mertansine treatment therefore remains an important next step for phase II clinical studies.

In conclusion, the immunoconjugate cantuzumab mertansine can be feasibly and safely administered to patients with advanced malignancies at doses that exceed those that portend antitumor activity in preclinical models. The preliminary biologic evidence of tumor localization, minor antitumor activity, and prolonged disease stability in patients with chemotherapy-refractory disease warrants broad clinical development of cantuzumab mertansine in CanAg-expressing malignancies.

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCION DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado
HUMERTO RUBIO CAMACHO

ASUNTO	Radicación	07-115803	
	Trámite	311	
	Evento	378	
	Actuación	519	1 0 5 0 0 0
	Oficio		
	Folios	06	

Patente de Invención	☒	Patente de Modelo de Utilidad	☐
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Vía PCT (fase nacional)	☒	Cap. I	☒	Cap. II	☐
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No. Sol. Internacional: PCT/US2006/014411
Fecha de Presentación Internacional: 14 de abril de 2006

Como resultado del examen de fondo, realizado con fundamento en el artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

Descripción:	Folios: 7 a 67	como se radicó inicialmente
	Folios: 113 a 188	como se modificó el 02/11/2007
Reivindicaciones:	Folios: 68 a 86	como se radicó inicialmente
	Folios: 189 a 211	como se modificó el 02/11/2007
Figuras:	Folios: 88 a 107	como se radicó inicialmente
Prioridad:	US 60/671.498,	15 de abril de 2005
	US 60/781.722,	14 de marzo de 2006

2. Objeto de la invención

Título de la invención:
Eliminación de población celular heterogénea o mezclada en tumores (folio 1).

El problema planteado en esta solicitud es la necesidad de proporcionar una mayor eficacia terapéutica de fármacos anticancerígenos citotóxicos.

Para solucionar este problema técnico la solicitud en estudio proporciona un método para eliminar una población celular heterogénea o mezclada AB, en donde A representa una población celular que expresa de manera selectiva una ligando y B representa una célula que carece del ligando expresado en A.

Clasificación Internacional de Patentes (IPC) es C07K16/46, A61K38/19, 39/395

3. De la solicitud de Patente

Reivindicaciones (artículo 30 D 486)

- El capítulo reivindicatorio presenta falta de concisión por cuanto presenta un elevado número de reivindicaciones (115), las cuales además no cumplen con el requisito de suficiencia ya que definen la materia objeto de la patente. La materia reclamada es tan amplia que no presenta sustento en la descripción, dado que no cualquier fármaco puede presentar los resultados descritos en la solicitud, por lo tanto es necesario restringir a los maitainoides evaluados. Así mismo, los enlazadores, sustituyentes y otros grupos químicos expresado en las reivindicaciones no aportan claridad al capítulo reivindicatorio y no permiten que un técnico medio en la materia pueda llevar a cabo la invención.
- Tampoco existe claridad en las reivindicaciones que definen los compuestos por su actividad, cada compuesto debe estar definido por su estructura química o secuencial.
- No existe concisión ni claridad entre las reivindicaciones independientes de método 1 y 57, dado que el preámbulo de cada una de estas reivindicaciones es totalmente diferente entre sí, lo cual no es correcto. El preámbulo normalmente coincide con el título de la invención y sirve de introducción informativa sobre el objeto que va a ser protegido. Contiene la información técnica conocida en el estado de la técnica del objeto que se pretende proteger. De acuerdo a lo anterior, la solicitud presenta diferentes objetos a ser protegidos, lo cual debe corregirse.

En la redacción de las reivindicaciones, se aconseja iniciar con la reivindicación menos limitativa, es decir más amplia, que contenga el menor número de características técnicas posibles pero sí suficientes para cumplir con el requisito de novedad, e incluir, una a una, dichas características limitativas en las reivindicaciones posteriores, que en cualquier caso serán dependientes de la primera. El grado de importancia de las características técnicas de la invención a los efectos de redacción de las reivindicaciones deberá determinarse en función del estado técnica más próxima a la invención. De esta forma podrá determinarse que características deberán incluirse necesariamente en la reivindicación principal y cuales podrán formar parte de las reivindicaciones dependientes.

En conclusión, el capítulo reivindicatorio no cumple con el requisito de claridad, sustento en la descripción y concisión de acuerdo con el artículo 30 de la Decisión 486.

4. Excepción a la patentabilidad (artículo 20 D486 literal a)-d))

La reivindicación 107 implica un método para eliminar células tumorales de un tumor. Dado que el método contempla el tratamiento in vivo, dicha reivindicación se encuentra dentro de la excepción a la patentabilidad, de acuerdo a los literales c) y d) del artículo 20 de la Decisión 486.

De lo anterior la reivindicación 107 no puede ser considerada patentable.

5. Reivindicaciones relativas a un uso o a un segundo uso (artículos 14 y 21 D 486)

La reivindicación 106 se refiere al método para utilizar la población celular reivindicada.

Por dicha razón, la reivindicación analizada no puede considerarse una invención y por lo tanto puede obtener patente.

6. Unidad de invención (artículo 25 D 486)

Esta invención presenta nueve diferentes conceptos técnicos inventivos así:

- Grupo I: Reivindicaciones 1-56. método para eliminar una población celular heterogénea o mezclada AS, en donde A representa una población celular que expresa de manera selectiva un ligando y S representa una célula que carece del ligando expresado en A.
- Grupo II: Reivindicaciones 108. Compuesto de la fórmula.
- Grupo III: Reivindicaciones 109. Compuesto de la fórmula.
- Grupo IV: Reivindicaciones 110. Un maintansinoide alquilado.
- Grupo V: Reivindicaciones 112. Lisina- N⁸-SMCC-DMI aislada.
- Grupo VI Reivindicaciones 113. Lisina- N⁸-SPDB-DM4 aislada.
- Grupo VII: Reivindicaciones 114. S-Cisteinil- DM4 ailado.
- Grupo VIII: Reivindicaciones 115. S-metil-DM4 aislado.

No existe un elemento técnico particular entre los diferentes compuestos y los compuestos pueden no ser obtenidos a partir del método reivindicado.

Se le sugiere al solicitante restringir la materia a patentar en un solo concepto común inventivo especificando el avance tecnológico sobre el estado de la técnica.

Esto se logra entre otras formas definiendo las características esenciales de la invención y mostrando las otras características particulares como dependientes, pero sin perjuicio de la concisión de las nuevas reivindicaciones tanto independientes como dependientes.

El solicitante también puede realizar una o más de las divisionales de acuerdo a los grupos de conceptos técnicos inventivos presentados en esta solicitud, esto claro, sin ampliar el objeto inicial de la invención.

7. Determinación del Estado de la Técnica

Considerando que la fecha de presentación de la primera solicitud de patente de la cual se reivindica prioridad es: 15 de abril de 2005, se buscaron documentos del estado de la técnica relacionados, con fecha de publicación anterior a esta.

Nº	Documento	Título	Fecha de publicación
D1	Art. 1 ¹	ERADICATION OF LARGE COLON TUMOR XENOGRAFTS BY TARGETED DELIVERY OF MAYTANSINOIDS	1996-08
D2	Art. 2 ²	Cantuzumab Mertansine, a Maytansinoid Immunoconjugate Directed to the CanAg Antigen: A Phase I, Pharmacokinetic, and Biologic Correlative Study	2003-01-15

¹ Art. 1. N L Kedersha, P D Ariniello, V S Goldmacher, J M Lambert, W A Blättler, y R V Chari. Proceedings of the National Academy of Sciences (PNAS), 1996. 93 (16) 8618-8623.
² Art. 2. Anthony W. Tolcher, Leonel Ochoa, Lisa A. Hammond, Amita Patnaik, Tam Edwards, Chris Takimoto, Lon Smith, Johann de Bono, Garry Schwartz, Theresa Mays, Zdenka L. Jonak, Randall, et.al. Journal of Clinical Oncology, Vol 21, N° 2 (Enero 15), 2003: 211-222.

D1 describe un medicamento maitansinoide DM1 plegado citotóxicamente desde 100 a 1000 veces mayores que los medicamentos corrientemente usados como anti cancerígenos. El inmuno conjugado C242-DM1 fue preparado por conjugación del DM1 en el anticuerpo monoclonal C242, el cual reconoce un tipo de mucina de glicoproteína expresado en varios tipos de cánceres monoclonales. Se encontró que el C242-DM1 es altamente tóxico hacia células cultivadas de cáncer de colon para un antígeno específico y muestra una eficacia remarcable in vivo (Resumen).

D2 describe un estudio para determinar la tolerancia máxima y la farmacocinética de cantuzumab mertansina, un inmuno conjugado del potente derivado maitansinoide (DM1) y un anticuerpo monoclonal humanizado (huC242) direccionado hacia el CanAg, en dosis intravenosas una vez cada semana y buscando evidencia de actividad antitumoral (Propósito).

8. Análisis de patentabilidad (según requisito) (artículo 14 D486)

Es importante mencionar que el siguiente análisis de patentabilidad se realizó teniendo en cuenta las objeciones de los numerales 3, 4, 5 y 6 de la presente comunicación, haciendo entonces el análisis para el primer grupo inventivo.

Novedad (artículo 16 D486)

Respecto a la reivindicación 1

La reivindicación 1 hace referencia a: Un método para eliminar una población celular heterogénea o mezclada AS, en donde A representa una población celular que expresa de manera selectiva un ligando y S representa una célula que carece del ligando expresado en A, el método que comprende:

- a) dirigir un conjugado de agente de enlace celular-fármaco a la población celular seleccionada A, caracterizado porque uno o más fármacos son enlazados de manera covalente al agente de enlace celular vía un enlazador que comprende un enlace disulfuro y el agente de enlace celular enlaza al ligando en la población celular A;
- b) internalizar el conjugado de agente de enlace celular-fármaco en las células que comprenden la población celular A;
- c) separar el fármaco desde el conjugado de manera que el fármaco experimenta una etapa de alquilación antes de eliminar las células en la población celular A; y
- d) liberar el fármaco alquilado desde la población celular A a fin de inducir la eliminación de células en la población celular B, eliminando de esta manera la población celular heterogénea.

Respecto la solicitud en estudio:

D1 muestra un método para tratar cáncer de colon rectal, que comprende administrar una cantidad efectiva de un medicamento maitansinoide, por ejemplo DM1, unido a un anticuerpo monoclonal C242 direccionado contra el cáncer humano de colon rectal (pág. 8619, 1ra. Columna, 3er. párrafo y pág. 8621). Con respecto a DM1, la referencia enseña que DM1 tiene una estructura como la mostrada en la figura 1 de la pág. 8619, el cual es un derivado de maitansinoide que tiene una N-metil-alanina que contiene un éster en la grupo hidroxilo C-3 del núcleo de la estructura; y además es unido a el anti cuerpo C242 por la vía de unión bisulfuro (pág. 8619, figura 1).

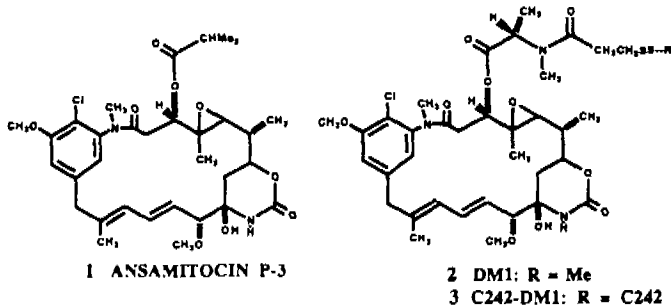
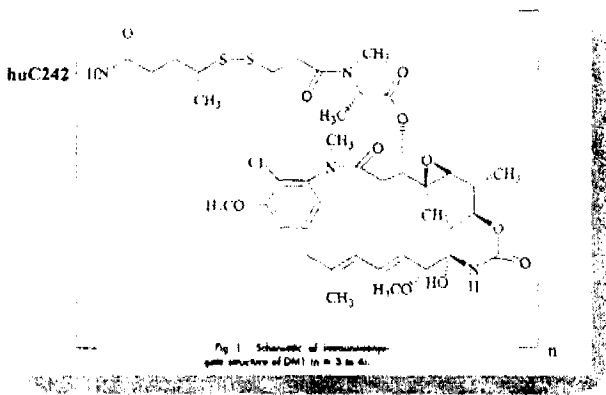


Fig. 1. Structural representation of Ansamitocin P-3 (compound 1), DM1 (compound 2), and C242-DM1 (compound 3).

El anticuerpo c242, en la referencia enseña que el anticuerpo se une a tumores específicos a CanAg-positivos (pág. 8618, columna segunda, último párrafo). Con respecto al cáncer colon rectal, la referencia enseña que tres líneas celulares del cáncer humano colon rectal, COLO 205, LoVo y HT-29 fueron inoculadas dentro del lado derecho de un ratón (pág. 8619, primera columna, y tercer párrafo).

D2 describe el tratamiento de pacientes que sufren de cáncer colon rectal, pancreático, entre otros, que comprende en administrarles una cantidad de cantusumab mertansina la cual es un inmuno conjugado que comprende DM1unido a un anticuerpo monoclonal tal como huC242 direccionado a CanAg (pág. 211, propósito, pág. 213, dosis y administración del medicamento y pág. 214 tabla 1). Con respecto a DM1, la anterioridad enseña que tiene una estructura como la mostrada en la figura 1(pág. 212), la cual es un derivado maitansinoide que tienen un N-metil-alanina que contiene un éster en el grupo hidroxilo del núcleo de la estructura, y además, es unido a el anticuerpo huC242 por la unión con en el bisulfuro (pág. 212, figura 1). Con respecto, a el anticuerpo huC242 la anterioridad enseña que el anticuerpo monoclonal humanizado se une específicamente por la afinidad a CanAg, con el cual es fuertemente expresado en la mayoría de los cánceres pancreáticos, biliares y colon rectales, entre otros. Por otra parte, enseña que una vez cantusumab mertansina es unido al dominio de CanAg, el complejo es internalizado y las moléculas de DM1 son liberadas intracelularmente por la hendidura de la unión bisulfuro (pág. 211, 1ra. Columna, 1er. Párrafo). Con respecto a los pacientes, la referencia enseña que la inmunohistoquímica de los tumores para CanAg revelaron que tienen una distribución repetida homogénea de especies de CanAg fueron observadas en 15 pacientes (44%), donde la mayoría de los pacientes tiene heterogéneos o focales especies (párrafo 219 y 220).



De acuerdo a lo descrito en las anterioridades D1 y D2 se observa que todas las características técnicas de un método para eliminar una población celular heterogénea o mezclada AS, en donde A representa una población celular que expresa de manera selectiva un ligando y S representa una célula que carece del ligando expresado en A, ya conocidos en el estado de la técnica.

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Dado lo anterior, el objeto de la invención recogido en la reivindicación 1, ha sido divulgado idénticamente en las anterioridades D1 y D2 por lo tanto esta reivindicación y sus dependientes no se pueden considerarse nuevas

9. Conclusión

Los requisitos de patentabilidad de la presente solicitud se encuentran afectados así:

Nº	Documento Nº	Reivindicaciones afectadas	Requisito que afecta
D1	Art. 1 ¹	1-56	Novedad
D2	Art. 2 ²	1-56	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de 60 días contados a partir de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única Nº 10 de 2001.



JOSÉ LUIS SALAZAR LOPEZ
Director de Nuevas Creaciones (C)

El anterior requerimiento fue notificado por fijación en lista, de fecha
_____ 01 ABR. 2007

CONCEPTO TÉCNICO Nº 844	EXAMINADOR TÉCNICO Código Nº 202090
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