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Targeting CD133 in an in vivo ovarian cancer model reduces ovarian cancer progression

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Abstract

Objectives—While most women with ovarian cancer will achieve complete remission after treatment, the majority will relapse within two years, highlighting the need for novel therapies. Cancer stem cells (CSC) have been identified in ovarian cancer and most other carcinomas as a small population of cells that can self-renew. CSC are more chemoresistant and radio-resistant than the bulk tumor cells; it is likely that CSC are responsible for relapse, the major problem in cancer treatment. CD133 has emerged as one of the most promising markers for CSC in ovarian cancer. The hypothesis driving this study is that despite their low numbers in ovarian cancer tumors, CSC can be eradicated using CD133 targeted therapy and tumor growth can be inhibited.

Methods—Ovarian cancer cell lines were evaluated using flow cytometry for expression of CD133. In vitro viability studies with an anti-CD133 targeted toxin were performed on one of the cell lines, NIH:OVCAR5. The drug was tested in vivo using a stably transfected luciferase-expressing NIH:OVCAR5 subline in nude mice, so that tumor growth could be monitored by digital imaging in real time.

Results—Ovarian cancer cell lines showed 5.6% to 16.0% CD133 expression. dCD133KDEL inhibited the in vitro growth of NIH:OVCAR5 cells. Despite low numbers of CD133-expressing cells in the tumor population, intraperitoneal drug therapy caused a selective decrease in tumor progression in intraperitoneal NIH: OVCAR5-luc tumors.

Conclusions—Directly targeting CSC that are a major cause of drug resistant tumor relapse with an anti-CD133 targeted toxin shows promise for ovarian cancer therapy.

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Conflict of interest statement

The authors declare that no competing interests exist.

Keywords

Ovarian cancer; CD133; Xenograft model; Cancer stem cells; Targeted toxin

Introduction

Although most women with ovarian cancer achieve complete remission with current treatment regimens, the majority of women relapse with chemoresistant disease [1,2]. Cancer stem cells (CSC) have been identified in many types of solid tumors as cells that are relatively quiescent, can self-renew, can grow as spheroids, and maintain the tumor by generating differentiated cells which make up the tumor bulk [3,4]. These characteristics, and the observation that CSC are resistant to conventional chemotherapeutics, suggest that CSC maybe the primary source of tumor recurrence. Thus, identification of strategies aimed at eliminating CSC in ovarian cancer could impact disease survival.

CSC markers vary depending upon the type of cancer studied and include: CD133, CD44, ALDH1, ALDH2, CD117, CD24, and ABCG2 [3,4]. In general, CSC comprise from 0.1 to 20% of the tumor [3]. Very few cancer cells that express these CSC markers are needed for a tumor to grow in vivo in NOD-SCID mice compared to tumor cells lacking CSC markers [4]. The quiescent nature of CSC allows them to resist standard chemotherapies which target rapidly proliferating cells. Furthermore, CSC have upregulated drug resistance genes [5] and express drug transporters, which allow CSC to “pump out” the chemotherapy [6].

CD133, a pentaspan membrane glycoprotein, has been identified as a CSC marker for various cancers [7,8]. Also known as prominin-1, CD133 was originally found on neuroepithelial stem cells in mice and later in human tissues [9]. The biological function of CD133 remains unclear, but it may be involved in primitive cell differentiation and epithelial-mesenchymal interactions [10–12]. Expression of CD133 in cancer-initiating cells is well documented for brain, prostate, colon, and breast cancers [13–17], and is indicative of a poor prognosis in many tumors [13,18]. Ovarian cancer cell lines and primary tumors have been characterized for the expression of CSC markers [5,19–29], with CD133 emerging as the most promising.

Targeted toxins serve as enzymatic inhibitors of protein synthesis [30] and represent a compelling alternative to conventional therapies since they synergize with chemotherapy [31–33]. Recently, we developed a monoclonal antibody (mAb) to a CD133 fusion protein that recognizes a non-glycosylated region of CD133 [34–36]. A derivative of the mAb was made [36] coupling the scFV from the mAb to a deimmunized PE-toxin using the endoplasmic reticulum retention sequence KDEL [37–39]. The toxin moiety of dCD133KDEL has been genetically deimmunized to permit multiple treatments with drug minimizing an anti-toxin response [40–46]. On a molecule/cell basis, targeted toxins are among the best killers of cancer cells when internalized [47]; CD133 serves as a highly internalized receptor when bound by ligand. We have shown that dCD133KDEL is reactive with CD133+ cells, is cytotoxic to cancer cell lines, and inhibits tumor growth in a mouse model system for head and neck cancer as well as breast cancer [48,49]. dCD133KDEL is the first such anti-CSC agent that shows remarkable anti-cancer effects despite the expression of CD133 in only a minority of the cancer cells.

In this paper, we study for the first time the ability of a CSC-directed drug to inhibit the growth and metastasis of human intraperitoneal ovarian carcinoma in vitro and in vivo.

Materials and methods

Cell lines

Human ovarian cancer lines NIH:OVCAR5, SKOV3, and A2780-s were obtained from Dr. Barbara Vanderhyden (Ottawa) [50,51], and MA148 was obtained from Dr. Ramakrishnan (University of Minnesota, Minneapolis) [52].

NIH:OVCAR5 cells were stably transfected with a vector containing the firefly luciferase (luc) gene, and a blastocidin resistance gene (Clontech Laboratories, Mountain View, CA), as previously described [49]. Transfection was initiated with Lipofectamine (Invitrogen, Carlsbad, CA) and stable clones were isolated using a FACS Diva flow cytometer (University of Minnesota Flow Cytometry Core Facility of the Masonic Cancer Center). NIH:OVCAR5-luc cells retained identical morphological and biological properties to the specific parental cell line and was maintained with additional 10 µg of blastocidin (InvivoGen, San Diego, CA).

mAb against CD133 (clone 7)

Clone 7 was the first mAb produced against the CD133 protein backbone, whereby mice were immunized with a fusion protein consisting of the two extracellular domains of CD133 and not the in-tracellular domain, as we have previously described [36]. In earlier studies, we had affirmed that mAb clone 7 was reactive with CD133 by transfecting cells that did not express CD133 with human CD133, and then performing flow cytometry binding studies [36].

Flow cytometry

Flow cytometry was performed using a FACSCaliber (University of Minnesota Flow Cytometry Core Facility). Cells were incubated with mAbs against CD133 (clone 7) [36], EGFR [53], the white blood cell marker CD45 (clone AHN-12; provided by Dr. Keith Skubitz, University of MN), and the B cell marker CD19 (clone BU-12) [54]. mAbs that are specific for CD45 and CD19 are used interchangeably as negative controls when performing flow cytometry experiments on carcinoma cell lines, since they both yield the same results. Data was analyzed using FLOWJO software (Tree Star Inc., Ashland, OR).

Construction and purification of dCD133KDEL

The construction of dCD133KDEL from the fusion of the scFV portion of the clone 7 mAb [36] and a deimmunized, truncated form of pseudomonas exotoxin 38, and its purification, have been previously described [48,49]. Briefly, the protein was expressed and purified from inclusion bodies using a Novagen pET expression system (Novagen, Madison, WI). Protein was refolded and then purified using ion exchange chromatography followed by size exclusion chromatography. Purity was determined by SDS-PAGE stained with Coomassie Brilliant Blue.

Time course viability assays

Trypan blue viability assays were performed by plating 10,000 NIH:OVCAR5 cells/well into 24-well plates as previously described [48,49]. dCD133KDEL, dCD19KDEL, and media were added at 1 nM and replaced daily. Cells were harvested from triplicate wells on days 2, 4, and 7 using trypsin and counted on a hemocytometer via trypan blue staining. Untreated wells typically became confluent around day 7 permitting the assessment of drug activity over time.

Mouse studies

Athymic nude mice (nu/nu) were purchased from the National Cancer Institute (Frederick Cancer Research and Development Center, Animal Production Area), housed in accredited, specific pathogen-free facilities at the University of Minnesota, and cared for by the Department of Research Animal Resources. Five to eight week old mice were injected intraperitoneally (i.p.) with 0.1 to 1 million NIH: OVCAR5-luc cells in PBS or saline, as indicated, on day 0 to initiate tumors. On day 3, mice were injected i.p. with dCD133KDEL, and then again multiple times during the week, as indicated. In Experiment 1, the drug was tested in male mice because of their larger size and the parameters for i.p. dosing of male mice had been optimized in our model of metastatic breast cancer [49]. In Experiment 2, the drug was tested for the first time in female mice.

Tumor growth was measured weekly in a minimally invasive manner using bioluminescent imaging. Mice were lightly anesthetized with isoflurane gas and received 100 μ l of a 30 mg/ml D-luciferin aqueous solution (Gold Biotechnology, St. Louis MO) i.p. 10 min before imaging to provide substrate for the luciferase enzyme. Images were captured using the Xenogen IVIS 100 imaging system and analyzed with Living Image 2.5 software (Xenogen Corporation, Hopkington, MA). All images represent a 2-min exposure time. Regions of interest (ROI) are expressed in units of photons/s/cm²/sr. Major relevant organs were removed and imaged in 6-well tissue culture plates lined with black tape. In each study, the individual body weights of the mice were measured since loss of body weight is a common adverse effect of most targeted toxins [55].

Histology

Organs that were excised from the mice following ex vivo imaging were fixed in 10% buffered formalin and sent to the University of Minnesota Cancer Center's Comparative Pathology Shared Resource for paraffin embedding. Representative slides were cut and stained with hematoxylin and eosin, and then examined for the presence of tumors.

Statistical analyses

Statistical analysis was performed using Prism 4 (Graphpad Software, San Diego, CA). Groupwise comparisons of single points were made by Student's *t*-test. *p*-Values <0.05 were considered significant.

Results

CD133 expression

The level of CD133 expression on the surface of the ovarian cancer cell lines was determined by flow cytometry. For NIH:OVCAR5, SKOV3, A2780-s, and MA148 ovarian cancer cell lines, the CD133 expression ranged from 5.6% to 16.0% (Fig. 1; top panel). The cells were also probed with negative control mAbs against CD45 (a marker for white blood cells) or mAbs against CD19 (a marker for B cells) which showed no reactivity against the four cell lines (Fig. 1; top panel). A representative analysis of NIH:OVCAR5 graphed as a dot plot (Fig. 1; bottom panel) shows a distinct population of CD133+ cells which was absent when the cells were stained with the mAb against CD19 or no antibody. Essentially all of the NIH:OVCAR5 cells expressed EGFR.

Time course viability assays show inhibition of proliferation

If CD133 marks CSC, then killing this minority subpopulation of cells should inhibit proliferation. To determine if this was the case, dCD133KDEL was added to cells in a trypan blue viability time course assay. Growth of the cells was significantly inhibited (*p* <

0.01) by the addition of dCD133KDEL at day 4 compared to cells that received no treatment or were treated with dCD19KDEL (Fig. 2).

In vivo studies in a human ovarian cancer mouse xenograft model

The human ovarian cancer cell line NIH:OVCAR5 has been shown to grow very well in nude mice and mimics the pattern of tumor growth seen in humans [50]. Coupling this with our flow cytometry data showing the existence of a CD133+ subpopulation, all subsequent experiments focused on NIH:OVCAR5 cells. We developed stably transfected luciferase-expressing cell lines by genetically altering NIH:OVCAR5 cells by transfection with a dual luciferase reporter gene.

To determine the effect of dCD133KDEL on animals with ovarian cancer, nude mice were injected i.p. with 500,000 NIH:OVCAR5-luc cells. Bioluminescent imaging was performed weekly to monitor tumor formation and response to treatment. In previous studies, dCD133KDEL was shown to be effective at inhibiting tumor growth in nude mice using human head and neck cancer cell lines as well as breast cancer cell lines [48,49]. In Experiment 1, male mice were used, since the parameters for i.p. dosing of male mice had been optimized in our model of metastatic breast cancer [49].

In Experiment 1, mice were injected with NIH:OVCAR5-luc cells, and treatment began 3 days later. A single course of treatment was one i.p. injection given four consecutive days a week. Mice M6–M10 were given 7 weekly courses of 20 µg/injection in 100 µl of saline; mice M6 and M7 were given 3 additional weekly courses of treatment. Five control mice that received no treatment (M1–M5) exhibited tumor masses (Fig. 3A) that remained large throughout the entire experiment, as shown by their bioluminescent images. In contrast, four of the five mice treated with dCD133KDEL responded to the treatment (Fig. 3B). Interestingly, one of the mice (M6) responded to dCD133KDEL after the 25-day image was taken; suggesting that dCD133KDEL can affect longer-term established tumors. Four of the mice treated with dCD133KDEL were relatively disease-free for over two months after treatment had ended, when the experiment was terminated. The bioluminescent values for each mouse at each time point were documented and shown to correspond to the digital images; i.e. the mice that were not treated showed high bioluminescent values throughout the entire 55-day period (Fig. 3C). Four of the mice that were treated with dCD133KDEL showed dramatic decreases in bioluminescent values throughout the entire 55-day period, while one mouse (M7) had a tumor that did not respond to treatment (Fig. 3D). The bioluminescent values for the four mice that responded to treatment with dCD133KDEL in Fig. 3D were significantly lower on day 32 ($p = 0.0006$), day 39 ($p = 0.0007$), day 55 ($p = 0.0004$), and day 95 ($p = 0.0071$) than the values for the mice that received no treatment (Fig. 3C). Weekly whole body weight measurements confirmed that dCD133KDEL at this dose was not toxic (Fig. 3F) when compared to the mice that did not receive treatment (Fig. 3E). Only two of the five mice that were not treated survived to day 95.

In another experiment, treatment began on day 3 after an i.p. injection of 100,000 NIH:OVCAR5-luc cells in 500 µl saline to female mice. A single course of treatment was one i.p. injection given five consecutive days a week, with whole body in vivo imaging performed weekly. Mice were given 5 weekly courses of 20 µg dCD133KDEL/injection in 100 µl of saline. The five control mice that received no treatment (M11 – M15) exhibited tumor growth which increased over time (Fig. 4A); mouse M13 succumbed to the tumor at day 20. In contrast, four of the five mice treated with dCD133KDEL responded to the treatment, with the tumors regressing by day 33 (Fig. 4B); again indicating that dCD133KDEL can affect longer-term established tumors. The bioluminescent values for each mouse at each time point were documented and shown to correspond to the digital images (Figs. 4C and D). The bioluminescent values for the four mice that responded to

treatment with dCD133KDEL in Fig. 4D were significantly lower on day 11 ($p = 0.0067$), day 18 ($p = 0.0084$), day 26 ($p = 0.0027$), and day 41 ($p = 0.0126$) than the values for the mice that received no treatment (Fig. 4C). Furthermore, treatment with dCD133KDEL did not have a substantial effect on the weight of the mice (Figs. 4E and F).

Mouse organs were removed on day 41 for ex vivo digital imaging (Fig. 5A). Representative digital images of the organs of the mice that responded to the dCD133KDEL treatment (M16 and M18) showed little if any bioluminescence, indicating that treatment slowed the spread of the tumor to peripheral organs including ovary (reproductive tract); in comparison to digital images of a representative of the untreated mice (M11) in which most of the organs contained bioluminescent tumor cells.

The organs of these mice were fixed in formalin and processed into paraffin blocks. Slides were stained with H&E and examined by light microscopy for the presence of NIH:OVCAR5 tumors. Tumors were observed in many of the organs of the mice that had not been treated (Fig. 5B). For example, a tumor was observed attached to the muscle of the colon wall. In another case, a tumor was found attached to muscle and appeared to have begun to penetrate the diaphragm. In both cases, the tumors were well established, vascularized, and lobular. In Fig. 5B, a vascularized tumor had attached to the exterior muscle of the small intestine and then penetrated through the wall. Tumors were also observed in the uterine horn and adjacent to the ovary in the untreated mice (not shown). In contrast, mice that had been treated with dCD133KDEL did not exhibit visible tumors in any of the organs examined, including the ovary.

Discussion

The original contribution of this study shows that, in an intraperitoneal ovarian cancer model, a drug targeting only 5% of ovarian cancer cells is capable of a robust anti-tumor effect. dCD133KDEL inhibited the progression of tumor growth after xenografted mice were given multiple i.p. injections of drug over a period of 4–6 weeks. At necropsy, ex vivo digital imaging of the major organs confirmed that tumors were less prevalent in those mice treated with dCD133KDEL compared to mice that were not treated. H&E stained slides of the same organs further verified that dCD133KDEL treatment inhibited tumor growth and progression in this mouse model for ovarian cancer. These results suggest that dCD133KDEL could serve as a novel treatment regimen for targeting CD133+ ovarian cancer and that it might be delivered intraperitoneally.

The findings in this model add to findings in head and neck cancer as well as breast cancer models, demonstrating that dCD133KDEL can effectively halt the progression of tumor growth. This raises an interesting possibility that targeting this established CSC marker may destroy the drug resistant root cells that fuel tumor recurrence. In ovarian cancer, as in many other cancers, the occurrence of carcinoma drug resistance and subsequent relapse is a major problem. CSC are notorious for their resistance to current chemotherapeutic agents [56–59]. dCD133KDEL is uniquely designed to target the cells that are responsible for drug resistance. First, as a targeted toxin, dCD133KDEL functions by a different mechanism than standard chemotherapy and second, dCD133KDEL selectively targets CSC. By selectively targeting CSC, dCD133KDEL may serve a very important role in tackling the problem of cancer recurrence when used in conjunction with current chemotherapies for ovarian cancer [60,61].

Some have suggested that tumor derived CD133+ cells may represent endothelial precursors [62], while others believe that CD44+/ckit+ cells are ovarian CSC [29]. Our data suggest that the elimination of CD133+ ovarian cancer cells with a targeted toxin designed to

selectively eliminate CD133+ cells results in long-term disease free tumor survivors, despite low levels of CD133+ expression. This same anti-CD133 scFV has been used to sort and eliminate tumor initiating cells in other models [48,49], providing further evidence that it is affecting CSC.

In this study we used dCD19KDEL as a negative control toxin, since we observed a subpopulation of CD133+ NIH:OVCAR5 cells (5.57%), while virtually no NIH:OVCAR5 cells expressed CD19 (a B cell marker). Thus, dCD133KDEL was effective at targeting the CD133+ cells, and due to the plasticity of CSC and their in vivo growth attributes, we believe that more CD133+ cells were generated during self-renewal. In contrast, since virtually all of the cells were CD19-, the dCD19KDEL toxin did not affect the in vitro or in vivo growth of the NIH:OVCAR5 cells.

This paper further indicates that peritoneal therapy may be a valid therapeutic strategy for toxin delivery. In another study using an intrasplenic MDA-MB-231-luc (breast cancer cell) model, we showed that i.p. administration of dCD133KDEL caused regression or inhibition of tumor growth in mice. The experiment was a model for metastatic disease [49]. A shortcoming of the nude mouse model is that it is highly artificial; but is currently the best model for evaluating efficacy of human drugs in vivo.

Data from Olin et al. [63] have shown that CD133 expression is dynamic and may be heterogeneous on CSC, such that a subset of CD133+ cells may be targeted by dCD133KDEL at any given time. They showed that the levels of CD133 in breast cancer cell lines increased in response to low O₂ (1% O₂ overnight or a gradual O₂ reduction over a period of a week) [63]. This suggests that our drug may work better in vivo than it does in vitro [63]. CD133 is also a glycosylated protein and changes in glycosylation may have an effect on tumor growth and recurrence, as well as the ability of dCD133KDEL to target the CSC within the tumor. The advantage of this mAb, which targets the non-glycosylated form of CD133, is that it may be able to target many forms of CD133, while other mAbs could not.

In this study, CD133+ expression ranged from 5.6% to 16.0% in the four ovarian cancer cell lines tested. Other groups have analyzed ovarian cancer cell lines for the presence of stem cell markers, and the level of CD133 expression ranged from 0.33 to 40% or more of the population [21,27]. Other putative CSC markers that were analyzed in these studies, such as CD44, ALDH1, and CD117, showed similarly variable, and sometimes high levels of expression in ovarian cancer cell lines [27]. In studies that have examined the expression of CD133 in primary ovarian cancer tissues and ascites, the levels of CD133+ cells ranged from 1.0–51.6% of cells [21,27,64]. Importantly, Steg et al., found that levels of CD133+ expression were higher in recurrent tumors than in matched primary tumor samples [64].

Our in vitro studies showed that treatment of NIH:OVCAR5 cells with dCD133KDEL caused a decrease in cell proliferation over time, compared to cells treated with dCD19KDEL or not treated. These results may be due to the killing of CD133+ cells by dCD133KDEL in combination with the lack of substantial proliferation by the CD133- cells. In previous studies using other carcinoma cell lines, we observed this same effect of dCD133KDEL on cell proliferation [48,49]. There are also reports in the literature of CD133- cells that become CD133+, and vice versa, as a function of time in culture and/or hypoxia [21,63], and the plasticity of the CSC phenotype is being recognized [65]. Many attributes of CSC still need to be elucidated; however, the nature of CSC may make it possible to substantially reduce or even eradicate tumor cells by targeting only CSC, even though they represent a minority population of cells.

Similarly, our in vivo studies showed that treatment with dCD133KDEL caused a decrease in ovarian cancer tumor growth. This effect is likely due to the targeting of CD133+ cells, since we have observed this effect in vivo in other xenograft model systems [48,49]. Previously, we showed that dCD133KDEL eliminated tumor initiating cells [48], and tumor initiation is considered a hallmark of CSC [66]. Also, the use of CD133+ cells as a marker for ovarian CSC is well established, and the findings that CD133+ ovarian cancer cells have CSC properties have been documented by others [23,27,64]. The results observed may be due to the killing of CD133+ cells by dCD133KDEL in combination with the lack of substantial proliferation by the CD133- cells. In previous in vivo studies, others have shown that tumors grow more rapidly when CD133+ cells are injected into animals, compared to when CD133- cells are injected [21,23]. The plasticity of CSC cannot be ruled out, and their ability to self-renew and differentiate is not fully understood [65–68]. As mentioned above, there are reports of CD133- cells that become CD133+, and vice versa, and this may occur in vivo as well. Furthermore, studies by Steg et al. have shown that primary ovarian cancer tumors contain 7% CD133+ cells, while tumors that were removed after recurrence of the cancer contained ~30% CD133+ cells [64]. Their studies would suggest that the CD133 profile increases over time.

Potential problems that have been attributed to targeted toxins, such as immunogenicity and toxicity, have been dealt with in the design of dCD133KDEL. Earlier studies show that by mutating 7 immunogenic epitopes, accounting for the majority of antibodies produced against this form of *Pseudomonas endotoxin*, there was reduced antibody production in animals [48].

Mice tolerate high doses of drug, but we may have approached the maximal tolerated dose, since we observed a slight decrease in weight of the female mice that received five consecutive doses of 20 µg of dCD133KDEL per week, for a total of over 20 doses, but not in the males. The male mice used in Experiment 1 were substantially larger (~32 g) than the female mice used in Experiment 2 (~22 g), which may have contributed to the males being more tolerant of the treatment. Drug will be given in future experiments based on meters squared since additional experiments will be needed to optimize the dose and dose schedule and determine toxicology. Nonetheless, we are encouraged since studies show that mice are an “on-target” model for dCD133KDEL [69] and they tolerate high doses of drug (single injections of 2 mg/kg). CD133 is expressed in several normal tissues in mice and humans; albeit, the level of expression of CD133 in normal tissues seems to be significantly lower than in tumors [49,70]. In addition, dCD133KDEL recognizes both human and mouse CD133, rendering it particularly useful for simultaneously determining its therapeutic potential and adverse reactions. Importantly, studies show that dCD133KDEL does not inhibit normal human hematopoietic progenitor cells [48].

In summary, dCD133KDEL can arrest the proliferation of ovarian cancer cells in vitro and in vivo. Future studies that are beyond the scope of this manuscript are planned to more fully elucidate the role of CD133 in ovarian cancer progression and recurrence. dCD133KDEL is a novel deimmunized toxin that appears to be targeting and eliminating CD133+ tumor-initiating cells, which some believe are CSC. dCD133KDEL is effective at preventing tumorigenesis and treating established tumors. Thus, dCD133KDEL can be used to study CSC populations and warrants further study as a potential adjunct to chemotherapy to prevent drug-resistant relapse in ovarian cancer patients.

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Abbreviations

dCD133KDEL	deimmunized pseudomonas exotoxin fused to anti-CD133 scFv with a KDEL terminus
aa	amino acid
Ab	antibody
CD19	cluster of differentiation 19
CD45	cluster of differentiation 45
ER	endoplasmic reticulum
FITC	fluorescein isothiocyanate
KDEL	Lys-Asp-Glu-Leu
mAb	monoclonal antibody
PE	pseudomonas exotoxin
photons/s/cm²/sr	photons per second per square centimeter per steradian
scFv	recombinant single chain VH and VL domain

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HIGHLIGHTS

- Cancer stem cells appear to be directly targeted by use of an antibody against CD133.
- An anti-CD133 targeted toxin, dCD133KDEL, shows promise for ovarian cancer therapy.
- dCD133KDEL inhibits growth of ovarian carcinoma in vitro and in vivo in a mouse model.

Cell Line	CD133	EGFR	CD45	CD19	No Aby
NIH:OVCAR5	5.57	98.5	ND	0.67	0.54
SKOV3	8.16	83.4	0.54	ND	0.36
A2780-s	16	1.43	0.21	ND	0.12
MA148	6.91	11.6	0.43	ND	0.21

Fig. 1.

Flow cytometry analysis of human ovarian cancer cell lines for CD133 expression. Four human ovarian cancer cell lines (NIH:OVCAR5, SKOV3, A2780-s, and MA148) were analyzed for the presence of markers on their cell surface by flow cytometry. Primary antibodies against CD133, EGFR, CD45, and/or CD19 were incubated with the cells, as described in the Materials and methods. No antibody (cells alone) was used as a control. Top panel: Percentage of cells positive for expression of each marker. Bottom panel: Flow cytometry data for the NIH:OVCAR5 cell line, showing dot plots for expression levels of CD133; EGFR, positive control; CD19, negative control; and No Aby, negative control.

Fig. 2. Trypan blue cell viability assay. NIH:OVCAR5 cells were incubated in 1 nM of dCD133KDEL (circle), 1 nM of dCD19KDEL (triangle), or media alone (square) for up to 7 days, as described in the Materials and methods.

Fig. 3. Effect of dCD133KDEL treatment on NIH:OVCAR5/Luc cells in nude mice. Whole body in vivo digital imaging of male mice that were (A) not treated or (B) treated with dCD133KDEL. Mice M6–M7 were given 10 weekly courses of treatment that ended on day 61, while mice M8–M10 were given 7 weekly courses of treatment that ended on day 45. Bioluminescence values of mice that were (C) not treated [M1, black square; M2, black triangle; M3, open circle; M4, black diamond; M5, black circle], or (D) treated with dCD133KDEL [M6, black square; M7, black triangle; M8, open circle; M9, black diamond; M10, black circle]. Average weight of mice that were (E) not treated or (F) treated with dCD133KDEL.

Fig. 4.

Effect of dCD133KDEL treatment on NIH:OVCAR5/Luc cells in nude mice. Whole body in vivo digital imaging of female mice that were (A) not treated or (B) treated with dCD133KDEL. Mice were given 5 weekly courses of treatment that ended on day 35. Bioluminescence values of mice that were (C) not treated [M11, black square; M12, black triangle; M13, open square; M14, black diamond; M15, black circle] or (D) treated with dCD133KDEL [M16, black square; M17, black triangle; M18, open square; M19, black diamond; M20, black circle]. Average weight of mice that were (E) not treated or (F) treated with dCD133KDEL.

Fig. 5. Effect of dCD133KDEL treatment on NIH:OVCAR5-luc cells in nude mice. (A) Ex vivo digital imaging of organs that were removed from female mice that were not treated (M11) or treated with dCD133KDEL (M16, M18). (B) Histopathology of tissues from mice. At day 41, organs were removed from mice that had been injected with NIH:OVCAR5-luc cells. FFPE blocks were stained with H&E and examined by light microscopy for the presence of NIH:OVCAR5-luc tumors. Tumors were observed in the colon, diaphragm, and small intestine of mice that were not treated, whereas tumors were not observed in mice that were treated with dCD133KDEL. Tumors are indicated by an asterisk. Bar = 1 mm.