Armed Antibodies Targeting the Mucin Repeats of the Ovarian Cancer Antigen, MUC16, Are Highly Efficacious in Animal Tumor Models

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Abstract

MUC16 is a well-validated cell surface marker for serous adenocarcinomas of the ovary and other gynecologic malignancies that is distinguished by highly repetitive sequences ("mucin repeats") in the extracellular domain (ECD). We produced and compared two monoclonal antibodies: one (11D10) recognizing a unique, nonrepeating epitope in the ECD and another (3A5) that recognizes the repeats and binds multiple sites on each MUC16 protein. 3A5 conjugated to cytotoxic drugs exhibited superior toxicity against tumor cells in vitro and in tumor xenograft models compared with antibody-drug conjugates of 11D10. Importantly, drug conjugates of 3A5 were well tolerated in primates at levels in excess of therapeutic doses. Additionally, the presence of circulating CA125 in a rat model did not exacerbate the toxicity of 3A5 drug conjugates. We conclude that targeting the repeat MUC16 domains, thereby increasing cell-associated cytotoxic drugs exhibited superior toxicity against tumor cells in vitro and in vivo without compromising safety. [Cancer Res 2007;67(10):4924–32]

Introduction

Epithelial ovarian adenocarcinomas constitute the largest class of gynecologic malignancies in the United States in terms of incidence and mortality and are the fifth leading cause of cancer deaths among women nationwide (1). In the 1980s, a serum antigen, CA125, was identified as a marker of these cancers, being elevated most notably in serous adenocarcinomas (2–4). Presently, CA125 has wide use as a marker for disease progression and response to therapy. Despite the long-standing importance of CA125 in the management of ovarian cancers, the gene encoding this antigen has only recently been described. CA125 is the extracellular domain (ECD) of a cell surface protein, MUC16, a member of the mucin family (5, 6). MUC16 has a single transmembrane domain and a short cytoplasmic domain. The ECD is predicted to comprise more than 14,000 residues. The most distinctive feature of the MUC16 ECD is tandem repeats of 156 amino acids each, referred to as mucin domains. Multiple proposed sequences have been reported; depending on the submitted sequence, there are at least 12 such repeats and possibly 60 repeats in tandem.

CA125 apparently enters the circulation by being shed from the full-length MUC16 at the cell surface. Antibodies against CA125 selectively bind ovarian carcinomas versus normal tissues, with expression levels the highest in serous adenocarcinomas (4). Immunohistochemistry and in vitro studies show surface expression of MUC16, a property that makes MUC16 an ovarian cancer target for antibody therapeutics, and indeed several such approaches have been explored (7).

Therapeutic antibodies that inhibit the proliferation of cancer cells on binding to surface antigens are used in the treatment of several malignancies (8). However, the targets for these antibodies are generally known to be critical for the progression of the disease, such that disrupting their functions would have a therapeutic effect. The role of MUC16 and/or CA125 in ovarian cancer pathogenesis is unknown nor does the antigen has a well-established physiologic function. If MUC16 expression is not crucial for ovarian cancer genesis or progression, an unmodified antibody against MUC16 may have little effect on the disease on binding to the cancer cells, although some therapeutic benefit has been attributed to antibodies binding to circulating CA125 and eliciting an immune response (9, 10).

An alternative antibody-based therapeutic approach involves conjugating cytotoxic drugs to antibodies such that the drug is stably associated with the antibody in circulation but is released when the antibody binds to the antigen-expressing tumor cell and is internalized and transported to lysosomes. This so-called "armed antibody" or "antibody-drug conjugate" (ADC) approach combines the powerful antiproliferative activities of chemotherapeutic small molecules with the targeting capabilities of antibodies (11–13). Importantly, disruption of antigen function is not required for activity; the target antigen need only be expressed at the plasma membrane and be internalized by the tumor cells. However, this technology may be limited by the inefficient delivery of the cytotoxic drug to its intracellular target, relative to systemic administration of the free drug. The number of antibody binding sites per cell and the rate of antigen internalization will dictate the amount of drug that can be delivered; an antibody that binds to multiple epitopes per target antigen would deliver more drug and possibly exhibit greater efficacy than an antibody that binds to a unique epitope on the target antigen. To test this hypothesis, we compared two monoclonal antibodies (mAbs) one (11D10) binding...
to a unique site on MUC16 and the other (3A5) binding to mucin repeats (therefore binding more sites per cancer cell than 11D10) and able to bind more avidly to CA125 (therefore subject to potential liabilities from binding to CA125 in circulation). We report here that 3A5 has superior in vivo and in vitro efficacy with no apparent deleterious effects from CA125 binding. We argue that the number of binding sites per cell can be a major determinant of the utility of armed antibodies.

Materials and Methods

Cloning. A MUC16 sequence comprising bases 41,587 to 43,816 at the 3’ end of NM_024690 and a sequence comprising several tandem mucin repeats (bases 38,551–40,607) were cloned separately from a pool of oligo(dT) primed human cDNA libraries (14). To generate MUC16TMshort, the 3’ clone was ligated into pRkkn neo encoding an NH2-terminal herpes simplex virus glycoprotein D epitope tag and a COOH-terminal FLAG tag, incorporating a NotI restriction site between the glycoprotein D and the MUC16 sequences. To generate the longer sequence (“MUC16TMlong”), the mucin repeats were ligated with MUC16TMshort using the NotI restriction site following the glycoprotein D epitope tag. For expression of soluble MUC16 ECD protein in baculovirus (“MUC16ECDshort”), bases 41,632 to 43,548 were inserted in a modified pVL1393 vector (BD Pharmingen) between a cleavable signal sequence and a COOH-terminal 8xHis tag.

mAb production. BALB/c mice were immunized with either MUC16ECDshort (mouse 11D10 or “mu11D10”) or commercially available purified CA125 (USBiological; murine 3A5 or “mu3A5”) resuspended in monophosphoryl lipid A/trehalose dicorynomycolate adjuvant (Bibi Immunochemicals). Three days after the final boost, popliteal lymph node cells were fused with cells derived from the murine myeloma cell line P3X63Ag.1 (CRL1397; American Type Culture Collection) using 50% polyethylene glycol. Hybridomas were selected using reverse transcription-PCR (RT-PCR). The forward primers were specific for the NH2-terminal amino acid sequence of the variable light and variable heavy region. Respectively, the light chain and heavy chain reverse primers were designed to anneal to regions in the constant light and constant heavy domain 1 that are highly conserved across species. Amplified variable light was cloned into a mammalian expression vector containing the human κ constant domain. Amplified variable heavy was inserted to a mammalian expression vector encoding the full-length human IgG1 constant domain. The chimeric antibodies (“ch3A5” and “ch11D10”) were transiently expressed as described (15).

Cells and antibodies. All cell lines were from the American Type Culture Collection and grown in the recommended medium unless otherwise indicated. OVCAr-3/luc cells were generated by stable transduction with a construct encoding firefly luciferase using retroviral transfer according to standard protocols. OVCAr-3/luc and P3C/MUC16 cells were maintained under 500 μg/ml G-418 selection. ADcs were made at Seattle Genetics, Inc. All secondary antibodies were from Jackson ImmunoResearch.

Flow cytometry analysis. Cells for flow cytometry analysis were resuspended with PBS + 5 mmol/L EDTA and washed with buffer consisting of PBS with 1% fetal bovine serum and 1 mmol/L EDTA. All subsequent steps were carried out at 4°C. Incubations of 5 × 10^6 cells were for 1 h each with 5 μg/mL primary antibody followed by the appropriate phycoerythrin (PE)-conjugated secondary antibody. Cells were then analyzed with a FACSCalibur flow cytometer (BD Biosciences).

Inhibition of in vitro proliferation. Proliferation in the presence of armed antibodies was assessed using cells plated at 2,000 per well in 50 μL of normal growth medium in 96-well clear-bottom plates. Twenty-four hours later, an additional 50 μL of culture medium with serial dilutions of armed antibodies were added to triplicate wells. Three (PC3) or 5 (OVCAr-3) days later, cell numbers were determined using CellTiter-Glo II (Promega Corp.) and with an EnVision 2101 Mutliabel Reader (Perkin-Elmer). To detect selective inhibition of in vitro proliferation (Fig. 4C), 3 × 10^5 OVCAr-3 cells were plated on each of three 100-mm dishes. Twenty-four hours later, cells received fresh medium containing 100 ng/mL antibodies in a total volume of 15 mL. Five days later, cells were resuspended and stained with mu11D10 and PE-conjugated goat anti-mouse secondary antibodies for flow cytometric analysis.

ELISA to estimate stoichiometry. Immunosorbtant plates (Nunc Immuno) were coated with 2 μg/ml of mu3A5 or mu11D10 in PBS for 1 h and blocked with Casein Blocker (Pierce Biotechnology) for 2 h. CA125 was diluted 183-fold with ELISA buffer (0.5% bovine serum albumin, 0.05% Tween 20 in PBS), and 100 μL/well (≈ 500 units/well) were added for 1 h at room temperature. Plates were washed with ELISA buffer, and serially diluted ch3A5, ch11D10, or a chimeric control IgG were added to wells containing either mu3A5- or mu11D10-captured CA125 for 1 h. Plates were washed with PBS containing 0.05% Tween 20, and the relative amount of bound chimeric antibody was determined with anti-human Fc horseradish peroxidase (Jackson ImmunoResearch) and TMB Peroxidase Substrate (KPL, Inc.).

Determination of antibody binding sites per cell (Scatchard analysis). The affinity constant and the number of cell surface binding sites for each antibody were estimated by incubating OVCAr-3 cells for 4 h on ice with a fixed concentration of 125I-labeled antibody combined with increasing concentrations of unlabeled antibody (16). The data were analyzed by nonlinear curve fitting using the New Ligand program, an in-house analysis method based on Ligand (17). In vivo efficacy. Efficacy studies were done using female CB17 ICR severe combined immunodeficient mice (Charles River Laboratories). All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (18).

To evaluate efficacy against i.p. tumors, mice were inoculated i.p. with OVCAr-3/luc cells (20 million cells per mouse). Bioluminescence was measured by injection with 5-luciferin at 250 mg/kg (Molecular Probes) and detection with an ultrasensitive cooled intensified charge-coupled device camera. Mice with similar bioluminescence signals 2 weeks after injection of 10 million cells into the right dorsal flank. When tumors showed signs of impending ulceration. Data collected from each experimental group were expressed as mean ± SE.

The PC3/MUC16TMlong efficacy model studies were done with s.c. injection of 10 million cells into the right dorsal flank. When tumors reached a volume of approximately 150 to 200 mm3, mice were separated into five groups of 10 each and dosed as described. Tumor volume was measured using calipers according to the following formula: \( V = \frac{4}{3} \pi (L A/B)^2 \), where \( A \) and \( B \) are the long and short diameters, respectively. Mice were euthanized before tumor volume reached 3,000 mm^3 or when tumors showed signs of impending ulceration. Data collected from each experimental group were expressed as mean ± SE.

The P3C/MUC16TMlong efficacy model studies were done with s.c. injection of 10 million cells into the right dorsal flank. When tumors reached a volume of approximately 150 to 200 mm3, mice were distributed into groups and dosed as described in Results. Nude rat OVCAr-3 toxicity model. All experimental procedures were done at Oncodesign (Dijon, France). NIH-OVCAr-3 ascitic tumors were serially transplanted in three steps into the peritoneal cavities of nude rats after whole body γ irradiation. Serum CA125 was determined using a standard ELISA assay. One hundred tumor-bearing nude rats were
randomized into five groups of 10 rats according to body weight and CA125 level (range, 16–2,290 units/mL). Twenty healthy nude rats were randomized in four groups of 5 rats according to body weight; these rats had no detectable circulating CA125. Rats received vehicle, drug-conjugated 3A5, or control ADC on treatment day 0. Blood sampling was done immediately before and 5 and 12 days after dosing.

Results

MUC16 is a type I transmembrane cell surface protein comprising between 12 (Genbank accession no. NM_024690) and 60 (Genbank accession no. AF414442) extracellular mucin repeats, in addition to nonrepeating sequence. The NM_024690 sequence is used as the reference for clones and constructs described below. Two approaches were taken to generate mouse mAbs against the ECD of MUC16. First, mice were immunized with a sequence encoding the juxtamembrane portion of MUC16 from the end of the major mucin repeats to the transmembrane domain (MUC16ECDshort; Supplementary Fig. S1). Among a panel of mAb, 11D10 exhibited the highest affinity for the antigenic protein. This antibody recognized a truncated form of MUC16 ectopically expressed in PC3 cells ("MUC16ETMshort"; Supplementary Fig. S1) but did not bind to cells lacking detectable MUC16 expression (Fig. 1A). 11D10 also binds to OVCAR-3 cells, a cell line that expresses high levels of cell surface MUC16 (Fig. 1C). By mutagenesis, we defined the 11D10 epitope within an 82-amino acid sequence C-terminal to the mucin repeat domain (data not shown).

The second panel of mouse mAbs was generated using purified CA125 from a commercial source as the immunogen to obtain antibodies recognizing the mucin repeats. Among this panel, mu3A5 was superior in terms of binding affinity and specificity for MUC16. This antibody interacted only weakly with the truncated MUC16 that was recognized by the first panel of antibodies. Insertion of five mucin repeats of 154 to 156 amino acids each NH2 terminus to MUC16ETMshort (creating MUC16ETMlong; Supplementary Fig. S1) allowed for the generation of a robustly binding, selective mAb.

### Table 1. Scatchard analysis of anti-MUC16 binding to OVCAR-3 cells

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Affinity (pmol/L)</th>
<th>Sites per cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>11D10</td>
<td>52</td>
<td>31,700</td>
</tr>
<tr>
<td>3A5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measurement #1</td>
<td>433</td>
<td>243,400</td>
</tr>
<tr>
<td>Measurement #2</td>
<td>288</td>
<td>107,800</td>
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conferred 3A5 binding (Fig. 1A). When tested for binding to cells stably expressing MUC16TMlong, 11D10 and 3A5 both gave flow cytometry shifts comparable with an antibody against the glycoprotein D epitope tag (Fig. 1A; data not shown). It is likely that both 3A5 and 11D10 bind once per copy of MUC16TMlong protein. Specificity of 3A5 for MUC16 was also shown by reduced binding to OVCAR-3 cells treated with small interfering RNA oligonucleotides targeting MUC16 message (data not shown). Assembly of the MUC16TMlong sequence introduced a non-MUC16 sequence (encoding [-SHLFTLGGR-]) between the repeats and the MUC16TMshort sequence. Subsequent to these studies, a construct encoding a continuous MUC16 sequence (amino acids 13,340–14,507 of NP_078996.2 encoded by NM_024690) was analyzed for 3A5 and 11D10 binding, yielding essentially identical results to those obtained with MUC16TMlong (data not shown).

MUC16TMlong encodes only a portion of the predicted full-length MUC16 sequence, particularly lacking the majority of the repeats (but including all of the unique COOH-terminal sequence). 3A5 binds much more extensively than does 11D10 to ovarian cancer cells that express the full-length MUC16 (Fig. 1A; data not shown). Scatchard analysis confirms that 3A5 binds to >3-fold more sites per OVCAR-3 cell than does 11D10, albeit with a lower apparent affinity (Table 1). $K_d$ values in Table 1 are consistent with $K_d$ values determined using a soluble substrate (data not shown). To determine if 3A5 recognizes multiple sites on MUC16, purified CA125 was captured onto plates coated with mu3A5 or mu11D10, and then the captured CA125 was interrogated with ch3A5, ch11D10, or an irrelevant antibody (“control”). Ch3A5 recognized CA125 immobilized by either mu3A5 or mu11D10, but ch11D10 only recognized CA125 immobilized by mu3A5 (Fig. 1B). Above 1 nmol/L, ch3A5 binding is ~8-fold greater than ch11D10 binding.
to CA125 immobilized by mu3A5, consistent with the Scatchard binding data on OVCAR-3 cells. This result shows that 3A5 binds to multiple epitopes on CA125 (therefore on MUC16), whereas 11D10 recognizes a unique epitope. Preliminary analyses have identified a sequence that is critical for 3A5 binding and is well conserved among the repeats and in mouse, rat, and primate MUC16 (data not shown).

Importantly, 11D10 and 3A5 both are specific for MUC16 and stain only cell lines, tumors, and normal tissues known to express significant levels of MUC16. Consistent with the stoichiometric data, 3A5 stains a higher proportion of human ovarian tumors and with greater intensity than 11D10 by immunohistochemistry (data not shown); for both antibodies, staining is membranous and is most prominent in serous adenocarcinomas, in agreement with published reports.

To evaluate if 11D10 and 3A5 antibodies could be used therapeutically, in vitro and in vivo studies were done using OVCAR-3 cells (19, 20). MUC16 expression in OVCAR-3 cells is typical of the expression in serous adenocarcinomas based on microarray, quantitative real-time RT-PCR, and immunohistochemical analyses (data not shown). The proliferation of OVCAR-3 cells in vitro and colony formation in soft agar are not sensitive to any unmodified anti-MUC16 antibodies among the 16 Genentech and commercial antibodies tested. To explore the drug-targeting potential of anti-MUC16 antibodies, 3A5 and 11D10 were conjugated to the auristatins monomethylauristatin F (MMAF) through a maleimidocaproyl (MC) linker (yielding Ab-MC-MMAF) and monomethylauristatin E (MMAE) through a cathepsin B-labile MC-[valine-citrulline]-para-aminobenzoate (“MC-VC-PAB”) linker (yielding Ab-MC-VC-PAB-MMAE, abbreviated “Ab-VC-MMAE”); refs. 21, 22). These drug-conjugated antibodies are designed to carry the cytotoxic payload in an inactive form until the antibody binds the target antigen at the cell surface, is internalized, and enters a degradative compartment, such as lysosomes, where active drug is released (23).

Proliferation of OVCAR-3 cells in vitro over 5 days was much more strongly inhibited by each 3A5-auristatin conjugate compared with the 11D10 conjugates (Fig. 2A). Irrelevant antibody conjugates were inactive against OVCAR-3 cells under these conditions (data not shown). The greater potency of the 3A5 drug conjugates is not due to a higher rate of transport to lysosomes, as 11D10 is internalized and delivered to lysosomes at least as efficiently as is 3A5 (data not shown), nor does 3A5 have a higher affinity for the target (Table 1). Rather, the greater activity of 3A5 conjugates is probably due to increased ADC binding per cell, resulting in increased overall delivery of drug into the cell. Indeed, 11D10 conjugates show slightly stronger activity versus 3A5 conjugates when added to the PC3/MUC16Tm long cells that bind the two antibodies to a similar degree (Fig. 2B).

OVCAR-432 (2) is a serous cystadenocarcinoma line that expresses MUC16, although less than OVCAR-3 (Fig. 3A). Additionally, the rates of internalization of 3A5 into these cells are comparable, with lysosomal staining apparent only after prolonged incubations (data not shown). Although the proliferation of OVCAR-3 cells is almost completely inhibited by 3A5-VC-MMAE in vitro (Fig. 3B) above 100 ng/mL, OVCAR-432 proliferation was incompletely inhibited even at the highest concentrations tested. When OVCAR-432 in a Petri dish was incubated with 100 ng/mL 3A5-VC-MMAE over 5 days, only ~50% of cells survived compared with untreated cells and cells incubated with an irrelevant MMAE conjugate (data not shown). The surviving OVCAR-432 cells were analyzed by flow cytometry with 11D10, revealing a selection for lower MUC16 expression (Fig. 3C). No selection for MUC16 expression was observed using the irrelevant conjugate (Fig. 3C) or unconjugated 3A5 (data not shown) nor with free MMAE at the approximate IC50 dose (data not shown). This result indicates that ADCs can be
Figure 4. In vivo efficacy of armed anti-MUC16 antibodies against OVCAR-3/luc mouse xenografts. Animals were dosed once weekly for four total doses after tumors were established and bioluminescence values were stabilized: dosing on days 0, 7, 14, and 21. All conjugates were dosed at 56 μg/kg drug, corresponding to 2.8 mg/kg 3A5-VC-MMAE, 2.3 mg/kg 3A5-MC-MMAF, 2.6 mg/kg 11D10-VC-MMAE, and 2.5 mg/kg 11D10-MC-MMAF. Irrelevant control Ab-VC-MMAE conjugate was dosed at an equivalent drug exposure.

A, tumor burden plotted as relative bioluminescence at day 0 (immediately before first dose), day 28 (1 wk after final dose), and day 63 (6 wks after final dose). LOD, limit of detection [1,000 relative luciferase units (RLU)]. Note the sustained elimination of tumors in the two 3A5 groups but not in other groups. B, Kaplan-Meier plot of survival of mice; symbols as in (A). 3A5 conjugates are significantly more efficacious than the corresponding 11D10 conjugates across both comparisons (P < 0.01). C, OVCAR-3 transplanted into the mammary fat pad: dosing at days 1, 8, and 15. Animals were dosed with 2 mg/kg 3A5-VC-MMAE (40.6 μg/kg drug = 118 μg/m² drug exposure; filled triangles and solid line), 6 mg/kg 3A5-MC-MMAF (133 μg/kg drug = 388 μg/m² drug exposure; filled squares and solid line), irrelevant VC-MMAE conjugate (118 μg/m² drug exposure; open triangles and dashed line), irrelevant MC-MMAF conjugate (388 μg/m² drug exposure; open squares and dashed line), or vehicle (PBS; filled circles and solid line).
selective for cells expressing high surface levels of target antigen \textit{in vitro}, sparing “neighboring” cells with lower or no antigen expression, and further underscores the role of antigen expression level in determining the activity of an ADC. Although in principle the MMAE released within higher expressing cells could diffuse into and kill the lower expressing cells, the absolute amount of free MMAE generated in this setting is presumably insufficient to produce an extensive “bystander” effect (24).

To determine if the superior \textit{in vitro} potency of 3A5 drug conjugates versus 11D10 conjugates would be retained \textit{in vivo}, we compared their efficacies in rodent tumor models. OVCAR-3 cells inoculated into the peritoneum of nude mice exhibit certain features in common with the human malignancy (20, 25). The cells proliferate within the peritoneum, form ascites, establish solid lesions on the peritoneal wall, and over time metastasize to the lung, liver, and heart (data not shown). Serum CA125 becomes elevated somewhat before the detection of metastases. Such an “OVCAR-3 i.p.” model would be an appropriate setting for testing anti-MUC16 ADCs. However, tumor burden is not easily measured in the peritoneum. To allow for measurement of tumor burden over time, OVCAR-3 cells were stably transduced with firefly luciferase (“OVCAR-3/luc”) so that bioluminescence on luciferin injection could be used as a surrogate reporter. Mice bearing OVCAR-3/luc tumors were dosed with 3A5 or 11D10 drug conjugates at a point when tumors were established and stable in the peritoneum, but metastases and overt signs of disease were absent. In preliminary studies, CA125 was markedly elevated in the peritoneum (>1,000 units/mL) and moderately elevated in the general circulation (<100 units/mL) at that stage. Both drug conjugate formats of 3A5 produced a reduction of bioluminescence (Fig. 4A) and a commensurate improvement in survival (Fig. 4B); the response as measured by bioluminescence correlated with life span. Two of the seven mice in the 3A5-VC-MMAE group had no bioluminescence at study termination and seemed to be free of tumor cells. In contrast, between the 11D10 conjugates administered at comparable doses, only the VC-MMAE format exhibited efficacy, producing a transient reduction in bioluminescence and a statistically significant survival benefit. A log-rank test showed significantly improved survival of 3A5 conjugates versus the corresponding 11D10 conjugates \((P = 0.002\) for VC-MMAE; \(P = 0.023\) for MC-MMAF). This study establishes drug conjugates of the repeat-binding 3A5 as more potent than the 11D10 antibody that binds to a unique site on MUC16. Conjugates of 3A5 are also better tolerated in normal Sprague-Dawley rats (data not shown).

The OVCAR-3/luc \textit{i.p.} model showed superior efficacy of 3A5-VC-MMAE compared with 3A5-MC-MMAF. To compare the relative efficacies of the two 3A5 drug conjugates in other models, we tested the conjugates in topical mouse xenograft models using the PC3/MUC16TMLong and OVCAR-3 cell lines. With weekly dosing at comparable drug exposure levels, 3A5-VC-MMAE most potently inhibited the growth of s.c. PC3/MUC16TMLong tumors (Supplementary Fig. S2A), achieving 100% complete responses at 3.73 mg/kg (220 \(\mu\)g/m\(^2\) drug). At 3.48 mg/kg (224 \(\mu\)g/m\(^2\) drug), 3A5-MC-MMAF had only a modest inhibitory effect on tumor growth. Using the PC3/MUC16TMLong \textit{s.c.} model (Supplementary Fig. S2B) and OVCAR-3 tumors grown in the mammary fat pads (Fig. 4C), similar efficacies were achieved with weekly dosing at 2 mg/kg 3A5-VC-MMAE (40.6 \(\mu\)g/kg drug = 118 \(\mu\)g/m\(^2\) drug exposure) and 6 mg/kg 3A5-MC-MMAF (133 \(\mu\)g/kg drug = 388 \(\mu\)g/m\(^2\) drug exposure). This result shows that appropriate dosing with 3A5 ADC can achieve eradication of xenograft tumors. Compared with vehicle-treated tumors, MUC16 expression seems to be unchanged by ADC doses that achieved partial tumor inhibition or in tumors that have returned after cessation of dosing, indicating that the drug-conjugated antibodies do not select against MUC16 expression in these models (data not shown).
Armed Anti-MUC16 Antibodies Are Efficacious

Preliminary safety assessments in Sprague-Dawley rats and cynomolgus monkeys showed that 3A5-MC-MMAF is better tolerated than 3A5-VC-MMAE. Of note, immunohistochemistry with 3A5 showed that the expression of MUC16 in normal tissues of cynomolgus monkeys is quite comparable with the normal human tissue expression, with antigen detected in bronchus and fallopian tube. However, toxicity due to damage to these organs by either 3A5 drug conjugate was not observed on histopathologic examination and was not suggested by changes in hematology or serum chemistry variables. The safety findings in primates were not confounded by any detectable host immune response to the 3A5 drug conjugates. In both species, the most significant toxicity was transient neutropenia produced by supraeficacious doses of 3A5-VC-MMAE (6.2 mg/kg = 95.9 μg/kg = 1,100 μg/m²; Supplementary Fig. S3; data not shown). The neutropenia is likely a target-independent toxicity, as MUC16 is not expressed on granulocytes.

The normal rat and monkey models do not present circulating CA125 as would be the case in ovarian cancer patients. An antibody such as 3A5 that binds to repetitive epitopes of a circulating antigen might be distributed and metabolized differently than an antibody that remains unbound in circulation. In the case of an armed antibody, this could in principle yield increased toxicity specifically in major organs of clearance, such as the liver. To evaluate the safety effect of drug-conjugated 3A5 binding to circulating CA125, nude rats harboring serum CA125 (range, 16–2,290 units/mL; median, 57 units/mL) due to peritoneal OVCAR-3 tumors were dosed with 3A5-VC-MMAE at two dose levels (685 and 2,056 μg/m² drug exposure) or with an irrelevant Ab-VC-MMAE conjugate (2,056 μg/m² drug exposure). Rats were evaluated for changes in body weight, hematology, and blood chemistry; data showing leukopenia (Fig. 5B), elevation of liver enzymes in the serum (Fig. 5B), and thrombocytopenia (Fig. 5C) are shown. The toxicity due to the higher dose of 3A5-VC-MMAE did not seem to be related to the circulating CA125, as the control conjugate (not binding to CA125) was at least as toxic. In addition, the rats tolerated the lower dose of 3A5-VC-MMAE, which would remain in excess of CA125 and form as much immune complex as the higher dose (data not shown). Histopathologic examination failed to reveal any lesions that could be attributed to 3A5-VC-MMAE exposure in CA125-positive rats (data not shown). This result suggests that toxicity from circulating 3A5-CA125 complexes will not be limiting for armed 3A5 therapy. The selection of the VC-MMAE or MC-MMAF format for further study will hinge on more precise determinations of relative safety and efficacy.

Discussion

Armed antibodies have great promise as cancer therapeutics by delivering a cytotoxic payload specifically to the tumor cells that overexpress target antigens at the cell surface (11). In principle, the level of cytotoxic drug delivered to its molecular target (e.g., microtubules for auristatins) inside the tumor cell is a function of the flux of antibody into the cell per unit time, which in turn is determined by antigen copy number and rate of turnover. For most targets, this mechanism of drug delivery will likely be much less efficient than systemic delivery of the free cytotoxic drug, although more specific for the tumor cells (and so presumably safer). To achieve sufficiently high levels of cytotoxic drug inside the tumor cells, either the target antigen should be rapidly turned over or the absolute level of surface antibody binding sites should be high.

MUC16 is a slowly internalizing cell surface protein that is expressed in a large proportion of ovarian cancers more strongly than in any normal human tissues. The absolute levels of MUC16 expression on relevant cell lines, such as OVCAR-3, are rather modest, possibly compromising the efficacy of anti-MUC16 ADC. Indeed, when we evaluated mAb 11D10 against a unique epitope of MUC16, the drug-conjugated antibody exhibited only partial activity against OVCAR-3 cells in vitro and in OVCAR-3 xenograft models. To increase cytotoxic drug delivery, we developed mAb 3A5 against the mucin repeats of MUC16, allowing multiple antibody binding per MUC16 molecule. Flow cytometry and Scatchard binding analyses showed increased binding of 3A5 to OVCAR-3 cells versus 11D10, a property also reflected in the superior staining of ovarian cancer tissues by 3A5.

As predicted from binding studies, armed 3A5 was much more potent than armed 11D10 in vitro and in vivo. Significantly, in a model expressing endogenous MUC16 and generating CA125, armed 3A5 achieved partial and, in some cases, complete responses conferring a marked survival advantage to the 3A5 treatment groups versus 11D10 groups and controls. Both 3A5 drug conjugates produce antitumor activity at doses that are well tolerated by rats and nonhuman primates, with transient neutropenia after exposure to 3A5-VC-MMAE being the primary toxicity. 3A5-VC-MMAE has been more efficacious than 3A5-MC-MMAF in multiple xenograft tumor models, whereas 3A5-MC-MMAF is better tolerated. 3A5 drug conjugates have exhibited excellent activity with dosing every 3 weeks (data not shown), indicating that these conjugates can be dosed with a schedule that achieves efficacy while allowing recovery from neutropenia. In our in vivo models, we have not observed any toxicities that could be attributed to armed 3A5 binding to circulating CA125. In addition, a phase I trial evaluating drug-conjugated antibodies against MUC1 did not find any correlation of toxicities with the levels of circulating MUC1 ECD (26).

Epithelial ovarian cancers are frequently diagnosed at a late stage. Although most patients respond initially to chemotherapy (commonly paclitaxel combined with a platinum drug), the majority will relapse and experience diminishing responses to therapy, with a poor prognosis. Treatment of this disease would benefit greatly from a novel therapeutic modality. The impressive efficacy of armed 3A5 in rodent tumor models, combined with the selectivity of the antibody for MUC16 and its consequent relative safety in rats and nonhuman primates, makes this strategy a promising approach for the treatment of epithelial ovarian cancers. The preclinical data suggest an encouraging therapeutic index, and those patients who might benefit from armed 3A5 would be readily identified by the presence of circulating CA125 and/or immunohistochemical detection of MUC16. We are presently extending these studies to other models and evaluating the effects, if any, of checmotherapeutic resistance.

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References


Correction: Armed Anti-MUC16 Antibodies Are Efficacious

In the article describing the efficacy of anti-MUC16 antibody-drug conjugates in ovarian cancer models in the May 15, 2007 issue of Cancer Research (1), an author, Sarajane Ross, was inadvertently omitted from the author list. The correct author list should read as follows:

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Armed Antibodies Targeting the Mucin Repeats of the Ovarian Cancer Antigen, MUC16, Are Highly Efficacious in Animal Tumor Models

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