

# A novel human monoclonal antibody that binds with high affinity to mesothelin-expressing cells and kills them by antibody-dependent cell-mediated cytotoxicity

Yang Feng,<sup>1</sup> Xiaodong Xiao,<sup>1</sup> Zhongyu Zhu,<sup>1,2</sup> Emily Streaker,<sup>1</sup> Mitchell Ho,<sup>3</sup> Ira Pastan,<sup>3</sup> and Dimiter S. Dimitrov<sup>1</sup>

<sup>1</sup>Protein Interactions Group, Center for Cancer Research Nanobiology Program, National Cancer Institute-Frederick, NIH and <sup>2</sup>Basic Research Program, Science Applications International Corporation-Frederick, Inc., National Cancer Institute-Frederick, Frederick, Maryland; and <sup>3</sup>Laboratory of Molecular Biology, CCR, National Cancer Institute-Frederick, NIH, Bethesda, Maryland

## Abstract

Mesothelin is a potential new target for cancer immunotherapy because it is present at relatively low levels only in mesothelial cells of pleura, peritoneum, and pericardium of healthy people, but is significantly elevated in a number of tumors, including mesothelioma, ovarian, pancreatic, and lung cancers. However, all currently available antibodies against mesothelin are either murine or chimeric, which could limit their use because of increased likelihood of immunogenicity compared with fully human antibodies. Here, we report the identification and characterization of a novel fully human monoclonal antibody, m912, which was isolated from a human Fab library by panning against recombinant mesothelin. This antibody in scFv, Fab, and IgG1 formats bound specifically and with high affinity (equilibrium dissociation constant in the nmol/L range) to cell surface-associated human mesothelin and to recombinant mesothelin. It specifically lysed cancer cells engineered to express mesothelin in the presence of peripheral blood mononuclear cells isolated from healthy donors most likely by antibody-dependent cellular cytotoxicity. M912 is the first

reported fully human monoclonal antibody to mesothelin, which has potential for cancer treatment and diagnosis. [Mol Cancer Ther 2009;8(5):1113–8]

## Introduction

Mesothelin was identified as antigen to an antibody Mab K1, which specifically recognized several ovarian cancers (1). It is encoded as a 628-amino acid glycoprotein and cleaved by furin into a membrane-attached 40-kDa form, mesothelin, and a smaller form released from cells (2). Mesothelin is attached to cell surface through glycosyl-phosphatidyl inositol link to its carboxyl terminus. Presently, limited knowledge about its function is available. Mice with both copies of mesothelin genes inactivated seem to have normal growth and reproduction ability (3). It has been reported that mesothelin interacts to CA125 (or MUC16), an ovarian cancer antigen, and the interaction may play a role in metastasis of ovarian cancers to the peritoneal cavity (4, 5). The downstream signals activated by the interaction of CA125 are still not clear.

The unique distribution pattern of mesothelin in human bodies suggests its potential as a cancer target. In healthy people, mesothelin expression is limited to mesothelial cells lining the pleura, peritoneum, and pericardium. Other normal tissues tested do not express mesothelin protein (1). However, mesothelin is overexpressed in a high percentage of ovarian cancers, pancreatic cancers, non-small lung cancers, and mesothelioma (6–8). It has been reported that a majority of serous carcinomas of the ovary and adenocarcinomas of the pancreas express high levels of mesothelin (9). In addition, high levels of mesothelin have been detected in >55% of lung cancers and >70% ovarian cancers (7, 10). In mesothelioma patients, mesothelin protein is not only readily detectable on tumors, but it is also present in patient serum (11). Furthermore, the mesothelin-positive lung cancer cells die upon exposure to a recombinant immunotoxin targeted to mesothelin (10). Because of its limited distribution in normal tissues and elevated expression in cancers, mesothelin has been considered as an excellent target for cancer therapy. Various methods have been used to deliver cytotoxic drugs to mesothelin-positive cells or elicit cell-mediated and humoral responses to mesothelin and, in turn, eliminate tumors. DNA vaccines against mesothelin have been shown to inhibit tumor growth in a mouse model (12, 13). A fusion protein of mesothelin-specific single chain and immunotoxin (SS1P) is currently in phase I trial (14). A chimeric monoclonal antibody specific to mesothelin, MORAb-009, is being tested in a phase I trial. In a xenograft model, MORAb-009 synergizes with chemotherapy drugs Taxol and gemcitabine, although it has little effect when used

Received 9/30/08; revised 1/9/09; accepted 2/19/09; published OnlineFirst 5/5/09.

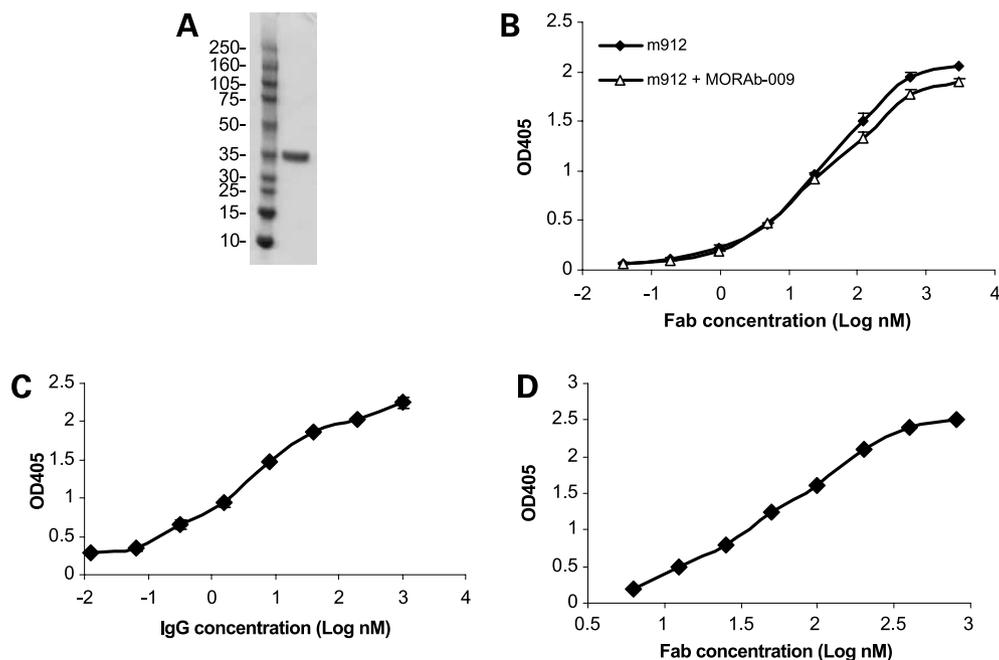
**Grant support:** Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, and by federal funds from the National Cancer Institute, NIH, under contract N01-CO-12400.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

**Requests for reprints:** Yang Feng, Center for Cancer Research Nanobiology Program, National Cancer Institute-Frederick, NIH, Bldg 469, Rm 139, Frederick, MD 21702-1201. Phone: 301-846-5629; Fax: 301-846-5598. E-mail: yfeng@ncifcrf.gov and Dimiter S. Dimitrov, Center for Cancer Research Nanobiology Program, National Cancer Institute-Frederick, NIH, Building 469, Room 150B, Frederick, MD 21702-1201. Phone: 301-846-1352; Fax: 301-846-5598. E-mail: dimitrov@ncifcrf.gov

Copyright © 2009 American Association for Cancer Research.

doi:10.1158/1535-7163.MCT-08-0945



**Figure 1.** High affinity binding of Fab m912 and IgG1 m912 to recombinant mesothelin. **A**, a sample of recombinant human mesothelin purified from insect cell supernatant was run on 4% to 12% NuPAGE Bis-Tris gel. Molecular weight standards are in kDa. **B**, binding of Fab m912 to recombinant mesothelin coated on ELISA plate (—◆—). In competition ELISA, 3 nmol/L MORAb-009 was added to all Fab m912 solutions (—△—). **C**, binding of IgG1 m912 to recombinant mesothelin on ELISA. **D**, binding of Fab m912 to mouse recombinant mesothelin on ELISA.

alone in these models (15). Given the potential of targeting mesothelin as an effective treatment for mesothelin-positive tumors, a fully human therapeutic antibody could provide additional options in terms of immunogenicity and better tolerance. Here, we describe a high-affinity fully human mesothelin antibody with a potential as a cancer therapeutic.

## Materials and Methods

### Cell Cultures

A431 cells, human epidermoid carcinoma cells, were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and penicillin/streptomycin (complete growth medium). A431 cells do not express mesothelin. H9 cells were stable clone cells established from A431 cells that have been transfected with a vector carrying full-length mesothelin cDNA. H9 cells were maintained in complete RPMI1640 growth medium supplemented with 0.75 mg/mL G418. OVCAR-3 cells were purchased from American Type Culture Collection and maintained in RPMI1640 complete growth medium.

### Expression of Recombinant Mesothelin Protein

Human mesothelin fragment including amino acids 296 to 600 (the numbers are based on sequence in AY743922 in the National Center for Biotechnology Information database) was cloned from pcDNA3.2 to a baculovirus transfer vector pAcGP67 through Sma I and Not I sites. The recombinant product had extra residues ADPG on the NH<sub>2</sub> terminus and 6 histidines on the COOH terminus. It was cotransfected with BaculoGold viral DNA into SF9 insect

cells according to the manufacturer's instruction. Mesothelin protein was purified from conditioned medium with a nickel-chelating column, and further polished with a Superdex75 gel filtration column in PBS. Purity of mesothelin was examined with SDS-PAGE.

### Antibody Selection by Phage Display

Purified mesothelin was labeled with biotin first and used for panning of a human naïve Fab phage library (16). Briefly, amplified phage ( $\sim 10^{12}$  plaque-forming unit) preabsorbed with MyOne streptavidin T1 beads (Invitrogen) was incubated with 4  $\mu$ g of biotin-mesothelin for 2 h. Specific phages were captured by fresh streptavidin beads. After extensive washes of the beads with PBS+0.05% Tween 20, phage was rescued by exponentially growing TG1 bacteria and helper phage. Pannings were repeated for three more times with more stringent washes at the latter two rounds. Three hundred colonies were picked from the last two rounds of panning and rescued with helper phage for screening. Two unique clones were selected, of which m912 had higher affinity and was selected for further characterization.

### Antibody Expression and Purification

The Fab fragment was expressed in HB2151 cells as described previously (17). A single chain form of m912 was made by cloning VH and VL from Fab, connected by linker 3(GGGGS), into pComb3x (18). Expression and purification of scFv were similar to that of the Fab. Fab was converted into IgG1 by subcloning the heavy chain variable region and the light chain into pDR12. Free Style 293 cells were transfected with pDR12-m912, and IgG was secreted into medium. Fab and IgG were purified

with a protein G column, and scFv was purified with a nickel-chelating column. All preparations were dialysed against PBS.

#### ELISA Binding Assay

Antigen (mesothelin) diluted in PBS is coated on narrow-well 96-well plate at 50 ng per well overnight at 4°C. Wells are blocked with 100  $\mu$ L of 4% milk/PBS for 1 h at 37°C. For Fab binding kinetics, Fab is titrated from 3,000 to 0.038 nmol/L (1:5 serial dilutions). Diluted Fab (50  $\mu$ L) is added to duplicate wells. In competition ELISA, designated concentration of competing IgG was included in all Fab solutions. After 2 h of incubation at 37°C, the wells are washed with PBST (PBS+0.05% Tween 20) for 4 times. Bound Fab was detected with anti-FLAG-horseradish peroxidase monoclonal antibody (1:1,000; Sigma) for 1 h at 37°C. Wells are washed again with PBST. Substrate ABTS is added (50  $\mu$ L per well), and the reaction is read at 405 nm. For ELISA with IgG, a goat anti-human Fc IgG conjugated with horseradish peroxidase was used at 1:1,000.

#### Flow Cytometry

A413 cells and H9 cells were detached by cell dissociation buffer and rinsed in PBS. Aliquots of cells were incubated with primary antibody (m912, or isotype controls) at indicated concentrations in 250  $\mu$ L of RPMI+10% fetal bovine serum for 1 h on ice. Unbound antibodies were washed away with medium. Secondary antibody goat anti-human IgG conjugated with FITC (Sigma) was incubated with cells at 8  $\mu$ L/mL for 30 min. For detection of Fab or scFv, 1.6  $\mu$ g/mL of anti-His6 monoclonal antibody (Qiagen) and 8  $\mu$ L/mL of goat anti-mouse IgG-FITC (Sigma) were incubated with cells. Cells were washed and resuspended in PBS +0.5% bovine serum albumin for flow cytometry on FACS-Calibur (Becton Dickinson).

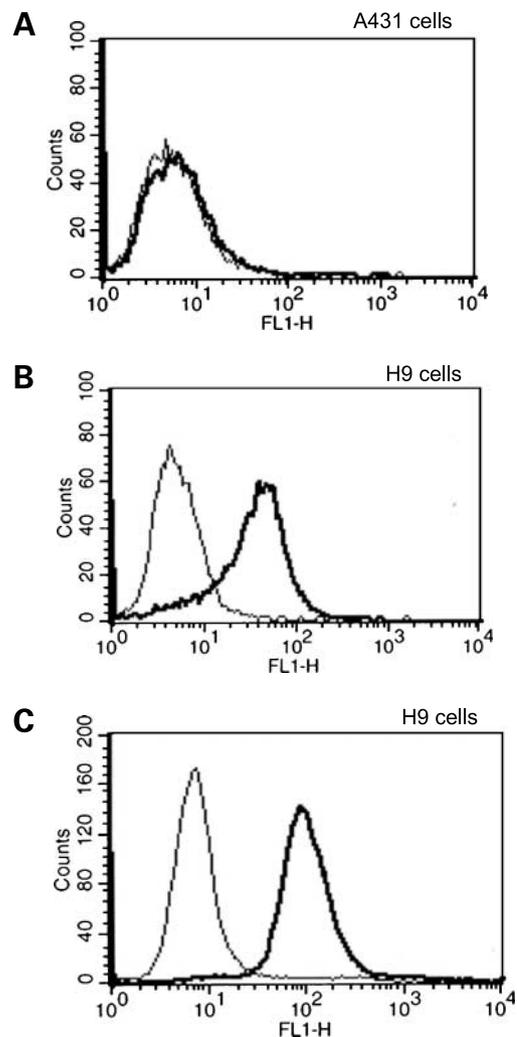
#### Cell Lysis by Antibody-Dependent Cell-Mediated Cytotoxicity

Peripheral blood mononuclear cells (PBMC) were isolated from healthy donors with Ficoll-Paque Plus (GE Healthcare). Collections of blood from donors were approved by National Cancer Institute-Frederick Research Donor Program. The viability of isolated cells was >95%. PBMC were seeded in a 96-well plate in RPMI+10% fetal bovine serum at 500,000 cells per well. Cells were incubated at 37°C and allowed to attach to the plate for 3 h. Unattached cells were rinsed off by two washes of warm PBS; cells attached in the wells were used as the effector cells. Target cells, A431 or H9 cells, were trypsinized and resuspended into single-cell suspensions. The target cells were incubated with various concentrations of antibody at room temperature for 30 min then added to effector cells at 10,000 cells per well. The ratio of effector and target cells is 50:1. The plate was centrifuged at 300  $g \times 5$  min and incubated at 37°C for 24 h. Supernatant (100  $\mu$ L) was transferred to an all-white plate, and 100  $\mu$ L of CytoTox-ONE reagent (Promega) were added to each well. The lactate dehydrogenase released from lysed cells converted CytoTox substrate to fluorescent resazurin, which was measured in fluorometer (Ex 560 nm/Em 590 nm). The percentage of specific lysis was calculated as following: (experimental treatment – effector cell control)/(high con-

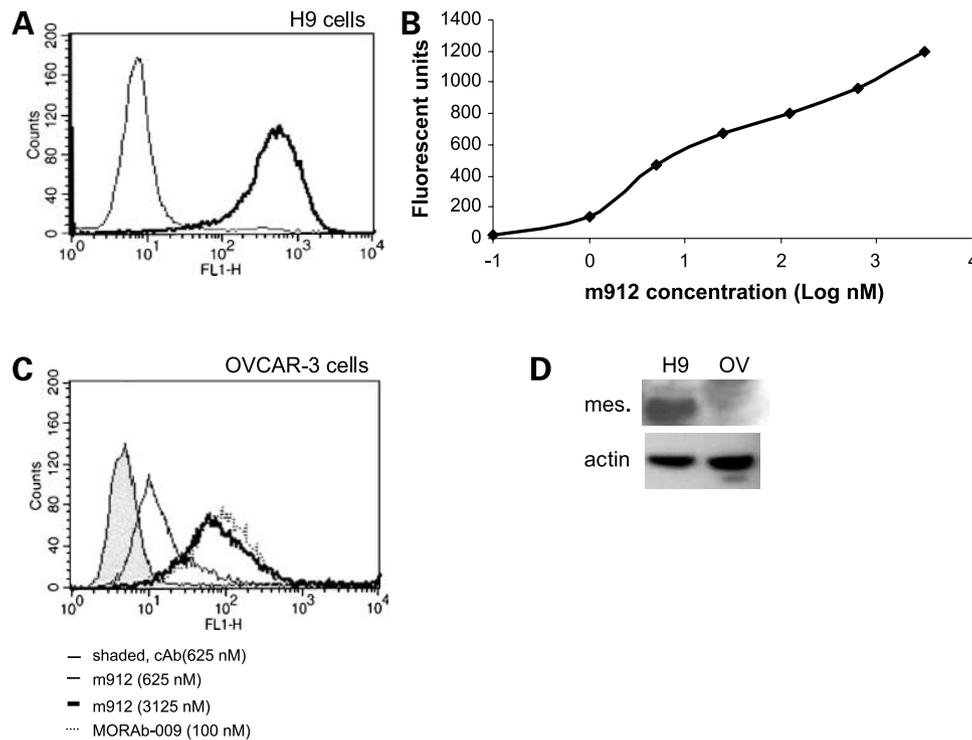
trol-target cell control)  $\times 100\%$ . Measurement of target cells alone treated with 1% Triton X-100 was used as high control. Each treatment was carried out in six duplicated wells. Each assay plate included control wells.

#### Western Blot

H9 and OVCAR-3 cells were lysed in radioimmunoprecipitation assay buffer [50 mmol/L Tris (pH 7.4), 1% Triton X-100, 0.1% SDS, 1% deoxycholate, 150 mmol/L NaCl, 5 mmol/L NaF, and 5 mmol/L EDTA]. After centrifugation at 20,000  $g \times 20$  min, the clear supernatant was resolved on 4% to 12% NuPAGE and transferred to polyvinylidene difluoride membrane. Primary antibodies used for blotting were MORAb-009 at 0.5  $\mu$ g/mL (for mesothelin) and goat polyclonal antibody against actin (clone



**Figure 2.** Specific, high-affinity binding of Fab m912 and scFv m912 to cell surface-associated mesothelin. **A**, lack of Fab m912 binding to mesothelin-negative (A431) cells. **B**, binding of Fab m912 but not of a control Fab to mesothelin-positive H9 cells. **C**, binding of scFv m912 but not of a control scFv to mesothelin-positive H9 cells. *Thick lines*, binding of m912 in Fab or scFv formats; *thin lines*, for control Fab and scFv. The concentrations of all Fabs and scFv are 40 nmol/L and 100 nmol/L, respectively.



**Figure 3.** High avidity binding of IgG1 m912 to cell surface-associated mesothelin. **A**, IgG1 m912 at 5 nmol/L was incubated with mesothelin-positive H9 cells, the cells were washed and further incubated with goat anti-human IgG conjugated with FITC, then washed and analyzed by flow cytometry. An isotype control IgG (*thin line*) at 100 nmol/L was used as negative control. **B**, the same flow cytometry analyses were done at different concentrations of IgG1 m912, and medium fluorescent units plotted as function of the antibody concentration. **C**, IgG1 m912 at 625 nmol/L (*thin line without shade*) and 3,125 nmol/L (*thick line*) was incubated with OVCAR-3 cancer cells, as done similarly in **A**. MORAb-009 was used at 100 nmol/L (*dotted line*). An isotype control human IgG was used at 625 nmol/L (*thin line, shaded area*). **D**, levels of mesothelin proteins in H9 and OVCAR-3 cells as shown by Western blot. Equal amount of lysates were loaded as shown by actin blot.

I-19; Santa Cruz Biotechnology; for monitoring equal loading) at 1  $\mu\text{g}/\text{mL}$ , respectively. Corresponding secondary antibodies were goat anti-human Fc-horseradish peroxidase and donkey anti-goat IgG-horseradish peroxidase at 1  $\mu\text{g}/\text{mL}$  for detection.

## Results

### Expression of Recombinant Human Mesothelin in Insect Cells and Its Purification

To efficiently select antibodies from phage libraries, a purified recombinant antigen, in this case mesothelin, is needed. To include as much as possible of the mesothelin molecule, we expressed its entire extracellular domain, including the GPI linkage site serine-598 (19). To achieve relatively high yields and glycosylation in eukaryotic cells, mesothelin was cloned in a baculovirus expression vector. The transfer vector pAcGP67 has a signal peptide that directs secretion of recombinant protein into medium. Indeed, recombinant mesothelin (296-600 amino acid) expressed in SF9 insect cells was secreted into the culture medium. The mesothelin purified by a 2-step procedure was >95% in purity and migrated at  $\sim 35$  kDa (Fig. 1A) on polyacrylamide gel. It migrated slightly faster than the same fragment that was expressed in mammalian cells (data not shown), reflecting differences in posttranslational

modifications, such as glycosylation. The purified mesothelin was recognized by MORAb-009 (20), a mouse/human chimeric IgG, and was used for panning of a naïve human Fab phage display library.

### High-Affinity Binding of m912 to Recombinant Mesothelin

Mesothelin produced in insect cells was labeled with biotin and used for panning of our large naïve Fab library as described in Materials and Methods. After the third and fourth rounds of panning, 300 clones were screened and 2 positive clones with different sequences were identified. Clone m912 bound with higher affinity to mesothelin than the other clone and was selected for further characterization. Fab m912 was converted to single chain and IgG1 formats. Fab m912 bound to mesothelin with an  $\text{EC}_{50}$  of 20 nmol/L as measured by ELISA (Fig. 1B). Including MORAb-009 at a concentration higher than its  $\text{IC}_{50}$  in Fab m912 ELISA did not reduce binding signals, suggesting that the two antibodies have different epitopes on mesothelin (Fig. 1B). The IgG1 exhibited higher effective binding affinity (avidity) of  $\sim 1.5$  nmol/L as measured by ELISA (Fig. 1C). Deglycosylation of mesothelin by PNGase F reduced its size by at least 3 kDa, but it did not change the binding ability of m912 (data not shown), indicating that the m912 epitope is independent of mesothelin glycosylation. Fab m912 also

recognized mouse mesothelin with about the same affinity (Fig. 1D).

#### Specific Binding of m912 to Cell Surface-Associated Mesothelin

It is essential that a therapeutic or diagnostic antibody recognize the native protein. Therefore, Fab m912 was tested for binding to native mesothelin associated with cell surfaces. We used H9 cells that are derived from parental cells (A431), which do not express mesothelin, and stably express mesothelin protein. Fab m912 bound to H9 cells but not to A431 cells (Fig. 2B), indicating that it is highly specific and does not recognize other membrane proteins on these cells.

Various clinical applications require different sizes and valences of antibodies for best effect. For example, small sizes are preferred for targeting and imaging, whereas full-size antibodies (IgGs) have much longer half-life in circulation, and some are able to mediate effector functions. Therefore, Fab m912 was converted to scFv and IgG formats, and tested for binding to cells by using flow cytometry. We found that m912 in both formats, scFv and IgG1, can bind specifically to cell surface-associated mesothelin (Figs. 2C and 3A).

To estimate the binding ability (avidity) of IgG1 m912 to native cell surface-associated mesothelin, we used antibody concentrations ranging from 0.1 to 3,125 nmol/L and H9 cells in a flow cytometry experiment (Fig. 3B). Even at the lowest concentration of 0.1 nmol/L, IgG m912 exhibited significant binding to H9 cells. Based on the medium fluorescent units bound to H9 cells at each m912 concentration, a 50% binding of m912 was estimated to be at ~5 to 10 nmol/L for these cells.

The binding of m912 was further tested in nontransfected cancer cells, ovarian cancer cell line, OVCAR-3. These cells have been reported to be positive with cell surface mesothelin. In flow cytometry, m912 showed specific binding in a dose-dependent fashion (Fig. 3C); however, higher concentrations were required to reach the same level of binding seen with H9 cells. This was partially due to the much lower levels of mesothelin protein expressed by the OVCAR-3 cells than by H9 cells as shown by the Western blot (Fig. 3D). MORAb-009 still showed high avidity on OVCAR-3 cells.

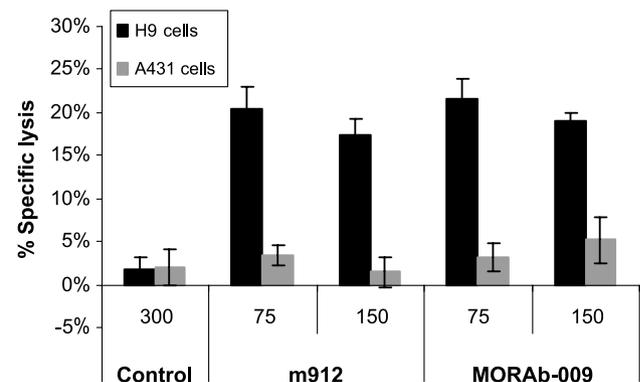
#### Specific Lysis of Mesothelin-Positive Cells by m912 in Presence of PBMC

Therapeutic antibodies can kill cancer cells through antibody-dependent cell-mediated cytotoxicity mediated by the Fc portion of the IgG. To test whether IgG1 m912 has such activity, we used a cell growth assay based on A431 and H9 cells. Incubation of IgG1 m912 alone with these cells did not affect their growth (data not shown). However, in the presence of PBMCs isolated from peripheral blood of healthy donors, IgG1 m912 specifically lysed mesothelin-positive (H9) cells likely by antibody-dependent cell-mediated cytotoxicity but not mesothelin-negative (A431) cells. The mouse-human chimeric antimesothelin antibody MORAb-009 (15) used as a positive control exhibited similar activity, whereas a control isotype antibody had a baseline lysis activity (Fig. 4). These results indicate that IgG1 m912 lysed the H9 cells by antibody-dependent cell-mediated cytotoxicity through specific binding to cell surface-associated mesothelin.

## Discussion

Mesothelin is an attractive target for cancer therapy because of its differential cell surface-associated expression in normal and tumor cells. It is differentially expressed not only in mesothelioma but also in ovarian, pancreatic, and lung cancers (6–8). Although the cellular function of mesothelin is not clear at present, mesothelin-targeted therapies have shown benefits in a number of studies in animal models (12, 13) and several early clinical trials are ongoing (14, 15). Among 33 patients (with mesothelioma, ovarian, and pancreatic cancers) treated with SS1P, a recombinant antimesothelin immunotoxin, at least 2 patients had complete resolution of ascites, and 50% of the patients had stable disease (14). In a separate clinical study, pancreatic cancer patients vaccinated with autologous tumor cells have shown increased mesothelin-specific CD8<sup>+</sup> T-cell response, including CD8<sup>+</sup> T cells that are able to lyse mesothelin-expressing tumor cells (21). These encouraging clinical results support the notion of targeting certain types of cancer cells through mesothelin and justify further studies on mesothelin therapeutics. A fully human mesothelin antibody seems to be a promising addition to mesothelin-related therapeutics currently under studies. In addition, it has been reported that mesothelin may also be released into serum through deletion at its carboxyl terminus or by proteolytical cleavage from its membrane bound form (19). An increase in the soluble form of mesothelin could be detected several years before malignant mesotheliomas occur among workers exposed to asbestos (22). Patients with ovarian, pancreatic, and lung cancers also have elevated soluble mesothelin in serum (11, 23, 24). Thus, mesothelin could also be a valuable biomarker for diagnosis and prognosis. New antibodies to mesothelin, therefore, could be also used for detection of mesothelin.

The antibody we have developed, m912, is fully human and in Fab, scFv, and IgG formats was able to specifically



**Figure 4.** IgG1 m912 induced antibody-dependent cell-mediated cytotoxicity in mesothelin-positive cells but not in mesothelin-negative cells. Freshly isolated PBMCs were incubated with target cells (H9 or A431) at ratio of 50:1, in the presence of IgG1 m912 or MORAb-009 at 2 different concentrations, 75 or 150 nmol/L, or an isotype control IgG1 at 300 nmol/L. Antibody-dependent cell-mediated cytotoxicity was detected with CytoTox-ONE reagent, which measures the lactate dehydrogenase released by lysed target cells. The percentage (%) of specific lysis was calculated as described in the Materials and Methods.

recognize cell surface-associated human mesothelin. It also bound to mouse mesothelin, which would facilitate its use in mouse models of cancer. We have not localized its epitope. However, m912 and MORAb-009 do not compete for binding to mesothelin (Fig. 1B) and MORAb-009 does not recognize mouse mesothelin (15), suggesting that m912 binds to an epitope that does not overlap the MORAb-009 epitope. In addition, the cross-reactivity of m912 to human and mouse mesothelin provides an advantage compared with MORAb-009 because it could be used in mouse models.

MORAb-009, which was derived from a mouse immune library and further improved by *in vitro* maturation, binds with high avidity even to nontransfected cells, such as OVCAR-3, which express relatively low levels of mesothelin (15). M912 was selected from a nonimmune human library and was not further matured *in vitro*. This is why it was not unexpected that its binding to OVCAR-3 cells requires higher concentrations than MORAb-009 to reach the same antibody surface concentration (Fig. 3C). Currently, we are improving the m912 affinity by *in vitro* maturation. As mentioned above, MORAb-009 does not bind to mouse mesothelin and may not be used in mouse models of cancer. In addition, it binds to an epitope that does not overlap the m912 epitope. Thus, the two antibodies may have different areas of application and could be used in combination.

The m912 was selected from a naïve library and its sequence does not deviate significantly from the germ line sequences. Its VL and JL regions together have only one mutation compared with the closest germ line gene IGKV1-39 (accession number X59315) and IGKJ4 (J00242). The heavy chain V gene of m912 is identical to the germ line gene IGHV4-61 (M29811) and its J gene is identical to IGHJ3\*02 (X86355). The D gene region has two mutations and an N2 insertion compared with IGHD3-16\*01 (X93614).

In the presence of PBMC, IgG1 m912 specifically lysed mesothelin-positive cells likely by antibody-dependent cell-mediated cytotoxicity but did not affect mesothelin-negative cells. Thus, even nonconjugated m912 could be a potential candidate therapeutic for treatment of mesothelin-positive tumors. Its tumor killing activity could be further improved by conjugation to toxins or small molecule drugs. The antibody could also be used for guiding liposomes or other carriers of anticancer drugs or imaging agents to cancer cells, and as an agent for detection of soluble mesothelin in serum of cancer patients.

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

## Acknowledgments

We thank members of our group for helpful discussions.

## References

- Chang K, Pastan I, Willingham MC. Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer* 1992;50:373–81.
- Chang K, Pastan I. Molecular cloning of mesothelin, a differentiation

antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci U S A* 1996;93:136–40.

- Bera TK, Pastan I. Mesothelin is not required for normal mouse development or reproduction. *Mol Cell Biol* 2000;20:2902–6.
- Rump A, Morikawa Y, Tanaka M, et al. Binding of ovarian cancer antigen CA125/MUC16 to mesothelin mediates cell adhesion. *J Biol Chem* 2004;279:9190–8.
- Gubbels JA, Belisle J, Onda M, et al. Mesothelin-MUC16 binding is a high affinity, N-glycan dependent interaction that facilitates peritoneal metastasis of ovarian tumors. *Mol Cancer* 2006;5:50.
- Argani P, Iacobuzio-Donahue C, Ryu B, et al. Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* 2001;7:3862–8.
- Hassan R, Kreitman RJ, Pastan I, Willingham MC. Localization of mesothelin in epithelial ovarian cancer. *Appl Immunohistochem Mol Morphol* 2005;13:243–7.
- Li M, Bharadwaj U, Zhang R, et al. Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. *Mol Cancer Ther* 2008;7:286–96.
- Yen MJ, Hsu CY, Mao TL, et al. Diffuse mesothelin expression correlates with prolonged patient survival in ovarian serous carcinoma. *Clin Cancer Res* 2006;12:827–31.
- Ho M, Bera TK, Willingham MC, et al. Mesothelin expression in human lung cancer. *Clin Cancer Res* 2007;13:1571–5.
- Cristaudo A, Foddis R, Vivaldi A, et al. Clinical significance of serum mesothelin in patients with mesothelioma and lung cancer. *Clin Cancer Res* 2007;13:5076–81.
- Chowdhury PS, Pastan I. Analysis of cloned Fvs from a phage display library indicates that DNA immunization can mimic antibody response generated by cell immunizations. *J Immunol Methods* 1999;231:83–91.
- Chang CL, Wu TC, Hung CF. Control of human mesothelin-expressing tumors by DNA vaccines. *Gene Ther* 2007;14:1189–98.
- Hassan R, Bullock S, Premkumar A, et al. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. *Clin Cancer Res* 2007;13:5144–9.
- Hassan R, Ebel W, Routhier EL, et al. Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. *Cancer Immun* 2007;7:20.
- Zhu ZY, Dimitrov DS. Construction of a large naïve human phage-displayed Fab library through one-step cloning. *Methods Mol Biol* 2009;525:129–42.
- Feng Y, Zhu Z, Xiao X, Choudhry V, Barrett JC, Dimitrov DS. Novel human monoclonal antibodies to insulin-like growth factor (IGF)-II that potentially inhibit the IGF receptor type I signal transduction function. *Mol Cancer Ther* 2006;5:114–20.
- Scott JK, Barbas CF, III. Phage-display vector. In: Barbas CF, III, editor. *Phage display: a laboratory manual*. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 2001.
- Hassan R, Bera T, Pastan I. Mesothelin: a new target for immunotherapy. *Clin Cancer Res* 2004;10:3937–42.
- Chowdhury PS, Viner JL, Beers R, Pastan I. Isolation of a high-affinity stable single-chain Fv specific for mesothelin from DNA-immunized mice by phage display and construction of a recombinant immunotoxin with anti-tumor activity. *Proc Natl Acad Sci U S A* 1998;95:669–74.
- Thomas AM, Santarsiero LM, Lutz ER, et al. Mesothelin-specific CD8 (+) T cell responses provide evidence of *in vivo* cross-priming by antigen-presenting cells in vaccinated pancreatic cancer patients. *J Exp Med* 2004;200:297–306.
- Creaney J, Robinson BW. Detection of malignant mesothelioma in asbestos-exposed individuals: the potential role of soluble mesothelin-related protein. *Hematol Oncol Clin North Am* 2005;19:1025–40.
- Hassan R, Remaley AT, Sampson ML, et al. Detection and quantitation of serum mesothelin, a tumor marker for patients with mesothelioma and ovarian cancer. *Clin Cancer Res* 2006;12:447–53.
- Corso CD, Stubbs DD, Lee SH, Goggins M, Hruban RH, Hunt WD. Real-time detection of mesothelin in pancreatic cancer cell line supernatant using an acoustic wave immunosensor. *Cancer Detect Prev* 2006;30:180–7.