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Review

Molecular Cancer Therapeutics

Mesothelin-Targeted Agents in Clinical Trials and in Preclinical Development

Ronan J. Kelly[1], Elad Sharon[1], Ira Pastan[2], and Raffit Hassan[2]

Abstract

Mesothelin is a tumor differentiation antigen that is highly expressed in several malignant diseases in humans, including malignant mesothelioma and pancreatic, ovarian, and lung adenocarcinomas. The limited expression of mesothelin on normal human tissues and its high expression in many common cancers make it an attractive candidate for cancer therapy. Several agents, including an immunotoxin, monoclonal antibody, antibody drug conjugate, and tumor vaccine, are in various stages of development to treat patients with mesothelin-expressing tumors. This review highlights ongoing clinical trials, as well as other approaches to exploit mesothelin for cancer therapy, that are in preclinical development. *Mol Cancer Ther; 11(3); 517–25. ©2012 AACR.*

Introduction

Mesothelin is a 40-kDa cell surface glycoprotein that is present on normal mesothelial cells lining the pleura, peritoneum, and pericardium (1). Mesothelin was originally identified as the antigen recognized by the mAb K1 that was produced by immunization of mice with the human ovarian cancer cell line OVCAR3 (2). The mesothelin gene encodes a precursor protein of 71-kDa that is processed to yield a 31-kDa shed protein named megakaryocyte-potentiating factor (MPF) and the 40-kDa cell bound fragment mesothelin (3). MPF was isolated from the culture supernatant of a pancreatic cancer cell line, and in mouse bone marrow cultures it showed megakaryocyte colony-forming activity in the presence of interleukin-3 (4). By immunohistochemistry using anti-mesothelin mAbs, mesothelin expression in normal human tissues is noted only in the single layer of mesothelial cells that line the pleura, peritoneum, and pericardium, surface epithelial cells of the ovary, tunica vaginalis, rete testis, and the tonsillar and fallopian tube epithelial cells (2, 5). However, mesothelin is highly expressed in several human tumors, including epithelial mesotheliomas (∼100% of cases) and lung (∼50% of cases), ovarian (∼70% of cases), and pancreatic/biliary (∼100% of cases) adenocarcinomas (5–8). In the majority of these cancers there is diffuse, homogeneous cell-surface mesothelin expression. However, in contrast to other tumors, which show mostly membranous staining, the pattern of mesothelin reactivity in lung cancer shows mostly cytoplasmic staining (9). Mesothelin is also expressed in many other cancers, including gastric cancer, biphasic synovial sarcoma, and uterine adenocarcinoma (5). Previous reviews have described the frequency and characterization of mesothelin expression in different tumor types (3, 10).

The normal biological function of mesothelin is unknown. In one study, mutant mice that lacked both copies of the mesothelin gene had no detectable phenotype, and both male and female mice produced healthy offspring, suggesting that mesothelin is not involved in normal growth and development (11). Mesothelin may play a role in cell adhesion, and preclinical studies have shown that mesothelin is the receptor for the tumor antigen CA-125 (also known as MUC16), and that they bind to each other with a high specificity (12, 13). Tumors expressing CA-125 may bind to mesothelin expressed on the surface of mesothelial cells that line the pleural or peritoneal cavity, leading to an increase in heterotypic cell adhesion and promoting metastatic spread (12, 13). Studies in pancreatic cancer suggest that mesothelin plays a role in tumorigenesis by increasing cellular proliferation, migration, and S-phase cell populations (14). The exact biological role of MPF is also unclear. Wang and colleagues (15) recently showed that in cells overexpressing MPF, phosphorylation of the MAP kinase ERK1/2 was enhanced and the rate of cell death was decreased, leading to an increase in cell numbers. Although the function of mesothelin in cancer is not fully understood at this time, its limited expression on normal human tissues and high expression in many cancers makes it an attractive tumor-associated antigen for cancer therapy.

Clinical Trials of Mesothelin-Targeted Agents

Several different therapeutic agents are being evaluated for treatment of patients with mesothelin-expressing cancers (Table 1; refs. 16–25). These include antibody-based

Authors' Affiliations: [1]Medical Oncology Branch and [2]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

Corresponding Author: Raffit Hassan, Laboratory of Molecular Biology, 37 Convent Dr., Rm. 5116, National Cancer Institute, Bethesda, MD 20892-4264. Phone: 301-451-8742; Fax: 301-402-1344; E-mail: hassanr@mail.nih.gov

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Table 1. Phase I/II clinical trials of mesothelin-targeted agents

Agent	Type of clinical trial	Dose and schedule	Patient population	Number of patients	Outcome	References
SS1P (immunotoxin)	Phase I single-agent bolus study	Dose escalation of SS1P given as i.v. infusion QOD	Mesothelin-positive mesotheliomas, and ovarian and pancreatic cancers	34	MTD of SS1P was 45 μg/kg i.v. QOD × 3 doses. DLT was pleuritis. 4 MR and 18 SD out of 33 pts.	(16)
	Phase I single-agent c.i. study	Dose escalation of SS1P given as c.i. over 10 days	Mesothelin-positive mesotheliomas, and ovarian and pancreatic cancers	24	MTD was 25 μg/kg/d given as c.i. over 10 days. No CR, 1 PR.	(17)
	Phase I SS1P plus pemetrexed and cisplatin	SS1P dose escalation with fixed standard doses of pemetrexed and cisplatin	Chemotherapy-naïve patients with malignant mesothelioma who are not candidates for curative surgical resection	19	Study open to patient accrual. At the MTD, 5 out of 7 evaluable patients have had a PR.	(18)
	SS1P plus paclitaxel, carboplatin, and bevacizumab	SS1P dose escalation with fixed dose of chemotherapy and bevacizumab	Newly diagnosed stage IV lung adenocarcinoma that is mesothelin-positive	2	Study closed because the incidence of mesothelin positivity was less than the expected 50% positivity in lung adenocarcinoma and the mesothelin staining was predominantly cytoplasmic (R. Hassan, unpublished data).	N/R
MORAb-009 (chimeric mAb)	Phase I single-agent study	Dose-escalation study	Mesothelioma, pancreatic cancer and mesothelin positive lung and ovarian cancer	24	MTD 200 mg/m^2 In patients with mesothelioma, treatment with MORAb-009 led to an increase in	(19)

(Continued on the following page)

Table 1. Phase I/II clinical trials of mesothelin-targeted agents (Cont'd)

Agent	Type of clinical trial	Dose and schedule	Patient population	Number of patients	Outcome	References
					serum CA-125 levels.	
	Phase II MORAb-009 plus gemcitabine	Patients randomized to either gemcitabine alone or gemcitabine plus MORAb-009	Locally advanced and metastatic pancreatic cancer	N/R	Study closed. Data not yet available.	(20)
	Phase II MORAb-009 plus pemetrexed and cisplatin	Single arm study	Newly diagnosed unresectable pleural mesothelioma	N/R	Study is ongoing but closed to new patient accrual.	(21)
CRS-207 (tumor vaccine)	Phase I single agent	Dose-escalation study	Patients with mesothelin-expressing cancers	17	MTD 1×10^9 cfu Mesothelin specific immune response observed in 5 out of 10 evaluable patients.	(22)
	Phase II CRS-207 plus GVAX	Patients randomized to GVAX pancreas vaccine versus GVAX pancreas vaccine plus CRS-207	Previously treated metastatic pancreatic cancer	—	Clinical trial opened August 2011	(23)
Autologous CIR T cells transfected with anti-mesothelin messenger RNA	Phase I	Patients will receive 1–3 doses of autologous CIR T cells	Progressive malignant pleural mesothelioma	—	Clinical trial opened May 2011	(24)
ADC, BAY 94-9343	Phase I	BAY 94-9343 given i.v. every 3 weeks	Patients with advanced solid tumors	—	Clinical trial opened September 2011	(25)

Abbreviations: c.i., continuous infusion; CIR, chimeric immune receptor; CR, complete response; MR, minor response; N/R, not reported; PR, partial response; QOD, every other day; SD, stable disease.

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therapies with SS1(dsFv)-PE38 (SS1P, a recombinant immunotoxin that targets mesothelin), a high-affinity chimeric (mouse/human) mAb (IgG/κ, MORAb-009), and an anti-mesothelin antibody drug conjugate (ADC, BAY-94 9343). In addition, a mesothelin tumor vaccine (CRS-207) and adoptive T-cell immunotherapy using mesothelin-specific chimeric antigen receptors (CAR) are also being evaluated in clinical trials.

Anti-mesothelin immunotoxin SS1P

The immunotoxin SS1P [SS1(dsFv)PE38] consists of an anti-mesothelin Fv that was obtained from a phage display library of mice immunized with recombinant mesothelin, genetically fused to a truncated form of the *Pseudomonas* exotoxin PE38. After it binds to cell-surface mesothelin via the Fv, PE38 is internalized into the cell, undergoes processing, and kills the cell due to inhibition of protein synthesis by ADP ribosylation and inactivation of elongation factor 2 (26). SS1P, which has a high affinity for mesothelin (Kd, 0.72M), is highly active against several mesothelin-expressing cell lines and causes regression of mesothelin-expressing xenografts in nude mice (27, 28). SS1P also showed cytotoxicity against tumor cells directly obtained from patients with ovarian cancer and mesothelioma (29, 30).

Preclinical studies showed marked antitumor synergy when SS1P was combined with several commonly used chemotherapeutic agents, such as paclitaxel, gemcitabine, and cisplatin (31, 32). Although the combination of SS1P with chemotherapeutic agents did not result in synergy in cell culture, there was a marked increase in antitumor activity with the combination in tumor xenograft models. In a study using a mesothelin-expressing cell line (A431-K5) that was grown as a tumor xenograft in athymic nude mice, treatment with SS1P plus paclitaxel resulted in increased antitumor activity with durable complete tumor regressions compared with treatment with paclitaxel or SS1P alone (31). This enhancement of the cytotoxicity of SS1P by paclitaxel *in vivo* is not due to an indirect effect whereby paclitaxel increases tumor permeability by damaging tumor endothelial cells. Rather, it is due to the direct effect of paclitaxel on tumor cells. The mechanism for this synergy was evaluated with the use of a pair of mesothelin-expressing cervical cancer cell lines (KB) that are sensitive or resistant to paclitaxel as tumor xenografts in mice (33). The synergistic effect of paclitaxel and SS1P was only seen in paclitaxel-sensitive KB tumor xenografts. Killing of tumor cells by paclitaxel altered the tumor architecture and significantly decreased the concentration of shed mesothelin in the tumor extracellular fluid. These results suggest that the synergy between SS1P and chemotherapy is due to the ability of cytotoxics to decrease the shed mesothelin in the tumor extracellular space, which allows more of the administered SS1P to bind to tumor cells and results in increased cell killing. In addition, paclitaxel alters tumor architecture by killing tumor cells, allowing increased tumor penetration. On the basis of these preclinical studies, the combination of SS1P with chemotherapy is being evaluated in clinical trials involving mesothelin-expressing cancers.

A limitation of immunotoxin-based therapies such as SS1P is the development of neutralizing antibodies to the toxin portion of the molecule, which limits repeated administration of the drug to patients. Previous efforts to decrease the immune response to immunotoxins by various approaches, including the use of the anti–B-cell mAb rituximab, were unsuccessful (34). However, we recently showed that immune depletion using the regimen of pentostatin plus cyclophosphamide completely abrogated the anti-immunotoxin immune response in immunocompetent BALB/c mice when repeat injections of SS1P were administered (35). This regimen resulted in host B-cell and T-cell depletion with minimal myeloid cell depletion. A pilot clinical trial to evaluate this approach to decrease the immunogenicity of SS1P in patients has just opened to patient accrual (36).

Phase I clinical trials of single-agent SS1P. Two phase I trials of SS1P have been conducted. The two trials used different schedules of administration, either as a bolus intravenous infusion or as a continuous infusion. In a phase I dose-escalation study, 34 patients with advanced mesothelin-expressing cancers (20 with mesothelioma, 12 with ovarian cancer, and 2 with pancreatic cancer) who had failed standard therapy were treated with SS1P given as a 30 min i.v. infusion every other day for either 3 or 6 doses (16). The first cohort of 17 patients received 6 doses of SS1P every other day for 6 doses with a maximum tolerated dose (MTD) of 18 μg/kg/dose. The dose-limiting toxicities (DLT) were grade 3 urticaria (1 patient) and grade 3 vascular leak syndrome (2 patients). A second cohort of 17 patients received only 3 doses with an MTD of 45 μg/kg/dose, with the DLT being grade 3 pleuritis. At the MTD of 45 μg/kg/dose, the mean C_{max} of SS1P was 483 ng/mL and the half-life was 466 min. Of the 33 patients who were considered evaluable for response, 4 had minor responses, 19 had disease stabilization, and 10 had progressive disease.

In the second phase I trial, SS1P was administered as a continuous i.v. infusion over 10 days (17). Twenty-four patients (16 with mesothelioma, 7 with ovarian cancer, and 1 with pancreatic cancer) were enrolled at 5 different dose levels of SS1P. The MTD was 25 μg/kg/d as a 10-day continuous infusion, and the DLT was reversible vascular leak syndrome. As a single agent, SS1P has shown modest clinical activity, and continuous infusion shows no significant benefit over bolus dosing. Due to the ease of administration, as well as pharmacokinetic data that show high blood levels and a prolonged half-life of SS1P when given as a bolus dose, this schedule is being evaluated in combination studies with chemotherapy.

Phase I clinical trials of SS1P in combination with chemotherapy. On the basis of preclinical studies that showed that the activity of SS1P can be increased when it is given in combination with chemotherapy, an ongoing clinical trial is evaluating SS1P in combination with

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pemetrexed and cisplatin as frontline therapy for patients with advanced, unresectable malignant pleural mesothelioma. The primary objective of this study is to evaluate the MTD and safety of SS1P in combination with chemotherapy, with secondary endpoints of tumor response, progression-free survival (PFS), and overall survival. Enrolled patients receive 6 cycles of standard-care chemotherapy consisting of pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 on day 1. During the first 2 cycles, patients receive SS1P on days 1, 3, and 5 at escalating dose cohorts of 25 mcg/kg, 35 mcg/kg, 45 mcg/kg, and 55 mcg/kg. Results from the first 19 patients were recently reported. Investigators treated 5 patients at an SS1P dose of 25 mcg/kg, 3 patients at 35 mcg/kg, 10 patients at 45 mcg/kg, and 1 patient at 55 mcg/kg. Of the 14 evaluable patients treated at all dose levels, 7 had a partial response, 3 had stable disease, and 4 had progressive disease (18). However, of the 7 evaluable patients treated at the MTD of SS1P in combination with pemetrexed and cisplatin, 5 had partial response, 1 had stable disease, and 1 had progressive disease. Of the 10 patients whose serum mesothelin levels were evaluated before and at completion of treatment, all 5 patients who had a partial response showed a significant decrease in serum mesothelin (63–83%). Two of 3 patients with progressive disease had increased mesothelin levels (16–34%), and 1 patient had a slight decrease (7%). Two patients with stable disease had discordant responses (17% increase and 60% decrease). Overall, the combination of SS1P, cisplatin, and pemetrexed was well tolerated, with hypoalbuminemia, edema, and fatigue being the main side-effects. The trial is now in the expansion phase.

A phase I trial evaluating SS1P in combination with bevacizumab, carboplatin, and paclitaxel for treatment of patients with mesothelin-expressing stage IV lung adenocarcinoma was recently closed due to poor accrual, primarily because the incidence of mesothelin expression in the patients screened for this study was lower than the expected 50% positivity in lung adenocarcinoma. In addition, in similarity to findings in preclinical immunohistochemistry studies, the predominant pattern of tumor mesothelin staining was cytoplasmic rather than membranous reactivity (R. Hassan, unpublished data; ref. 9).

MORAb-009, a chimeric anti-mesothelin mAb

MORAb-009 consists of the heavy- and light-chain variable regions of a mouse anti-mesothelin single-chain Fv grafted to human IgG1 and κ constant regions. MORAb-009 has high affinity for mesothelin, with an affinity (K_D) of 1.5 nM. *In vitro* MORAb-009 inhibits the adhesion between cell lines expressing mesothelin and MUC16, and mediates ADCC against mesothelin-positive ovarian cancer, pancreatic cancer, and mesothelioma cell lines. In a tumor xenograft model using the mesothelin-positive cell line A431-K5, treatment with MORAb-009 in combination with gemcitabine or paclitaxel resulted in significant tumor shrinkage compared with MORAb-009 or chemotherapy alone (37). In toxicology studies in cynomolgus monkeys, repeated doses of MORAb-009 at 15 mg/kg were well tolerated. On the basis of these preclinical data, MORAb-009 was evaluated in clinical trials for patients whose tumors were mesothelin-positive.

Phase I dose-escalation study of MORAb-009. To determine the safety and MTD of MORAb-009, investigators conducted a phase I clinical trial in patients with mesothelin-positive cancers who had failed standard treatment options for their disease. MORAb-009 was administered as an i.v. infusion at doses ranging from 12.5 to 400 mg/m^2 on days 1, 8, 15, and 22. Patients with stable disease at day 35 could receive the next cycle of MORAb-009. A total of 24 patients (13 with mesothelioma, 7 with pancreatic cancer, and 4 with ovarian cancer) were enrolled in this study (19). The MTD of MORAb-009 was 200 mg/m^2 with 2 patients at the 400 mg/m^2 having DLT (grade 4 transaminitis and a grade 3 serum sickness). No objective response was noted, although 11 patients had stable disease.

An interesting observation during the phase I clinical trial was the effect of MORAb-009 on serum CA-125 kinetics. As noted above, laboratory studies suggest that mesothelin may be involved in tumor spread by binding to CA-125. In this phase I trial, 8 patients with mesothelioma had serum CA-125 levels measured at baseline and at different time points of MORAb-009 therapy (38). In all of these patients, and even in those who had normal CA-125 levels at baseline, treatment with MORAb-009 led to a marked increase in serum CA-125 levels. The increase in CA-125 levels was not due to inflammation or disease progression, because the CA-125 levels returned to baseline values once MORAb-009 treatment was stopped. These results suggest that MORAb-009 interferes with mesothelin CA-125 interaction in patients, and could have potential use for prevention of tumor metastasis in patients with mesothelioma and ovarian cancer.

Phase II clinical trials of MORAb-009 with chemotherapy. Given the favorable safety profile of MORAb-009 and the possibility of clinical benefit, investigators initiated 2 phase II clinical trials to evaluate its efficacy in combination with chemotherapy. In the first trial, MORAb-009 was administered with gemcitabine in patients with unresectable pancreatic cancer (20). This was a double-blind, placebo-controlled trial in patients with advanced pancreatic cancer who were not candidates for surgical resection. The patients were randomized to gemcitabine alone or gemcitabine plus MORAb-009. The primary outcome is overall survival, with PFS as a secondary endpoint. This trial has completed patient enrollment, but the results have not yet been published.

The second study is evaluating MORAb-009 as frontline therapy in combination with pemetrexed and cisplatin in patients with unresectable epitheloid malignant pleural mesothelioma. Patients receive pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 on day 1 with MORAb-009 5 mg/kg on days 1 and 8 of a 3-week cycle.

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Patients with tumor response or stable disease after 6 cycles of therapy will continue on MORAb-009 until disease progression occurs (21). The primary endpoint of this study is to evaluate whether combination treatment with MORAb-009 plus pemetrexed and cisplatin improves PFS compared with the PFS observed in the pivotal phase III clinical trial of pemetrexed and cisplatin. No preliminary data have been reported yet, and the study is ongoing.

CRS-207, a mesothelin tumor vaccine

CRS-207 is a mesothelin vaccine that uses a live attenuated strain of the bacterium *Listeria monocytogenes* (*Lm*), a facultative intracellular bacterium, as the vector (39). CRS-207 is based on CRS-100, which is an engineered vector that has deletions of the 2 genes that encode the virulence determinants actA and internalin B. These deletions result in a 1,000-fold decrease in virulence compared with the wild-type *Lm*. Preclinical studies showed that CRS-207 elicits human mesothelin-specific CD4+/CD8+ immunity in mice and cynomolgus monkeys, and exhibits therapeutic efficacy in tumor-bearing mice (39).

Phase I clinical trial of CRS-207. A phase I clinical trial of CRS-207 for the treatment of patients with mesothelin-expressing cancers was conducted to determine the safety and MTD of CRS-207. The results of this trial were recently reported (22). In this phase I study, CRS-207 was administered i.v. every 3 weeks for a total of 4 doses ranging from 1×10^8 to 1×10^{10} cfu. Seventeen patients (7 with pancreatic cancer, 5 with mesothelioma, 3 with lung cancer, and 2 with ovarian cancer) were treated. The MTD of CRS-207 was 1×10^9 cfu, with a DLT of hypotension observed at the 1×10^{10} cfu dose level. In this group of heavily pretreated patients, no objective antitumor response was observed. However, a mesothelin-specific T-cell response was observed in 5 of 10 patients tested. Based on the tolerability and immune activation, CRS-207 may be an attractive agent for treating mesothelin-expressing cancers either alone or in combination with other agents.

Phase II clinical trial of CRS-207 with GVAX pancreatic vaccine. A phase II clinical trial of CRS-207 for the treatment of patients with metastatic pancreatic cancer has just opened for enrollment (23). In this phase II study, previously treated patients with metastatic pancreatic cancer will be randomized to either GVAX (irradiated pancreatic cancer cell lines that have been genetically modified to secrete granulocyte-macrophage colony stimulating factor) alone every 3 weeks $\times$ 6 doses or sequential administration of 2 doses of GVAX vaccine and 4 doses of CRS-207. The primary endpoint of this clinical trial is to determine whether administration of CRS-207 will improve overall survival compared with GVAX alone. Secondary endpoints include the safety of combining CRS-207 with GVAX, and monitoring CRS-207–induced *Listeria* and mesothelin-specific immune response.

Adoptive T-cell immunotherapy using mesothelin-specific CARs

Because tumor-associated antigens are poorly immunogenic, the generation of a T-cell response to these antigens is limited in patients (40). Over the last several years, investigators have developed different approaches to exploit T cells for cancer therapy, such as the administration of tumor-infiltrating T cells and adoptive transfer of T cells expressing T-cell receptors against tumor antigens, which have resulted in clinical activity in some tumors (41). An attractive approach to increase the specificity of these T cells for tumors is to use CARs, which can kill these cells in a non-HLA–restricted manner. A CAR consists of an antigen-specific portion of a mAb, such as Fv, and the signaling component of the T cells (usually the ζ chain of the TCR/CD3 complex). T cells expressing the CARs are specifically directed to tumor cells, and then the activation of T cells kills the tumor (42). Adoptive transfer of T cells expressing CARs has shown promise in several cancers (43). Mesothelin is an attractive candidate for T-cell adoptive therapy using CARs, and preclinical studies have shown its efficacy in animal models. Carpenito and colleagues (44) generated a CAR that recognizes mesothelin using anti-mesothelin single-chain Fv fused to the T-cell receptor ζ signal transduction domain, as well as CD28 and CD137(4-1BB) domains. They then evaluated the T cells transduced with these CARs for efficacy using lentiviral vectors against mesothelin-expressing tumor xenografts in NOD/*scid*/*IL2rγ*$^{-/-}$ mice. Both intratumoral and i.v. administration of these transduced T cells resulted in significant shrinkage of these large, established mesothelin-expressing tumors. T cells expressing CARs that contained the CD28 and CD137 domains had greater efficacy than CARs expressing only the T-cell receptor ζ signaling domain (44). Recently, the same group described an alternative method that may be safer than the retroviral or lentiviral vector transduction method. The use of RNA encoding an anti-mesothelin CAR for T-cell transduction showed robust activity against mesothelin-expressing xenografts (45). One advantage of RNA CAR T-cell therapy is that it avoids the potential risk of malignant transformation from insertional mutagenesis using retroviral or lentiviral constructs. In addition, if toxicity due to CAR T-cell therapy occurs, it can be reduced by stopping the administration of RNA CAR T cells, which is not the case with stably transduced T cells using viral vectors. A phase I clinical trial of adoptive T-cell therapy using mesothelin-directed CARs has been initiated for treatment of patients with pleural mesothelioma (24).

ADCs targeting mesothelin

The limited expression of mesothelin on essential human tissues makes it a good target for ADCs. Although no mesothelin-targeted ADC is yet in clinical use, some of these compounds, including MDX-1204 and BAY 94-9343, have undergone preclinical development. The anti-mesothelin ADC MDX-1204 consists of the human anti-mesothelin mAb MDX-1382 conjugated to duocarmycin, a

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DNA alkylating agent (46). This compound showed antitumor efficacy against mesothelin-expressing xenografts in mice, and at clinically relevant doses was well tolerated by cynomolgus monkeys without any clinical toxicity due to the antibody binding to mesothelin. Another anti-mesothelin ADC is BAY 94-9343, which consists of the fully human anti-mesothelin IgG1 linked to DM4, a potent tubulin-binding drug (47). This drug conjugate showed cytotoxicity against mesothelin-positive cell lines with IC_{50} in the nanomolar range, as well as antitumor activity against mesothelin-expressing ovarian, pancreatic, and mesothelioma tumor xenografts. A phase I clinical trial of BAY 94-9343 recently opened for the treatment of patients with advanced solid tumors. The primary objectives of this trial are to determine the safety and MTD of BAY 94-9343, and to study its pharmacokinetics (25).

Mesothelin-Directed Therapies in Preclinical Development

Various approaches to target mesothelin for cancer therapy are currently in preclinical development, and below we discuss some of these methods.

Mesothelin cancer vaccines

Several lines of work support the use of mesothelin vaccination for cancer therapy. In one study, anti-mesothelin IgG antibodies were shown to be present in 39% and 41% of patients with advanced mesothelin-expressing mesothelioma and ovarian cancer, respectively (48). This suggests that at least in some patients, the tolerance to mesothelin breaks down. In addition, in a clinical trial of patients with advanced pancreatic cancer who were receiving vaccination with granulocyte macrophage colony-stimulating-secreting pancreatic cancer cell lines (CG8020/CG2505), a strong mesothelin-specific CD8+ T-cell response was observed in 3 of 14 patients who developed a delayed-type hypersensitivity response following vaccination (49). In a subsequent clinical trial of this whole-cell vaccine for metastatic pancreatic cancer, the investigators observed mesothelin-specific CD8+ T cells in most of the patients at baseline, but this response was augmented after vaccination (50).

In several preclinical studies, researchers have evaluated the use of mesothelin as a tumor vaccine using different strategies. Yokokawa and colleagues (51) identified 2 novel HLA-2 mesothelin epitopes recognized by cytotoxic T lymphocyte. T cells generated from these native or agonist mesothelin epitopes lysed mesothelin-expressing pancreatic cancer, ovarian cancer cells, and mesothelioma cell lines. In addition, using a DNA vaccine encoding human mesothelin, Chang and colleagues (52) showed efficacy in a mouse model by generating both CD4+ and CD8+ T cells as well as an antibody-mediated immune response to mesothelin. Other vaccine strategies, such as targeting mesothelin directly to dendritic cells using mAbs or using dendritic cells transduced with full-length mesothelin, have also shown enhanced induction of mesothelin-specific CD4+ and CD8+ T-cell immunity (53, 54). In another study, vaccination with chimeric virus-like particles that contained human mesothelin substantially inhibited tumor progression in mice, with increases in mesothelin-specific antibodies and cytotoxic T-lymphocyte activity (14). It is likely that some of these vaccination strategies will be evaluated in clinical trials in the near future.

Mesothelin-directed gene therapy

Given the potential toxicity of systemic gene therapy to nontarget tissues, such as the liver, a gene therapy with the ability to specifically target tumor cells is clinically desirable. Breidenbach and colleagues (55) conducted studies to evaluate mesothelin as a target for adenoviral vectors for gene therapy of ovarian cancer. Using established ovarian cancer cell lines and purified tumor cells obtained from patients, they showed high mesothelin gene and protein expression. Adenoviruses that contained the mesothelin promoter driving reporter gene expression showed that the mesothelin promoter was activated in ovarian cancer cell lines but not in normal control cells. In addition, in an *in vivo* study in mice, the adenovirus construct containing the mesothelin promoter had a low expression in the liver, making it potentially useful for the clinic. To further improve on targeting of adenoviruses for gene therapy of ovarian cancer, the authors conjugated an adenovirus vector containing an Fc-binding domain to a mouse anti-human mesothelin mAb. This transductional targeting approach resulted in increased transgene expression in ovarian cancer cells and showed the use of mesothelin targeting for ovarian cancer gene therapy.

The use of conditionally replicative adenoviruses (CRAd) that contain tumor-specific promoters restricts virus replication to cancer cells. Tsuruta and colleagues (56) tested the efficacy of a mesothelin promoter-based CRAd with a chimeric Ad5/3 fiber (AdMSLNCRAd5/3) against a human ovarian cancer xenograft model in immunodeficient mice. AdMSLNCRAd5/3 treatment resulted in significant tumor growth inhibition and improved the overall survival of these mice compared with no virus administration or wild-type adenovirus administration (56). Gene therapy has clearly shown some promise in preclinical models. Clinical testing will ultimately determine the efficacy of mesothelin-directed gene therapy, and future studies are planned.

Conclusions

Mesothelin is a tumor differentiation antigen that has limited expression on normal human tissues but is highly expressed in several malignant diseases, which makes it a good candidate for cancer therapy. Thus far, clinical trials of an anti-mesothelin immunotoxin and mAb, as well as a mesothelin vaccine, have shown encouraging activity without any untoward toxicity due to targeting of normal mesothelial cells that express mesothelin. In addition to the mesothelin-targeted therapies currently in the clinic

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(i.e., SS1P, MORAb-009, BAY 94-9343, CRS-207, and adoptive T-cell immunotherapy), several other agents are in various stages of preclinical development. Although the preclinical studies and clinical trials conducted to date have validated mesothelin as a drug target, the ongoing clinical trials, some of which have just been initiated, will determine whether mesothelin-targeted therapies benefit patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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