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Mesothelin Immunotherapy for Cancer: Ready for Prime Time?

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ABSTRACT

Mesothelin is a tumor antigen that is highly expressed in many human cancers, including malignant mesothelioma and pancreatic, ovarian, and lung adenocarcinomas. It is an attractive target for cancer immunotherapy because its normal expression is limited to mesothelial cells, which are dispensable. Several antibody-based therapeutic agents as well as vaccine and T-cell therapies directed at mesothelin are undergoing clinical evaluation. These include antimesothelin immunotoxins (SS1P, RG7787/LMB-100), chimeric antimesothelin antibody (amatuximab), mesothelin-directed antibody drug conjugates (anetumab ravtansine, DMOT4039A, BMS-986148), live attenuated *Listeria monocytogenes*–expressing mesothelin (CRS-207, JNJ-64041757), and chimeric antigen receptor T-cell therapies. Two antimesothelin agents are currently in multicenter clinical registration trials for malignant mesothelioma: amatuximab in the first-line setting and anetumab ravtansine as second-line therapy. Phase II randomized clinical trials of CRS-207 as a boosting agent and in combination with immune checkpoint inhibition for pancreatic cancer are nearing completion. These ongoing studies will define the utility of mesothelin immunotherapy for treating cancer.

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INTRODUCTION

Mesothelin (MSLN) is a tumor-differentiation antigen discovered by Ira Pastan and Mark Willingham at the National Cancer Institute more than 20 years ago.[1,2] It is a cell-surface glycoprotein with normal expression limited to mesothelial cells lining the pleura, peritoneum, and pericardium but is also highly expressed in many cancers, including malignant mesothelioma, pancreatic cancer, ovarian cancer, lung adenocarcinoma, endometrial cancer, biliary cancer, gastric cancer, and pediatric acute myeloid leukemia.[3-11] There are few tumor-specific antigens that are used to therapeutically target solid tumors, because most of them are also expressed on critical tissues. Because MSLN is expressed only on dispensable tissues, the risk of nonspecific toxicity is decreased. Since our initial report in 1999 that indium-111–labeled anti-MSLN monoclonal antibody K1 localizes to MSLN-expressing tumor xenografts in mice, it has been shown that anti-MSLN monoclonal antibodies do localize to MSLN-positive solid tumors in patients.[12-14] In addition, preclinical studies as well as results from initial clinical trials have validated MSLN as an attractive target for cancer therapy with antibody-based approaches as well as tumor vaccines.[15]

The first patient to receive an MSLN-targeted therapy was a patient with ovarian cancer treated by Raffit Hassan in 2000 with the anti-MSLN agent SS1P.[16] Since then, there has been a remarkable proliferation of drugs targeting MSLN. Many are in advanced stages of clinical testing, including randomized trials for mesothelioma and pancreatic cancer. The discovery of MSLN and its validation as a target for cancer therapy have been previously described.[15] This review will focus on important therapeutic approaches as well as ongoing clinical trials and discuss the broad implications of targeting MSLN for cancer therapy.

MSLN

MSLN is a glycophosphatidylinositol (GPI) –linked cell-surface glycoprotein. It is synthesized as a 71-kD precursor protein and is then cleaved by the endoprotease furin to release the secreted N-terminal region, called megakaryocyte potentiating factor (MPF), whereas the 41-kD mature MSLN remains attached to the membrane.[2,17] The remaining GPI-linked mature MSLN can also be shed from the cell through the action of the tumor necrosis factor α–converting enzyme protease.[18] The normal physiologic distribution of MSLN identifies it as

a differentiation factor for mesothelial cells, but the biologic role that MSLN plays in these cells remains unclear. Database searches reveal that MSLN is remotely homologous to two inner ear proteins of unknown structure and contains no conserved consensus domains.[19] Three-dimensional structure prediction programs have determined that MSLN consists of a superhelical structure with armadillo-type repeats.[20] No crystal structure has yet been determined for the whole protein, but the structure of an N-terminal fragment bound to a Fab of the SS1 antibody has been obtained.[21] Furthermore, *MSLN* knockout mice grow and reproduce normally and have no detectable phenotype.[22]

MSLN is expressed by many solid tumors, with particularly robust expression in mesothelioma, epithelial ovarian cancer, and pancreatic adenocarcinoma (Table 1).[23-46] Higher expression of MSLN has been correlated with poorer prognosis for patients with ovarian cancer,[35] cholangiocarcinoma,[41,42] lung adenocarcinoma,[8,9] triple-negative breast cancer,[10,45] and resectable pancreatic adenocarcinoma.[32,33] In the neoplastic setting, MSLN is known to bind to the ovarian cancer antigen MUC16 (cancer antigen 125).[47] The two proteins are frequently coexpressed, and binding of MSLN and MUC16 has been shown to induce cell-to-cell adhesion in these cell types.[48] MUC16 expressed on cancer cells can also facilitate cancer cell attachment to the MSLN-expressing serosal surfaces in the pleura and peritoneum, possibly contributing to peritoneal seeding and metastatic spread. In addition, signaling mediated by MSLN and MUC16 binding has been reported to increase cellular resistance to anoikis,[49] upregulate matrix metalloproteinases important in cellular invasion and metastasis,[50-52] and induce secretion of autocrine growth factors by constitutively activating nuclear factor kappa B (NF-κB).[53,54] However, it seems that MSLN expression may also trigger signaling events independent of MUC16 binding.[51] The exact mechanics of these pathways and how MSLN interacts with components of the tumor microenvironment, including stromal cells, the extracellular matrix, and immune-cell populations, are not known.

Why MSLN, a glycoprotein normally restricted to serosal cells of the pleura, peritoneum, and pericardium, is expressed in a wide variety of adenocarcinomas is not clear at this time. However, regulation of MSLN expression in tumors has been assessed in several studies and seems to be cell-type specific. At the epigenetic level, it was found that hypomethylation did not correlate with MSLN expression in ovarian or endometrial cancer specimens[44] or in mesothelioma.[27] However, hypomethylation of the promoter was noted in MSLN-expressing pancreatic cancer specimens, and treatment of a nonexpressing pancreatic cancer cell line with demethylating agents could induce expression of MSLN, suggesting that epigenetic mechanisms may regulate MSLN expression in this cell type.[55] Transcription of MSLN is driven by a TATA-less promoter located upstream of the transcriptional start site. Enhancers responsible for initiating strong expression in normal serosal cells and cancers derived from them (eg, mesothelioma and ovarian cancer) are unknown. Cancer-specific ectopic upregulation in pancreatic and cervical cancers has been attributed to the transcriptional enhancer factor 1 (TEF-1) transcription factor binding to a conventional MCAT sequence within an upstream enhancer region called CanScript. However, TEF-1 expression itself is necessary but not sufficient to induce MSLN expression,

Table 1. MSLN Expression in Solid Tumors

Tumor Type	No. of Patients With MSLN Expression–Positive Disease (%)	References	Correlation of MSLN Expression With Prognosis
Mesothelioma	290 of 352 (82)	3,23-28	NA
Epithelioid	248 of 261 (95)		
Sarcomatoid	0 of 23 (0)	26,27	
Pancreatic adenocarcinoma	303 of 357 (85)	3,6,7,26,28-32	Poor[32,33]
Epithelial ovarian cancer	346 of 494 (70)	3,5,26,28-30,34	Conflicting[34,35]
High-grade serous	248 of 332 (75)		
Endometroid	36 of 52 (69)	3,5,29,30	
Mucinous	2 of 19 (11)	5,29,30	
Clear cell	11 of 21 (52)	3,29	
NSCLC	1,157 of 2,036 (57)	3,8,9,24-27,30,36	
Adenocarcinoma	1,082 of 1,686 (64)		Yes, poor[8,9]
Squamous	40 of 188 (21)		
SCLC	0 of 55 (0)	25,27,30	NA
Esophageal cancer	25 of 88 (28)	3,37	NS
Gastric cancer	312 of 666 (47)	3,26,28,30,38-40	Conflicting[38,39]
Biliary cancer			Yes, poor[41,42]
Extrahepatic	93 of 98 (95)	7,31,41,43	
Intrahepatic	1 of 10 (10)	31	
Other or unspecified	36 of 85 (42)	3,31,42	
Colorectal cancer	27 of 90 (30)	3,26,30	NS
Cervical cancer	1 of 4 (25)	3	NS
Endometrial cancer	34 of 58 (59)	3,26,30,44	NS
Breast cancer			Yes, poor[10,45]
Triple negative	33 of 50 (66)	10,46	
Other	1 of 61 (1.6)	10,46	
Unspecified	11 of 118 (9)	3,26,30	

Abbreviations: MSLN, mesothelin; NA, not applicable (expression is 0% or 100%); NS, not studied; NSCLC, non–small-cell lung cancer; SCLC, small-cell lung cancer.

suggesting an unknown cofactor is also required.[56] Similarly, the yes-associated protein 1 (YAP1) transcription factor binds to an SP-1 motif within the CanScript and is also required but insufficient for MSLN expression.[57] Further study will be required to delineate this mechanism. More recently, it was discovered that MSLN is reciprocally regulated at the post-transcriptional level by mIR-198 as part of a feedback loop that involves NF-kB and the homeobox transcription factors octamer transcription factor 2 (OCT-2), pre–B cell leukemia homeobox 1 (PBX-1), and valosin-containing protein (VCP).[58]

STRATEGIES TO TARGET MSLN

The high cell-surface expression of MSLN in many cancers lends itself to tumor-specific targeting using monoclonal antibodies, by themselves or carrying protein toxins or low–molecular weight cytotoxic agents, as well as by chimeric T cells containing Fv fragments that recognize MSLN (Fig 1). Other approaches in advanced clinical trials include vaccines that can induce T-cell immune response to MSLN.

Immunotoxins

SS1P is a recombinant protein therapeutic that consists of a high-affinity disulfide-bonded Fv that targets MSLN fused to a *Pseudomonas* exotoxin A (PE) payload.[59,60] SS1P binds to MSLN and is internalized by the cell through endocytosis. The toxin is delivered to the cytosol, where it irreversibly modifies elongation factor-2 to halt protein synthesis and induce apoptosis.[61] SS1P has been tested as a single agent as well as in combination with chemotherapy.[16,62,63] The dose-limiting toxicities (DLTs) are capillary leak syndrome, which is a class effect of immunotoxin therapies, and pleuritis, from on-target, but off-tumor, SS1P binding to mesothelial cells causing inflammation of the normal pleura. The single-agent maximum-tolerated dose (MTD) is 45 mcg/kg when administered as a bolus infusion on days 1, 3, and 5.[16] Additive toxicity was not observed in combination with cisplatin plus pemetrexed chemotherapy.[63] Efficacy of SS1P is limited by antidrug antibody formation, which typically occurs by the end of the first cycle (three doses) of treatment. For this reason, SS1P is now being administered in combination with a lymphocyte-depleting conditioning regimen of pentostatin and cyclophosphamide. This delays antidrug antibody formation and allows patients to receive multiple effective cycles of SS1P.[64]

LMB-100 (previously RG7787 and Ro6927005) is a recently developed recombinant immunotoxin that also targets MSLN. It consists of a humanized anti-MSLN Fab fragment with a newly designed PE (PE24) engineered to be less immunogenic than SS1P.[65] It also has decreased toxicity in animal models and shows broad activity against different MSLN-expressing cancers.[66-68] Phase I clinical trials of LMB-100 have recently opened for treatment of patients with mesothelioma and pancreatic cancer.

Vaccine

Elizabeth Jaffee's laboratory at Johns Hopkins was the first to identify MSLN as a vaccine-induced $CD8^+$ T-cell target using immunized lymphocytes from patients responding to granulocyte-macrophage colony-stimulating factor–secreting whole pancreatic tumor cell vaccine (GVAX), which expresses multiple antigens. Using an unbiased genomics-based screening approach, they identified MSLN as a target of vaccine-induced T cells in patients who were treated with GVAX who also demonstrated long-term disease-free survival.[69,70] Subsequently, in collaboration with scientists at Aduro Biotech, they developed a *Listeria monocytogenes* vaccine expressing MSLN (LM-mesothelin) and demonstrated in preclinical models that GVAX was the best priming vaccine and LM-mesothelin was the best boosting vaccine for the treatment of pancreatic cancer. These studies led to the clinical development of an LM-mesothelin for other cancers expressing MSLN.

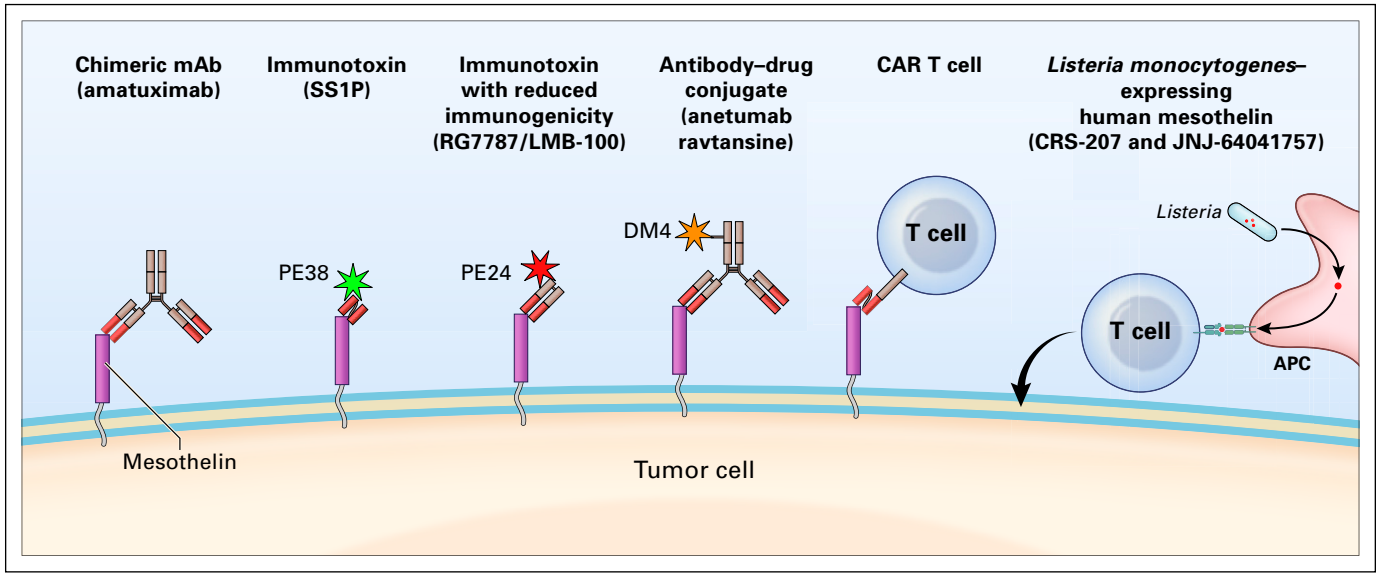

Fig 1. Approaches used to target MSLN in clinical trials. APC, antigen-presenting cell; CAR, chimeric antigen receptor; DM4, ravtansine; mAb, monoclonal antibody; PE, pseudomonas exotoxin.

CRS-207 is a recombinant live-attenuated, double-deleted *L monocytogenes* engineered to secrete MSLN into the cytosol of infected antigen presentation cells.[71] The bacteria retain the potency of the fully virulent pathogen but have vastly reduced toxicity as compared with wild-type *Listeria* because of the selective deletion of two virulence factors: ActA (ΔactA) and internalin B (ΔinlB). This blocks the direct internalin B–mediated infection of nonphagocytic cells, such as hepatocytes, and the indirect ActA-mediated infection by cell-to-cell spread from adjacent phagocytic cells. LM provides potent stimulation of innate immunity and also stimulates an adaptive immune response through recruitment and activation of $CD4^+$ and $CD8^+$ T cells specific for encoded heterologous antigens. *Listeria* ΔactA/ΔinlB-based vaccines were rapidly cleared from mice after immunization and induced potent and durable effector and memory T-cell responses with no measurable liver toxicity. In a phase I study, CRS-207 was well tolerated at doses up to a maximum planned dose of 1×10^{10} colony-forming units.[72] Immune activation and induction of MSLN-specific T-cell responses were observed.

Chimeric Monoclonal Antibody

Amatuximab (previously MORAb-009) is a chimeric high-affinity monoclonal immunoglobulin G1/k antibody targeting MSLN.[73] In vitro, amatuximab mediates inhibition of MSLN-dependent cell adhesion and antibody-dependent cellular cytotoxicity. Although it has moderate antitumor activity as a monotherapy against MSLN-expressing tumor xenografts in vivo, this effect is markedly increased in combination with chemotherapy. In a phase I trial, the DLTs were transaminitis and serum sickness; the MTD was determined to be 200 mg/m^2.[74]

Antibody–Drug Conjugates

Anetumab ravtansine (previously BAY 94-9343) is an antibody–drug conjugate consisting of a human anti-MSLN antibody conjugated to the maytansinoid tubulin inhibitor DM4 via a disulfide-containing linker. In preclinical studies, anetumab ravtansine was shown to be both potent and highly selective in killing MSLN-expressing tumor cells compared with MSLN-negative cells.[75] The drug is bound and internalized by MSLN-expressing tumor cells[75]; degradation of the BAY 94-9343 disulfide-based linker releases a cell-permeable DM4 metabolite with bystander killing potential. In vivo, anetumab ravtansine localizes specifically to MSLN-expressing tumors and inhibits tumor growth in pancreatic, ovarian, and mesothelioma cancer models. The MTD of anetumab ravtansine in a phase I trial was 6.5 mg/kg administered once every 3 weeks intravenously.[76] DLTs were keratitis and peripheral neuropathy. These adverse events were reversible and not life threatening, but they required dose reduction in approximately half of all patients. DMOT4039A, a humanized immunoglobulin G1 anti-MSLN monoclonal antibody conjugated to the antimitotic agent MMAE has completed phase I testing in patients with pancreatic and ovarian cancers (ClinicalTrials.gov identifier NCT01469793). The MTD was found to be 2.4 mg/kg when administered once every 3 weeks and 1 mg/kg when administered on a once-per-week schedule. DLTs were hyperglycemia, hypophosphatemia, and ileus.[77]

Chimeric Antigen Receptor T Cells

To make anti-MSLN chimeric antigen receptor (CAR) T cells, autologous patient T cells are modified to express an MSLN-binding T-cell receptor linked to appropriate cosignaling molecules, such that binding of these modified T cells to MSLN on cancer cells activates the cells to attack the tumor.[78,79] Given concerns that targeting MSLN could cause serositis, as is seen with the immunotoxin SS1P, an mRNA electroporation technique was used to engineer the anti-MSLN CAR T cells used in the initial clinical study, because this method produces only transient expression of the CAR (ClinicalTrials.gov identifier NCT1355965). Data from two patients in this study have been reported; both patients experienced transient responses, and neither developed serositis.[80] Given these promising results, two trials of anti-MSLN CAR T cells engineered by typical viral transduction methods have been initiated (ClinicalTrials.gov identifiers NCT01583686 and NCT02159716). More recently, it was also shown in preclinical studies that intrapleural infusion of anti-MSLN CAR T cells could enhance cell persistence and induce more durable responses[81]; a clinical trial of this delivery method is now open (ClinicalTrials.gov identifier NCT02414269). Because there have been recent reviews describing results from ongoing clinical trials as well as future directions of these therapeutics,[78,79] CAR T-cell clinical trials are not discussed in the remainder of this review.

CLINICAL DEVELOPMENT OF MSLN-TARGETED THERAPIES

Multiple clinical trials are currently evaluating different anti-MSLN agents for cancer therapy. Most of the efforts thus far have focused on malignant mesothelioma, pancreatic cancer, and ovarian cancer (Table 2).

Mesothelioma

Because a majority of malignant mesotheliomas have high and uniform cell-surface expression of MSLN, this disease is an especially good target for MSLN-directed therapies. However, sarcomatoid mesotheliomas, which constitute approximately 10% to 15% of all mesotheliomas, are not eligible for these therapies, because they lack MSLN expression. There are currently more than 10 clinical trials of anti-MSLN agents for treating mesothelioma, including one first-line registration trial and one registration trial in the second-line setting.

SS1P clinical trials. The anti-MSLN immunotoxin SS1P has been extensively evaluated in this tumor type. In the phase I clinical trials of SS1P, patients with mesothelioma were treated on an every-other-day bolus schedule[16] and on a continuous-infusion schedule.[62] These studies established the safety of MSLN as a target for cancer therapy. The DLT was pleuritis because of expression of MSLN on mesothelial cells lining the pleura. Surprisingly, patients did not develop pericarditis. However, antitumor activity was limited because most patients developed neutralizing antibodies against the toxin portion of SS1P and could not receive additional effective treatment cycles. On the basis of laboratory studies, two different approaches were pursued to increase the efficacy of SS1P. Given the remarkable antitumor synergy between SS1P and chemotherapy in vivo, SS1P was tested in combination with

Table 2. Selected Ongoing Clinical Trials Using MSLN-Targeted Therapies

ClinicalTrials.gov Identifier	Trial	Phase	Randomized	Investigational Agent	Disease Setting	Recruiting Centers
NCT02357147	Amatuximab or placebo plus cisplatin and pemetrexed	II	Yes	Amatuximab	Unresectable malignant pleural mesothelioma	United States, Europe, Australia
NCT02610140	Anetumab ravtansine *v* navelbine	II	Yes	Anetumab ravtansine	Second-line treatment of MSLN-expressing mesothelioma	United States, Canada, Europe
NCT02004262	GVAX plus CRS-207 *v* CRS-207 *v* chemotherapy	II	Yes	CRS-207	Previously treated metastatic pancreatic adenocarcinoma	United States, Canada
NCT01362790	MSLN-targeting immunotoxin plus pentostatin and cyclophosphamide	II	No	SS1P	Epitheloid mesothelioma, lung and pancreatic cancers	United States
NCT02798536	Reduced immunogenicity MSLN-targeted immunotoxin LMB-100	II	No	LMB-100	Previously treated epitheloid mesothelioma	United States
NCT02810418	Reduced immunogenicity MSLN-targeted immunotoxin LMB-100	II	No	LMB-100	Previously treated metastatic and/or locally advanced pancreatic ductal adenocarcinoma	United States
NCT02243371	GVAX pancreas vaccine and CRS-207 with or without nivolumab	II	No	CRS-207	Metastatic pancreatic cancer with progression after one prior chemotherapy	United States
NCT02575807	CRS-207 with or without epacadostat	I/II	No	CRS-207	Platinum-resistant ovarian, fallopian, or peritoneal cancer	United States
NCT01675765	CRS-207 plus pemetrexed and cisplatin	Ib	No	CRS-207	Unresectable malignant pleural mesothelioma	United States
NCT02592967	*Listeria monocytogenes*–based vaccine	I	No	JNJ-64041757	NSCLC with at least two prior lines of systemic therapy	United States
NCT02485119	Antibody–drug conjugate	I	No	BAY94-9343	Advanced solid tumors not amenable to standard therapy	Japan
NCT02341625	Antibody–drug conjugate	I/IIa	No	BMS-986148	Pancreatic, ovarian, or gastric cancer, NSCLC, or mesothelioma	United States, Australia, Canada
NCT02159716	CAR T-meso immunotherapy	I	No	Anti-MSLN CAR	Metastatic pancreatic adenocarcinoma; recurrent serous epithelial ovarian cancer or primary peritoneal carcinoma; epitheloid pleural mesothelioma; failure of at least one standard-of-care chemotherapy for advanced-stage disease	United States
NCT02580747	CAR T-meso immunotherapy	I	No	Anti-MSLN CAR	Chemotherapy-refractory or -relapsed MSLN-positive malignant mesothelioma, ovarian tumors, pancreatic cancer, triple-negative breast cancer, endometrial cancer, other MSLN-positive tumors	China
NCT02465983	CAR T-meso plus CAR T-19 immunotherapy	I	No	Anti-MSLN CAR plus CD19 CAR	Unresectable or metastatic pancreatic cancer	United States
NCT01583686	CAR T-meso immunotherapy	I/II	No	Anti-MSLN CAR	Recurrent or refractory MSLN-expressing metastatic or unresectable cancer	United States
NCT02414269	Intrapleurally administered MSLN-targeted T cells	I	No	Autologous T cells engineered to target MSLN	MSLN-expressing pleural mesothelioma, NSCLC, breast cancer metastatic to the pleura	United States

Abbreviations: CAR, chimeric antigen receptor; CAR T-meso, mesothelin-directed chimeric antigen receptor–modified T cells; MSLN, mesothelin; NSCLC, non–small-cell lung cancer.

pemetrexed and cisplatin as first-line therapy for pleural mesothelioma.[63] Patients who were not candidates for curative surgical resection received pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 on day 1 of a 21-day cycle for a maximum of six cycles; SS1P 45 μg/kg was administered on days 1, 3, and 5 during cycles one and two only. This study demonstrated the safety of the combination. The most common SS1P-related toxicities included hypoalbuminemia, fatigue, hypotension, and edema. Of the 20 evaluable patients treated in this study, 12 (60%) experienced objective tumor responses, and three had stable disease. This study also showed that serum MSLN and serum MPF levels correlated with radiologic tumor response. However, the addition of chemotherapy did not delay the formation of anti-SS1P neutralizing antibodies.

Because neutralizing antibodies to immunotoxins limited their clinical efficacy, strategies were developed to circumvent this. Single-agent immunosuppressive agents, such as steroids, cyclosporine, and rituximab, were not successful.[82] A preclinical study showed that coadministration of pentostatin and cyclophosphamide ended anti-SS1P antibody formation in immunocompetent mice.[83] On the basis of these results, a pilot study was designed in

which patients with mesothelioma for whom standard therapies had failed received pentostatin and cyclophosphamide before SS1P. Using this approach, only two of 10 patients developed anti-SS1P antibodies after the first cycle compared with 88% of patients in prior studies of SS1P alone.[64] This regimen induced lymphopenia without neutropenia, and none of the patients developed opportunistic infections. More importantly, three of 10 evaluable patients experienced major cancer regressions that were long lasting. To avoid use of an immunosuppressive regimen with immunotoxin therapy, a less immunogenic anti-MSLN immunotoxin, LMB-100 (RG7787), was developed and is now being evaluated in a clinical trial for treatment of patients with mesothelioma for whom prior chemotherapy has failed (ClinicalTrials.gov identifier NCT02798536).

Amatuximab for pleural mesothelioma. On the basis of the phase I study of amatuximab in patients with advanced solid tumors that established its safety[74] and laboratory data showing synergy with chemotherapy in preclinical in vivo models, a phase II study of amatuximab was initiated in patients with mesothelioma.[84] In this nonrandomized trial, 89 patients with malignant pleural mesothelioma who had received no prior chemotherapy and were not candidates for surgical resection were treated with pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 on day 1 of a once-every-3-weeks cycle for six cycles. Amatuximab 5 mg/kg was administered on days 1 and 8 of a 21-day cycle. The primary end point of this study was improvement of progression-free survival (PFS). The objective response rate by independent radiologic review was 40%, and 51% of patients had stable disease, for an overall disease control rate of 91%. Although the study did not meet its primary end point of a 6-month PFS response rate of 62%, the overall survival of patients was 14.8 months, which is better than historical controls with pemetrexed and cisplatin alone. Analysis of the pharmacokinetic data showed that serum amatuximab trough concentrations greater than the population median of 38.2 μg/mL were associated with significant improvement of both PFS as well as overall survival. Overall survival was 583 days for patients with amatuximab trough concentrations greater than 38.2 μg/mL versus 375 days for patients with amatuximab trough concentration less than 38.2 μg/mL.[85] Pharmacodynamic modeling shows that administering amatuximab 5 mg/kg once per week will allow 80% of patients to achieve amatuximab trough concentrations greater than 38.2 μg/mL. On the basis of these data, a randomized phase II registration clinical trial of amatuximab plus pemetrexed and cisplatin versus pemetrexed and cisplatin for patients with newly diagnosed unresectable mesothelioma has been initiated (ClinicalTrials.gov identifier NCT02357147).

Anetumab ravtansine trials. Anetumab ravtansine was evaluated in a phase I clinical trial in patients with MSLN-expressing cancers that included an expansion cohort of patients with mesothelioma.[76] In this trial, anetumab ravtansine was administered to patients in increasing doses once every 3 weeks; the MTD of anetumab ravtansine with this schedule was 6.5 mg/kg. The DLTs at 7.5 mg/kg were keratitis and neuropathy. The activity of anetumab ravtansine in patients with MSLN-positive malignant mesothelioma was evaluated in an expansion cohort. Of 16 patients with mesothelioma treated at the MTD, five patients (31%) experienced objective tumor responses, and seven (44%) had stable disease. However, in patients with pleural mesothelioma who received anetumab ravtansine as second-line therapy, five (50%) of 10 experienced objective partial responses, and four (40%) had stable disease. These results have led to a randomized phase II trial of anetumab ravtansine as second-line therapy for patients with mesothelioma (ClinicalTrials.gov identifier NCT02610140). In this registration clinical trial, patients are randomly assigned to anetumab ravtansine 6.5 mg/kg once every 3 weeks versus vinorelbine 30 mg/m^2 once per week, with progression-free survival as the primary end point.

CRS-207 study in pleural mesothelioma. On the basis of a phase I study showing the safety of CRS-207 and induction of MSLN-specific T-cell immune response in patients with mesothelioma,[72] a phase Ib clinical trial of CRS-207 plus pemetrexed and cisplatin was initiated in patients with pleural mesothelioma who were not candidates for surgical resection. Patients received two doses of intravenous CRS-207 2 weeks apart, followed 2 weeks later by standard doses of pemetrexed and cisplatin for four to six cycles. Patients achieving a response or stable disease received two more doses of CRS-207 once every 3 weeks as maintenance. As of December 2015, 38 patients had been enrolled in the study. The most common CRS-207 toxicities were similar to those seen in the phase I trial, consisting mostly of grade 1 fever (79%) and chills or rigor (82%). Of the 34 evaluable patients treated in the study, 20 (59%) experienced objective partial responses, and 12 (35%) had stable disease, for a disease control rate of 94%.[86]

Pancreatic Cancer

Because several studies have shown high expression of MSLN in pancreatic cancer, different approaches to targeting it are being evaluated in the clinic, including MSLN vaccine, chimeric antibody, and antibody–drug conjugate.

Clinical trials of CRS-207 in pancreatic cancer. A heterologous prime–boost strategy using cyclophosphamide with GVAX pancreas followed by CRS-207 was tested in previously treated patients with metastatic pancreatic cancer. Cyclophosphamide was administered the day before GVAX to inhibit regulatory T cells. Patients in the comparator arm received cyclophosphamide plus GVAX alone. Despite a heavily pretreated patient population, there was an overall survival benefit favoring the prime–boost arm (6.1 *v* 3.9 months; hazard ratio, 0.59; $P = .02$).[87] A follow-up study comparing the efficacy of CRS-207 alone versus prime–boost vaccine versus chemotherapy in patients who had received two or more prior regimens for metastatic disease was recently completed (ClinicalTrials.gov identifier NCT02004262). The primary end point examining overall survival was not reached, with a median overall survival of 3.8 months for patients treated with CRS-207 plus GVAX pancreas, 5.4 months for patients treated with CRS-207 alone, and 4.6 months for patients treated with chemotherapy (D. Le, personal communication, September 2016). Ongoing and future strategies are aimed at combinatorial strategies with other immune modulators. A second study testing the prime–boost strategy with or without programmed death 1 (PD-1) inhibition is almost complete (ClinicalTrials.gov identifier NCT02243371). Results of these studies will inform further development of these agents.

Antibody-based therapies for pancreatic cancer. The antibody–drug conjugate DMOT4039A was recently tested in a phase I study

that included patients with previously treated advanced pancreatic cancer. In this study, MSLN expression was not prospectively assessed in the majority of enrolled patients; however, the last 10 patients enrolled were required to have 2+ or 3+ expression to join. Responses were seen in two of 26 patients treated at the recommended phase II dose.[77]

On the basis of antitumor efficacy of the antimesothelin immunotoxin SS1P administered in combination with pentostatin and cyclophosphamide in patients with malignant mesothelioma, one study is currently enrolling patients with advanced previously treated pancreatic cancer (ClinicalTrials.gov identifier NCT01362790). In addition, based on preclinical studies showing remarkable synergy between nab-paclitaxel and LMB-100, a phase I clinical trial of this combination has just started accrual for patients with previously treated pancreatic cancer with progressive disease (ClinicalTrials.gov identifier NCT02810418).

Non–Small-Cell Lung Cancer

MSLN mRNA and protein are present in a substantial number of lung adenocarcinomas.[8,9,25,30,36] Recent studies have identified MSLN expression in more than 50% of both early-stage and advanced lung adenocarcinomas. Kachala et al[9] found MSLN expression in 69% of early-stage (stages I to III) lung adenocarcinomas, with approximately 20% of patients strongly expressing MSLN. Thomas et al[8] observed MSLN expression in 53% of advanced (stages IIIB to IV) lung adenocarcinomas, with high expression found in approximately 25% of patients. The studies also indicated that MSLN expression might be an independent driver of an aggressive tumor phenotype; in both series, high MSLN expression was independently associated with inferior overall survival. Tumors with high MSLN expression were also more likely to have *KRAS* mutations, compared with tumors with low MSLN expression. Given its frequent expression and possibly important role in lung adenocarcinoma growth and dissemination, MSLN is being actively investigated as a potential therapeutic target in patients with therapy-resistant lung adenocarcinoma. A phase I study is evaluating the safety and immunogenicity of JNJ-64041757, a live attenuated *L monocytogenes* vaccine in patients with non–small-cell lung cancer (ClinicalTrials.gov identifier NCT02592967).

Ovarian Cancer

The antibody–drug conjugate DMOT4039A was recently tested in a phase I study that included patients with platinum-resistant ovarian cancer.[77] In this study, MSLN expression was prospectively assessed, and 3+ expression by immunohistochemistry was required for eligibility. Responses were seen in three of 10 patients treated once every 3 weeks and one of 12 patients treated on the once-per-week administration schedule.

FUTURE DIRECTIONS

There has been substantial progress in the development of different approaches to target MSLN for cancer therapy. Although there was initial concern that pericardial toxicity could be limiting in the development of MSLN-targeted agents because of mesothelin expression on mesothelial cells lining the pericardium, this has not been seen in patients. Because MSLN is highly expressed in many cancers, these therapies could have broad implications for treatment of patients with solid tumors. In the case of malignant mesothelioma, two drugs are currently in multicenter registration clinical trials. These include amatuximab plus pemetrexed and cisplatin versus chemotherapy alone in untreated patients with unresectable pleural mesothelioma. In the second-line setting, a randomized clinical trial of anetumab ravtansine versus vinorelbine is currently ongoing in patients with pleural mesothelioma who experienced progression with prior pemetrexed and cisplatin therapy. Clinical trials of anti-MSLN immunotoxins are also ongoing for previously treated patients with mesothelioma. In the case of pancreatic cancer, a randomized clinical trial of GVAX pancreas vaccine and CRS-207 with or without nivolumab is ongoing in patients with metastatic disease. In addition, the combination of LMB-100 with nab-paclitaxel is being evaluated in patients with locally advanced and metastatic pancreatic cancers for whom prior therapies have failed. A new clinical trial of an attenuated *L monocytogenes*–expressing MSLN is currently ongoing in lung cancer.

Because some of the MSLN-directed therapies may have nonoverlapping toxicity with chemotherapy and immunotherapy agents and could potentially result in synergistic activity, combination clinical trials have just been initiated. These include anetumab ravtansine with chemotherapy for treatment of patients with mesothelioma and lung cancer and clinical trials of CRS-207 plus immune checkpoint inhibitors for pancreatic cancer. However, with regard to combination therapy trials, it will be important to have good scientific rationale before conducting large-scale clinical trials. For example, in the case of lung adenocarcinoma, to combine MSLN-directed agents with immune checkpoint therapy, it would be important to have a good understanding of coexpression of MSLN as well as programmed death ligand 1 (PD-L1). Furthermore, additional clinical trials for some of the agents we have described are being planned for assessment in additional cancers with MSLN expression, such as gastric cancer, endometrial cancer, cholangiocarcinoma, and triple-negative breast cancer. Finally, it is imperative to identify and validate companion assays to detect tumor MSLN expression.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Mesothelin Immunotherapy for Cancer: Ready for Prime Time?

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