Research Highlight

Mesothelin-targeted immunotherapies for malignant pleural mesothelioma

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Introduction

Despite the use of aggressive trimodality therapy consisting of neoadjuvant chemotherapy, surgery, and hemithoracic radiation, the median survival of patients with malignant pleural mesothelioma (MPM) is 12 to 18 months (1,2). Immune responses have been shown to be beneficial in MPM patients (3,4); therefore, focusing immunotherapeutic strategies on promoting these immune responses is an attractive approach. One such approach involves targeting cancer-associated antigens highly expressed on mesothelioma cells by use of monoclonal antibodies, recombinant immunotoxins, vaccines, or genetically engineered T cells. Targeted antigens can be either cell-surface antigens, such as mesothelin, or intracellular antigens, such as WT-1. Cell-surface antigens are favored for the immunotherapeutic approach because of their ease of targeting.

Mesothelin as a cancer-antigen target in MPM

Antigen expression

Mesothelin is a 70-KDa cell-surface glycoprotein expressed at very low levels by a single layer of mesothelial cells lining the pleura, peritoneum, and pericardium (5). High-level expression of surface mesothelin has been reported in multiple malignancies, including mesothelioma (5-8). While others have reported mesothelin expression in MPM (9), our group has documented uniform and high-level cell-surface expression of mesothelin in epitheloid and biphasic MPM, which was retained in tumor samples even after chemotherapy, as well as in metastatic lymph nodes (10).

Pathobiology

Studies suggest that mesothelin plays a role in tumorigenesis by increasing cellular proliferation and migration (11) and is a marker of neoplastic progression (12). Our group has demonstrated—both in an orthotopic MPM mouse model and in patients—that mesothelin contributes to the locoregional aggressiveness characteristic of MPM (10). Mesothelin overexpression is associated with the expression of metalloproteinase-9 (MMP-9), a protein involved in the degradation of extracellular matrix, facilitating cancer cell migration and local invasion (10).

Biomarker

Robinson *et al*. first reported that the determination of soluble mesothelin-related protein (SMRP) levels in the serum could be used as a marker for detection of MPM, with a sensitivity of 84% and a specificity of 100% (13). Preclinical and clinical studies have now demonstrated that serum SMRP levels correlate with stage and tumor burden and may be a useful marker for following the therapy response in patients with MPM (14,15).

Mesothelin-targeted immunotherapy

Given its high levels of expression in MPM and its limited expression in normal tissues, mesothelin provides a safe target for tumor-specific therapies. The following mesothelin-targeted immunotherapeutic approaches are currently the focus of clinical trials (*Table 1*).

Recombinant immunotoxin

SS1P is an immunotoxin that comprises a high-affinity antimesothelin mouse antibody chemically conjugated to a *Pseudomonas* exotoxin (26). In preclinical models, SS1P

Table 1 Mesothelin-targeted immunotherapies in clinical trials for malignant pleural mesothelioma

Agent	Phase	Intervention	Mechanism	References
SS1P	I	Alone (completed) Phase I with cisplatin and pemetrexed Phase I with pentostatin and cyclophosphamide	Antibody conjugated to a *Pseudomonas* exotoxin	(16-19)
MORAb-009	I/II	Alone (completed) Phase II with cisplatin and pemetrexed	Monoclonal antibody (ADCC)	(20,21)
BAY 94-9343	I	Alone	Antibody-drug conjugate	(22)
CRS-207	I	Alone	Vaccine	(23)
Adoptive T-cell therapy	I	CAR T cells alone and with fludarabine, cyclophosphamide, and aldesleukin	CAR-transduced T cells	(24,25)

Abbreviations: ADCC, antibody dependent cell-mediated cytotoxicity; CAR, chimeric antigen receptor

has shown regression of mesothelin-expressing tumors, both alone and in conjunction with chemotherapy and radiation therapy (26-29). Evaluation of two phase I single-agent clinical trials (consisting of 58 mesothelin-expressing solid tumor patients) showed 5 subjects with partial response and 18 with stable disease. A dose-limiting toxicity in these trials was pleuritis, possibly caused by the targeting of normal mesothelial cells in the pleura (16,17). A third phase I trial, treating MPM patients with SS1P plus a standard regimen of chemotherapy, demonstrated 7 patients with partial response, 3 with stable disease, and 4 with progressive disease; the trial is now in the expansion phase (18).

Monoclonal antibody

MORAb-009 is a high-affinity chimeric monoclonal antibody that consists of a mouse antimesothelin single-chain variable fragment that binds mesothelin, grafted to a human IgG1 and κ constant regions (30). MORAb-009 works by eliciting antibody-dependent cellular cytotoxicity in which cells of the immune system lyse target cells expressing the mesothelin-bound antibody. In a phase I single-agent clinical study in which patients with refractory mesothelin-expressing solid tumors were treated, no complete or partial response was noted; 11 subjects had stable disease, and 9 had progressive disease. MORAb-009 displayed a promising safety profile, with the most common adverse reaction being infusion-related drug hypersensitivity that was amenable to antihistamine (20). Currently, phase II clinical trial of MORAb-009 in combination with chemotherapy is ongoing in mesothelioma patients (21).

Antibody-drug conjugates

Two mesothelin antibody-drug conjugates that have undergone preclinical testing are MDX-1382 (Medarex), an antibody conjugated to a potent DNA alkylating agent, and BAY 94-9343 (Bayer Healthcare), an antibody linked to a DM4 moiety that binds to tubulin (31,32). A phase I clinical trial of BAY 94-9343 for patients with solid tumors recently opened (22), and the drug has been adopted by the United States Orphan Drug Act, which will encourage its development and shorten the regulatory review and approval process for MPM patients.

Antimesothelin vaccine

CRS-207 is a vaccine that uses a live, attenuated strain of the bacterium *Listeria monocytogenes* to present human mesothelin to the host immune system (33). Recently, 17 patients (including 5 patients with mesothelioma) were treated in a phase I clinical trial (23). Although no objective tumor response was seen, CRS-207 was well-tolerated, and immune activation was observed, with 5 of 10 patients showing mesothelin-specific T-cell response. A phase II clinical trial in pancreatic cancer patients treated with CRS-207 and granulocyte macrophage-colony stimulating factor-expressing cells showed promising results, with prolonged survival observed in patients with mesothelin-specific immune cell responses (34).

Adoptive T-cell therapy

One recent advance in immunotherapy is the ability to use peripheral blood lymphocytes to generate genetically

redirected T cells that target tumor antigens by the use of retroviral and lentiviral vectors (35). The safety and long-term persistence of these viral vectors were documented in a decade-long follow-up of HIV patients (36). These engineered T cells have the ability to circumvent tolerance to tumor antigens such as mesothelin (37). Furthermore, the ability to genetically reprogram T cells allows for the addition of costimulatory molecules, such as CD-28 and 4-1BB, to increase T-cell survival and expansion and to prevent anergy. In these cells, antigen recognition is usually achieved by expressing antigen receptors, which consist of either physiological, major histocompatibility complex (MHC)-restricted T cell receptors, or non-MHC-restricted chimeric antigen receptors (CARs) (35,38). Currently, there are two phase I clinical trials using antimesothelin CAR-transduced T cells. A National Cancer Institute clinical trial is treating metastatic or unresectable cancers that express mesothelin with CAR T cells, in conjunction with myeloablative chemotherapy and/or aldesleukin (an interleukin (IL)-2 analog), to augment CAR T-cell persistence (24). In the second phase I clinical trial, MPM patients are receiving 1 to 3 doses of mesothelin-targeting CAR T cells (25). In this trial, CARs are introduced into the T cells by electromicroporation, to enhance safety by limiting the survival of the T cells (39).

Current perspectives in mesothelin-targeted immunotherapy

Clinically relevant mouse model

The flank- and peritoneal-tumor animal models that have been used to date in preclinical studies of MPM do not reflect the clinical progression or outcomes seen in MPM patients. To recapitulate clinical MPM, our group has developed an orthotopic MPM mouse model (40). The resultant encasing tumors mimic human disease anatomically, with gross pleural and mediastinal tumor distribution and frequent chest wall and diaphragmatic invasion. In addition to metastasis to mediastinal lymph nodes, extensive angiogenesis and lymphogenesis attributable to high-level secretion of vascular endothelial growth factor were observed (10). Notably, tumor burden and progression can be reliably measured by magnetic resonance imaging, bioluminescence imaging, and serum SMRP levels (40). An orthotopic MPM mouse model that recapitulates human MPM pathobiology not only provides an appropriate *in vivo* tumor microenvironment in which to investigate novel therapeutic agents but also generates information regarding pharmacokinetics and tumor delivery of agents that will aid in designing future clinical trials.

Targeting tumor heterogeneity

Although epithelioid MPM expresses mesothelin uniformly, biphasic MPM tumors have a potential heterogeneity, which increases the complexity of targeting a single antigen. It is therefore critical to appropriately characterize the antigen expression of the targeted tumor, as well as that of other potentially exploitable targets. Targeting mesothelin-expressing cells may lead to a less-aggressive, more-manageable tumor that is possibly more susceptible to standard therapeutic agents, such as chemotherapy.

Targeted agent delivery to the tumor microenvironment

In clinical trials, shed mesothelin has been shown to cause low antitumor efficacy, because of binding of SS1P antibody to serum mesothelin, with a decreased amount of antibody reaching the tumor (16). A subsequent study found increased antitumor efficacy by combining taxol and SS1P, possibly attributable to taxol decreasing mesothelin shedding (41). Other strategies have focused on enhancing the tumor trafficking of the therapeutic agent by simultaneously targeting tumor-associated chemokines. Moon *et al.* added a chemokine receptor to a CAR, thereby increasing T-cell trafficking to the tumor (42).

On-target off-tumor toxicity

The potential targeting of normal cells expressing mesothelin may have been seen in the SS1P trial in which the dose-limiting toxicity was pleuritis. Therefore, strategies to protect against possible toxicities are needed. One approach to prevent adverse outcomes in CAR T-cell therapy is to incorporate a safety, or "suicide", gene in the transferred cells. In this strategy, a prodrug that is administered for use in the case of an adverse event is activated by the suicide-gene product and kills the transduced cell (43). Three promising suicide genes—herpes simplex virus thymidine kinase (HSV-TK), deoxycytidine kinase (dCK), and inducible caspase 9 (44,45)—are currently undergoing investigation by our group. An added translational advantage of HSV-TK and dCK is their ability to be imaged by positron emission tomography scan, which

may serve as a useful tool for future T-cell studies.

Host immune response

Immunogenicity to the SS1P immunotoxin may have inhibited maximal drug delivery to the tumor microenvironment; such a concern is the reason for pretreating patients with the lymphoablative drugs pentostatin and cyclophosphamide in a current clinical trial (19). In CAR T-cell therapy, immunomodulating cytokines, such as IL-12, have proven to be efficacious in decreasing the host immune response, thus decreasing the need for lymphoablation (46).

Conclusions

Mesothelioma-specific overexpression of mesothelin and its role in tumor aggressiveness provide an opportunity to target mesothelin with immunotherapeutic agents. The recent advances in the understanding of the kinetics of therapeutic-agent distribution and associated limiting factors, through noninvasive tumor and T-cell imaging, as well as the ability to measure serum SMRP levels, have enhanced our ability to obtain maximal information for clinical trials in rare diseases such as mesothelioma. Measures to counteract the immune-inhibitory environment, such as therapy with anti-PD1 or anti-CTLA-4, have not yet been investigated in MPM. This approach, which combines promoting an immune response with counteracting an immune-inhibitory environment, will play a significant role in the future course of immunotherapeutic treatment of MPM.

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