

Immunotherapy for Malignant Pleural Mesothelioma Current Status and Future Prospects

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Abstract

Malignant pleural mesothelioma (MPM) is a rare malignancy of the pleura that is frequently resistant to conventional therapies. Immunotherapy is a promising investigational approach for MPM that has shown some evidence of clinical benefit in select patients. However, tumor-induced immunosuppression is likely a major impediment to achieving optimal clinical responses to immunotherapeutic intervention. MPM contains a variable degree of infiltrating T-regulatory cells and M2 macrophages, which are believed to facilitate tumor evasion from the host immune system. Additional immunosuppressive factors identified in other human tumor types, such as tumor-associated programmed death ligand-1 expression, may be relevant for investigation in MPM. Conventional cytoreductive therapies, such as radiation, chemotherapy, and surgery, may play a critical role in successful immunotherapeutic strategies by ablating intratumoral and/or systemic

immunosuppressive factors, thus creating a host environment more amenable to immunotherapy. This article reviews the immunotherapeutic approaches being evaluated in patients with MPM and discusses how immunotherapy might be rationally combined with conventional tumor cytoreductive therapies for this disease.

Keywords: mesothelioma; immunotherapy; immunosuppression; multimodality.

Clinical Relevance

This is a concise review of recent advances in immunotherapy for malignant pleural mesothelioma. This article also provides guidance on how immunotherapy might be rationally combined with conventional tumor cytoreductive therapies to achieve improved tumor responses in patients.

Although the overall prevalence of malignant pleural mesothelioma (MPM) is low in the general population (~ 2,000–3,000 new cases in the United States annually), it can occur in anyone from shipyard workers to teachers to close relatives of those who have worked with asbestos (1). The prognosis for MPM is poor, with a median survival of ≤ 12 months and a 5-year survival rate of less than 5% (2). The only treatment shown to prolong overall survival in randomized phase III trials is pemetrexed/cisplatin combination chemotherapy, which prolongs median survival by 2.8 months (3). There is preliminary clinical evidence that

immunotherapy—in particular intrapleural IFN administration, mesothelin-targeted antibody, and immune checkpoint blockade—may produce tumor control in select patients with MPM. Anecdotally, early-stage disease and epithelioid histology appear to be most amenable to immunotherapeutic intervention.

Passive Immunotherapy: Cytokines and Mesothelin-Targeted Antibodies

Cytokines such as ILs and IFNs activate host immune responses against viruses and

tumors. IFNs also have direct cytotoxic/cytostatic effects against transplantable murine mesothelioma tumors and human mesothelioma cells *in vitro*. Astoul and colleagues reported that intrapleural IL-2 administration produced objective clinical responses in 12 of 22 (54%) patients with MPM, with all responders having early-stage epithelioid histology (4). Median survival for responders was 28 months, compared with 8 months for nonresponders (4). Clinical outcomes using other routes of IL-2 administration, however, are conflicting (reviewed in Ref. 5).

(Received in original form November 5, 2013; accepted in final form January 11, 2014)

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Am J Respir Cell Mol Biol Vol 50, Iss 5, pp 870–875, May 2014

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Originally Published in Press as DOI: 10.1165/rcmb.2013-0472TR on January 22, 2014

Internet address: www.atsjournals.org

IFNs have shown potential for treating MPM. Because systemic IFN administration has suboptimal toxicity and efficacy profiles, attempts have been made to improve the therapeutic index of IFNs through direct intrapleural administration. In a phase I trial of 89 patients with early-stage MPM, intrapleural IFN- γ administration showed clinical benefit, with an overall response rate of 20% (6). However, IFN- γ has been abandoned due to excessive toxicity. Clinical trials evaluating intrapleural instillation of adenovirus-encoding IFN- β (Ad-IFN- β) or adenovirus-encoding IFN- α 2b (Ad-IFN- α 2b) suggest that these cytokines have therapeutic potential for MPM. Serman and colleagues reported that intrapleural treatment with Ad-IFN- β or Ad-IFN- α 2b induced measurable antitumor immune responses in the majority of patients with MPM or other pleural malignancies (7–9). Disease stabilization or tumor regression was observed in a subset of patients (5 of 18 with Ad-IFN- β , compiled from two studies, and 5 of 9 with Ad-IFN- α 2b). Responses in patients with MPM were limited primarily to those with early-stage disease, and induction of antiadenovirus-neutralizing antibodies severely limited the effectiveness of repeated dosing (8). To potentially augment tumor responses in advanced MPM, an ongoing pilot trial conducted by Serman and colleagues is evaluating intrapleural Ad-IFN- α 2b treatment combined with chemotherapy, which might reverse tumor-induced immunosuppression (ClinicalTrials.gov identifier NCT01119664).

Mesothelin-targeted immunotoxin therapy, specifically when combined with host immune depletion via chemotherapy, has shown promise for treating MPM and peritoneal mesothelioma. Hassan and colleagues reported that systemic SS1P antimesothelin immunotoxin treatment combined with pentostatin/cyclophosphamide treatment produced significant tumor shrinkage in 3 of 10 patients and disease stabilization in another 3 of 10 patients (10). SS1P, a fusion of an antimesothelin antibody fragment linked to a portion of *Pseudomonas* exotoxin A, was originally believed to have direct cytotoxicity against mesothelioma. However, tumor shrinkage in two patients was not evident until 4 to 7 months after initiation of SS1P treatment. This observation resembles

delayed tumor responses seen in other immune-based therapies, possibly due to augmented endogenous antitumor immunity elicited by pentostatin/cyclophosphamide chemotherapy. Chemotherapy can selectively deplete immunosuppressive T-regulatory (Treg) cells, which might be relevant to the antitumor mechanism of action of SS1P + pentostatin/cyclophosphamide treatment. There is an ongoing phase II trial evaluating amatuximab, an ¹¹¹Indium-radiolabeled antimesothelin antibody, in patients with MPM (ClinicalTrials.gov identifier NCT00738582). Adjunctive pemetrexed/cisplatin chemotherapy is also being used in this study and thus will be an interesting comparator to SS1P + pentostatin/cyclophosphamide therapy.

Therapeutic Vaccines

Therapeutic cancer vaccines require effective antigen presentation, and the most frequent antigen presentation strategy uses dendritic cells (DCs) loaded with tumor-associated antigens. Large numbers of autologous DCs can be differentiated *ex vivo* from peripheral blood precursors, making this approach feasible for clinical translation. In a phase I trial of 10 patients with MPM, vaccination with autologous tumor lysate-loaded DCs induced tumor-specific immunity in some patients, albeit a minority (11). However, there was no correlation between immune responses and clinical outcomes.

Other therapeutic vaccines evaluated for MPM include those manufactured from autologous tumor cells or consisting of synthetic MPM antigen-derived peptides. In early-phase clinical trials, these vaccines induced measurable tumor-specific immune responses in the majority of patients (12, 13). Despite this, clinical responses were generally not observed, although disease stabilization may have occurred in some patients. There are four phase I/II trials testing manufactured vaccines for MPM. Two trials are evaluating Wilms-tumor-1 peptide vaccination after surgery (ClinicalTrials.gov identifier NCT01265433) or multimodality therapy (ClinicalTrials.gov identifier NCT01890980). One trial is testing mesothelin-encoding *Listeria monocytogenes*-based vaccination before pemetrexed/cisplatin chemotherapy

(ClinicalTrials.gov identifier NCT01675765), and one trial is testing allogeneic tumor cell vaccination combined with metronomic cyclophosphamide and celecoxib treatment (ClinicalTrials.gov identifier NCT01143545).

Adoptive Transfer of *Ex Vivo* Engineered Chimeric Antigen Receptor T Lymphocytes

Chimeric antigen receptors (CARs) specific for tumor-associated antigens can be stably introduced into bulk peripheral blood T lymphocytes *ex vivo*, thus redirecting their native specificities toward cognate antigen-expressing tumors. The most common forms of CARs are fusions of single-chain variable fragments derived from high-affinity antigen binding regions of murine antihuman antibodies and human CD3- ζ transmembrane/intracellular signaling domains. CARs are stably transduced into T lymphocytes using lentivirus, whereas mRNA transfection is used for transient CAR expression. CAR T lymphocytes are major histocompatibility complex (MHC) independent, which obviates the need for human leukocyte antigen matching. This allows a single manufactured CAR product to be used for modifying T lymphocytes in broad patient populations irrespective of genetic background.

Three groups at the University of Pennsylvania have shown that adoptive transfer of engineered antimesothelin human CAR T lymphocytes induces regression of large human MPM xenograft tumors in immunodeficient mice (14–16). Human CAR T lymphocytes have also been redirected against fibroblast activation protein (FAP), a cell surface antigen frequently expressed in the stromal and tumor cell compartments of all histological subtypes of human MPM (17). Anti-FAP CAR T lymphocytes can effectively lyse human MPM cells *in vitro* and inhibit establishment of human mesothelioma xenograft tumors in immunodeficient mice (17). Early-phase clinical trials in other cancer types have shown that infusion of antitumor CAR T lymphocytes can establish memory progeny that are detectable in circulating blood at least 6 months after treatment (18). Barrett and colleagues recently published a review of CAR T-cell biology, including discussion of

clinical manufacturing challenges and patient safety issues (19). Altogether, these data demonstrate the potential of CAR T lymphocytes for treating mesothelioma and providing durable protection against disease recurrence. Three phase I trials in patients with MPM are planned or are underway to evaluate the safety and effective doses of CAR T lymphocytes redirected against FAP and mesothelin (ClinicalTrials.gov identifiers NCT01722149, NCT01355965, NCT01583686).

Immune Checkpoint Blockade

Immune checkpoints are pathways that dampen inflammatory responses and mediate immune tolerance toward normal tissue. Because most immune checkpoints are initiated by ligand–receptor interactions, they can be readily blocked by antagonist antibodies or recombinant forms of cognate ligands/receptors. Dr. James Allison first discovered that a receptor on T lymphocytes called cytotoxic T-lymphocyte antigen-4 (CTLA-4) is vital for maintaining host immune tolerance to established tumors. The CTLA-4 receptor sequesters CD80 and CD86 immune costimulatory signals provided by antigen-presenting cells, thus raising the activation threshold for T lymphocytes. Allison’s group showed that systemic treatment with CTLA-4–blocking antibody—as monotherapy or combined with therapeutic tumor cell vaccination—induced regression of established melanoma and colon tumors in mice (20, 21). These studies paved the way for clinical trials of anti-CTLA-4 immunotherapy in multiple cancer types, culminating with FDA approval of ipilimumab for melanoma in 2011.

A phase II trial evaluating anti-CTLA-4 immunotherapy (tremelimumab) in 29 patients with chemotherapy-resistant advanced mesothelioma (28 pleural and 1 peritoneal) was recently reported by Calabrò and colleagues (22). Objective clinical responses were observed in only 2 of 29 patients. However, disease stabilization was noted in nine patients (31%), all with epithelioid histology. Overall survival rates were 48% at 1 year and 37% at 2 years, which was considered noteworthy. These preliminary results infer

that anti-CTLA-4 immunotherapy, although generally not effective at reducing MPM volume, may produce disease stabilization in a subset of patients, possibly resulting in extended survival. These observations parallel the overall survival benefit conferred by ipilimumab anti-CTLA-4 immunotherapy in patients with melanoma (23). There are two active clinical trials testing tremelimumab in patients with MPM, including a randomized double-blind study (ClinicalTrials.gov identifiers NCT01655888 and NCT01843374).

Another T lymphocyte–associated immune checkpoint receptor that has emerged as a promising cancer immunotherapeutic target is programmed death-1 (PD-1). PD-1 is expressed primarily on activated effector T lymphocytes, including those that infiltrate into human tumors (24, 25). The natural ligands for PD-1 are known as programmed death ligand-1 (PD-L1) and programmed death ligand-2 (PD-L2). Expression of PD-L1/PD-L2 on tumor cells or in stroma impairs effector T lymphocyte activity within the tumor

Table 1: Preclinical Multimodality Regimens Incorporating Tumor Immunotherapy

Multimodality regimen	Immunomodulatory/antitumor effects	Reference
Radiation + immunotherapy		
Fractionated local irradiation + CTLA-4 blockade	Enhanced induction of tumor-specific T lymphocytes; delayed growth of nonirradiated distant tumor	37
Fractionated local irradiation + therapeutic vaccination	Transient systemic MDSC depletion; enhanced tumor-specific T-lymphocyte induction	38
Fractionated local irradiation + adoptive T lymphocyte transfer	Transient systemic Treg depletion; enhanced proliferation of donor T lymphocytes in recipients	39
Chemotherapy + immunotherapy		
Intratumoral Ad-IFN- α immunogene therapy followed by cisplatin + gemcitabine.	Systemic Treg and MDSC depletion; enhanced tumor-specific T-lymphocyte induction; increased intratumor T-lymphocyte infiltration	41
Cyclophosphamide + therapeutic vaccination	Systemic Treg depletion	42
Gemcitabine + CTLA-4 blockade	Systemic and intratumor Treg depletion; induction of protective immunologic memory	43
Surgical tumor reduction + immunotherapy		
Preoperative intratumoral Ad-IFN- β immunogene therapy + complete resection	Increased intratumor T-lymphocyte infiltration; induction of protective immunologic memory	44
Complete resection + postoperative therapeutic vaccination	Increased intratumor T-lymphocyte infiltration; delayed growth of recurrent and unresected distant tumor	45
Partial resection + postoperative gemcitabine + anti-CD40 agonist immunotherapy	Induction of protective immunologic memory	46
Cryoablation + CTLA-4 blockade	Increased intratumor T lymphocyte infiltration; induction of protective immunologic memory	47

Definition of abbreviations: Ad-IFN- α , adenovirus-encoding IFN- α ; MDSC, myeloid-derived suppressor cell; Treg, T-regulatory.

microenvironment (26). *In situ* expression of PD-L1 is broadly found across diverse human tumor types, most frequently in melanoma and in ovarian, kidney, and lung cancers (26). Phase I/II trials in melanoma, kidney cancer, and lung cancer have shown that anti-PD-1 immunotherapy has durable clinical activity even after treatment cessation, possibly correlating with histological PD-L1 expression on primary tumors (27–29). A recent phase I trial evaluating anti-PD-L1 immunotherapy also showed clinical activity across a wide array of advanced cancers (30). Preliminary data reported by Wolchok and colleagues showed that combination anti-CTLA-4 + anti-PD-1 immunotherapy (at maximum tolerated doses) produced objective clinical responses, with at least 80% tumor reduction in 53% of treated patients with melanoma (31). The overall safety profiles of anti-PD-1/PD-L1 +/- anti-CTLA-4 immunotherapies are quite favorable, with high-grade immune-related adverse events being mostly reversible with steroids (27–31). Although there are no clinical trials testing anti-PD-1/PD-L1 immunotherapy in MPM, evaluation of MPM for histological PD-L1 expression is warranted because it may be a biomarker for predicting clinical response to anti-PD-1/PD-L1 immunotherapy (27).

Combining Immunotherapy with MPM Cytoreductive Therapies

Enhancing the therapeutic reach of immunotherapy via radiation and chemotherapy. Primary human MPM tissue can harbor significant levels of immunosuppressive Treg cells and M2 macrophages, which may impede the optimal clinical effectiveness of immunotherapy (reviewed in Ref. 32 and 33). Therefore, the most effective immunotherapy approaches might integrate multimodality regimens that also abrogate immunosuppressive circuits. Radiation and chemotherapy may deplete tumor-promoting immunosuppressive cells, thereby creating a host environment more amenable to immunotherapy.

Local tumor irradiation can alter multiple immunological parameters in the tumor microenvironment and enhance host antitumor immunity. Irradiation can increase the range of tumor antigen epitopes

presented on tumor cell surface MHC molecules while up-regulating MHC expression via IFN- γ -dependent signaling (34). Furthermore, local tumor irradiation can increase immunostimulatory chemokine (i.e., CXCL16) and type-1/type-2 IFN production within the tumor microenvironment (35, 36). Hence, targeted irradiation can potentially convert established tumors into “*in situ* vaccines.” Dewan and colleagues (37) and others have shown that local fractionated irradiation of established murine tumors can augment systemic T lymphocyte immunity that delays growth of distant, nonirradiated tumors, especially when combined with potent immunotherapies such as CTLA-4 blockade. Part of this effect results from transient intratumoral and/or systemic depletion of Treg cells and myeloid-derived suppressor cells (MDSCs; capable of converting to M2 macrophages), which creates a “window” for augmenting antitumor immune responses via immunotherapy (38, 39).

Chemotherapy can also augment tumor immunotherapy by depleting Treg cells and MDSCs while enhancing antigen presentation in tumor cells (40). Fridlender and colleagues showed that cisplatin + gemcitabine treatment can effectively reduce Treg cells and MDSCs in

mesothelioma tumor-bearing mice (41). The sequential use of intratumoral IFN- α immunogene therapy followed by cisplatin + gemcitabine chemotherapy induced complete regression of established mesothelioma tumors in 13 of 15 treated mice (41). Others have shown that chemotherapy combined with therapeutic vaccination or anti-CTLA-4 immunotherapy has synergistic antitumor effects in murine mesothelioma models and induces long-term protective immunity against disease recurrence (42, 43). Hassan and colleagues reported that immune depletion using pentostatin/ cyclophosphamide may have reversed tumor-induced immunosuppression in patients with mesothelioma treated with adjunctive SS1P antimesothelin immunotoxin. Such immune modulation by chemotherapy might have depleted Treg cells and/or MDSCs, possibly contributing to tumor control in the subset of clinical responders in that study (10). Sterman and colleagues are conducting a pilot trial evaluating intrapleural Ad-IFN- α 2b treatment combined with first- or second-line chemotherapy, which might also reverse tumor-induced immunosuppression (ClinicalTrials.gov identifier NCT01119664).

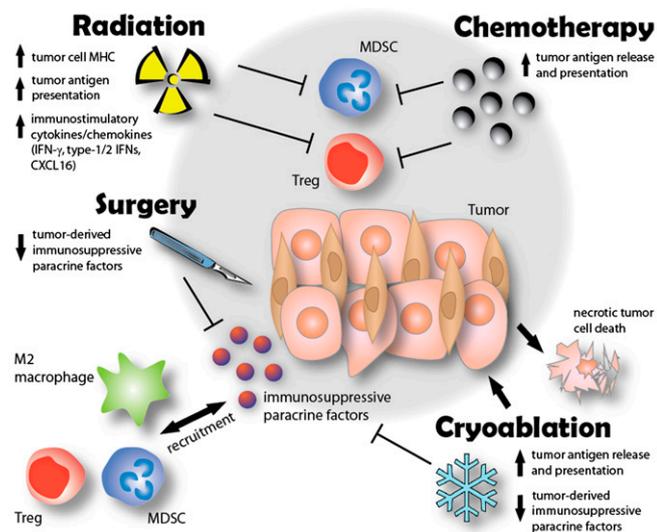


Figure 1. Immune modulation of the tumor microenvironment by cytoreductive therapies. Fractionated tumor irradiation enhances tumor antigen presentation and promotes immunostimulatory cytokine/chemokine expression within tumors. Radiation and chemotherapy can deplete T regulatory (Treg)-cells and myeloid-derived suppressor cells (MDSCs). Chemotherapy and cryoablation promote tumor antigen release and uptake by host antigen-presenting cells. Surgical tumor resection and cryoablation may reduce tumor-derived paracrine factors that promote recruitment of Treg cells, MDSCs, and/or M2 macrophages. MHC, major histocompatibility complex.

Tumor reduction via surgical resection or cryoablation to augment immunotherapy. Surgical tumor reduction may create a host environment more amenable to immunotherapy by (1) reducing the ratio of tumor cells versus antitumor effector T lymphocytes, (2) reducing the quantities of intratumor and/or systemic immunosuppressive cells, and (3) ablating tumor-derived paracrine factors that promote local recruitment of immunosuppressive cells. In subcutaneous mesothelioma tumor-bearing mice, preoperative intratumoral IFN- β immunogene therapy synergized with complete tumor resection to prevent disease recurrence in approximately 50% of treated mice (44). Mukherjee and colleagues showed in mice that complete resection of subcutaneous mesothelioma tumors followed by therapeutic tumor cell vaccination induced systemic immunity that delayed growth of recurrent and unresected distant tumors (45). Broomfield and colleagues reported that complete resection of subcutaneous mesothelioma tumors combined with postoperative sequential gemcitabine chemotherapy followed by anti-CD40 agonist immunotherapy cured approximately 80% of treated mice (46). Partial resection of primary tumor

(i.e., leaving residual gross tumor) can provide a source of tumor antigens for which subsequent chemotherapy + immunotherapy induced T-lymphocyte memory protecting against tumor rechallenge (46). These studies demonstrate that host antitumor immune responses can be augmented by surgical tumor reduction and that residual tumor (gross tumor or possibly microscopic margins) can be an *in situ* source of tumor antigens that can be leveraged with immunotherapy.

Cryoablation is an alternate surgical approach for reducing tumor volume. Murine studies have shown that *in situ* cryoablation of established solid tumors can create a depot of tumor antigens for effective presentation to the host immune system. Anti-CTLA-4 immunotherapy applied on tumor cryoablation can enhance immune-mediated protection against tumor rechallenge at primary and distant sites (47). Cryoablation has shown remarkable clinical efficacy in treating localized MPM (48), and the addition of anti-CTLA-4 immunotherapy might significantly augment its effects.

Conclusions

Immunotherapy offers a targeted approach for treating human cancers with generally

manageable toxicities. MPM clinical responses to immunotherapy, although infrequent, might be improved by abrogating intratumor and/or systemic immunosuppressive circuits. Our current understanding of immunosuppression in MPM has advanced significantly within the past decade. Treg cells and M2 macrophages can occupy a substantial proportion of the stromal space in primary MPM (32, 33) and may be pertinent suppressors of adaptive antitumor immunity in this disease. Conventional MPM treatments, such as radiotherapy, chemotherapy, and surgery, may effectively ablate immunosuppressive cell populations, thus creating a host environment more amenable to immunotherapy. Murine studies using multimodality regimens incorporating immunotherapy demonstrate that a wide array of treatment combinations can yield synergistic antitumor effects (Table 1; Figure 1). However, selection and optimization (i.e., dosage and timing) of each specific component of multimodality regimens needs to be carefully evaluated before planning clinical trials. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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