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Immune responses and immunotherapeutic interventions in malignant pleural mesothelioma

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Abstract

Malignant pleural mesothelioma (MPM) is an aggressive, primary pleural malignancy with poor prognosis, hypothesized to originate from a chronic inflammatory state within the pleura. Similar to what has been observed in other solid tumors (melanoma, ovarian and colorectal cancer), clinical and pre-clinical MPM investigations have correlated anti-tumor immune responses with improved survival. As such, a better understanding of the complex MPM tumor microenvironment is imperative in strategizing successful immunotherapies. Herein, we review the immune responses vital to the development and progression of MPM, as well as assess the role of immunomodulatory therapies, highlighting recent pre-clinical and clinical immunotherapy investigations.

Keywords

Mesothelioma; Immune response to cancer; Immunotherapy; Immunomodulation; Tumor antigens

Introduction

Malignant pleural mesothelioma (MPM) is an aggressive primary pleural malignancy with median survival of 9–12 months, hypothesized to originate from a chronic inflammatory process within the pleura. Currently, even with trimodality therapy (chemotherapy, surgical resection, and thoracic radiation), median survival is only 17 months [1, 2]. Despite poor prognosis even after aggressive therapies, MPM patients with anti-tumor immune responses survive longer, with improved survival associated with increased CD8⁺ tumor-infiltrating lymphocytes [3, 4]. Immunosuppressive cytokines and regulatory T cells (T_{regs}) are also hypothesized to infiltrate the tumor microenvironment, dampening anti-tumor immune function and promoting MPM tumor growth [5]. Understanding the interplay between pro- and anti-tumorigenic immune cells in the MPM microenvironment is vital in developing novel therapies in MPM. Our review highlights the immune responses that are vital to the development and progression of MPM, as well as assesses the complex interplay between immune cells and immunomodulatory therapies, in an attempt to provide investigators with key information in the quest to develop novel immunotherapies for MPM.

Frustrated phagocytosis and the development of MPM

The association between asbestos exposure and subsequent development of mesothelioma has been documented in multiple animal species via inhalation of, or subcutaneous, intrapleural, and intraperitoneal inoculation with, asbestos fibers [6-9]. Inhaled asbestos fibers present within the lung cause infiltration of circulating macrophages into the pleural space, which try to phagocytose the inhaled foreign bodies [10]. In the effort to clear asbestos fibers, reactive oxygen species are generated, with subsequent production of inflammatory cytokines and increased recruitment of immune cells to sites of inflammation within the pleura [11-14]. Macrophages' repeated phagocytotic efforts fail to clear the asbestos fibers, resulting in continued generation of reactive oxygen species and secretion of pro-inflammatory cytokines [14]. This process, often referred to as "frustrated phagocytosis," represents a chronic inflammatory state that has been shown to generate malignant transformation of mesothelial cells in vitro (Fig. 1) [10, 15]. Similar "frustrated phagocytosis" was observed following the introduction of carbon nanotubes into the peritoneal cavity of mice, subsequently inducing inflammation and fibrosis to an equal or greater extent than long asbestos fibers [16-18]. As single and multiwalled carbon nanotubes are increasingly being used in electronic and other consumer products, these studies raised concerns, although the inhalational threshold for the observed changes is not yet known.

Tumor-infiltrating immune cells in human MPM

Immune responses within the tumor microenvironment are increasingly implicated as a determining factor in tumor progression and aggressiveness. Higher degrees of tumor-infiltrating CD3⁺ in ovarian cancers and intratumoral CD45RO⁺ cells in colorectal cancer were associated with prolonged survival [19, 20]. In a recent investigation from our laboratory, the role of the inflammatory response in both tumor and stroma was assessed in 175 epithelioid MPM patient specimens [21]. Routine hematoxylin- and eosin-stained slides were evaluated for both acute and chronic inflammation within MPM tumor and stromal components. Patients with high stromal, chronic inflammatory responses were observed to

have improved median overall survival, as compared to patients with low chronic inflammatory responses (19.4 vs. 15.0 months, respectively). Importantly, on multivariate analysis, chronic stromal inflammation remained an independent predictor of survival within our study cohort ($P = 0.02$, HR = 0.659, 95% CI 0.464–0.937). For the first time, this investigation evaluated the inflammatory response within both the tumor and stroma, providing insight into the prognostic value of inflammatory responses in epithelioid MPM. The analysis of tumor specimens from MPM patients has revealed associations between the presence of immune cell infiltrates and patient outcome, allowing pro- and anti-tumorigenic activity to be attributed to specific immune cell types (Table 1). In this section, we highlight the roles of various immune cells as determined in patient specimens.

Cytotoxic CD8⁺ T-cell infiltration

The correlation of the presence of tumor-infiltrating CD8⁺ cells with MPM patient survival was demonstrated by immunohistochemistry in extrapleural pneumonectomy specimens. Yamada et al. [4], in a multivariate analysis examining 27 patients undergoing extrapleural pneumonectomy as initial treatment, demonstrated CD8⁺ lymphocyte infiltration as an independent prognostic factor. In addition, Yamada et al. observed positive MHC I expression in all MPM specimens, possibly accounting for the effective anti-tumor activity of CD8⁺ cells. The protective association of tumor-infiltrating CD8⁺ T cells has also been demonstrated in patients who have received induction chemotherapy. In an immunohistochemical evaluation of 32 patients who underwent induction chemotherapy followed by extrapleural pneumonectomy, a high density of tumor-infiltrating CD8⁺ cells was found to be an independent predictor of prolonged survival (3-year overall survival: 83% vs. 28% for high vs. low infiltration, respectively) [3]. Furthermore, this study observed that high levels of tumor-infiltrating CD8⁺ T cells were correlated with reduced frequency of mediastinal lymph node metastasis. Interestingly, the authors observed higher CD8⁺ lymphocyte infiltration in patients receiving cisplatin/pemetrexed (currently the most efficacious MPM chemotherapy regimen) versus cisplatin/vinorelbine. These results suggest that strategies that promote tumor-infiltrating CD8⁺ T cells may be beneficial to MPM patients.

Regulatory T cells (T_{regs}) and mast cells

Clinical studies examining the presence of CD4⁺ CD25⁺ FoxP3⁺ regulatory T cells (T_{regs}) in patients with MPM and their prognostic significance are quite scarce [5]. Hegmans et al. confirmed the presence of significant numbers of T_{regs} on immunohistochemistry; however, no correlation with survival was possible due to the small number of tumor samples examined. Additionally, the dual and seemingly contradictory role of tumor-associated mast cells has been detailed in breast, lung, and ovarian cancer [22–26], but a paucity of data exist on mast cell function in MPM and their role is unknown. Studies elucidating the natural history of T_{reg} and mast cell infiltration upon MPM progression are required.

Peripheral immune cell relative ratio as a predictive biomarker in MPM

Peripheral blood neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation shown to be prognostic in solid malignancies [27, 28], was also found to be an independent predictor of survival on multivariate analysis in the recent study examining 173 MPM patients undergoing systemic therapy [29]. One-year survival rate for NLR <5 and ≥5 was 60% versus 26%, with median overall survivals of 16.7 versus 6.6 months, respectively. Furthermore, subgroup analysis revealed a 3-month survival advantage in patients whose NLR normalized post-treatment. The clinical utility of NLR in mesothelioma, however, remains to be seen, as the promise of this simple, low-cost, peripheral blood test needs to be reproduced by other investigators.

Although MPM was initially thought to be a poorly immunogenic tumor due to a paucity of reported cases of spontaneous remissions [30], clinical investigation into MPM patient samples demonstrates that increased intratumoral CD8⁺ lymphocyte infiltration is beneficial in MPM patients' prognosis.

Murine immunotherapeutic interventions

As analysis of the correlation between immune cell infiltration in the tumor microenvironment and patient outcome is complex and difficult to interrogate due to the rarity of MPM, murine models have been used to evaluate disease characteristics and novel treatments. Investigators have attempted immunotherapy and immunomodulation as a rational means not only to promote the infiltration of tumors with cytotoxic immune cells but also to reduce the effects of immunosuppressive cells within the MPM tumor microenvironment.

Immune cell-modulating therapies (Table 2; Fig. 2)

T_{regs} depletion

CD25, a marker of activation on T lymphocytes, is constitutively expressed by T_{regs} [31]; the presence of CD4⁺ CD25⁺ T_{regs} has been implied in the inhibition of anti-tumor immunity and has also been associated with worse prognosis in several cancers [32-35]. Depletion of T_{regs} using intratumoral or intraperitoneally injected anti-CD25 antibody in mice prior to intraperitoneal inoculation with mesothelioma tumor resulted in the restriction of tumor growth and significantly increased median survival from 19 to 33 days [5, 36, 37]. Flow cytometric analysis of tumor cell suspensions showed that T_{reg} depletion resulted in a large accumulation of intratumoral interferon- γ (IFN- γ)-producing CD4⁺ and CD8⁺ cytotoxic T cells, which the investigators proposed to be responsible for the observed anti-tumor response [36]. CD4⁺ cells appeared to be important mediators that lure CD8⁺ cells into the tumor microenvironment, as depletion of CD4⁺ cells significantly reduced intratumoral CD8⁺ cell infiltration by approximately 2.5-fold [36].

Beneficial observations of T_{reg} depletion prior to tumor implantation were reproduced by Anraku et al. [38]. Enhanced survival was dependent on tumor infiltration by CD8⁺ cells, as CD8-depleted mice were ineffective at mounting a robust anti-tumor response despite T_{reg} blockade. T_{reg} depletion by anti-CD25 antibody did not improve survival, however, if administered as a monotherapy in *established* MPM tumors. Following the observation of increased T_{reg} percentage in response to pemetrexed therapy, Anraku et al. hypothesized that T_{reg} blockade in concert with pemetrexed may prove effective. In fact, combination of pemetrexed chemotherapy and anti-CD25 antibody administration—given intraperitoneally 4 days after tumor injection—functioned synergistically, improving survival in mice with established MPM tumors. In addition to pemetrexed, gemcitabine also eliminated established MPM tumors with the addition of T_{reg} depletion via cyclophosphamide or anti-CD25 antibody [39]. Again, anti-tumor response was demonstrated to be reliant upon CD8⁺ T cells, with nearly unaffected tumor growth occurring in CD8⁺ depleted mice. Cyclophosphamide, a chemotherapeutic with established immunostimulatory properties including the ability to selectively deplete T_{regs}, was found to sensitize MPM tumor cells to TRAIL-mediated apoptosis, suggesting that anti-tumor immune responses provoked by cyclophosphamide depend on increased tumor cell susceptibility, rather than effector cell proliferation [40]. A more recent investigation observed retarded MPM tumor growth when either anti-CD25 antibody or cyclophosphamide was given following cisplatin cycles [41]. These data implied that T_{reg} depletion between cisplatin cycles may hinder MPM tumor cell repopulation, potentially improving outcomes. Although T_{reg} depletion combined with chemotherapeutic agents was beneficial in MPM, T_{reg} depletion by anti-CD25 did not

improve the efficacy of intratumoral IL-2 immunotherapy in an investigation by Jackaman et al. [42].

Although human studies are scarce, the aforementioned investigations imply that the balance between cytotoxic T cells and T_{regs} within the MPM tumor microenvironment contributes to whether a tumor will be subject to (a) an immunosuppressive environment in which unrestrained tumor growth can occur or (b) an anti-tumor setting in which cytotoxic T cells can elicit an effective cytotoxic immune response.

Tumor-associated macrophage depletion

In various solid tumors, in the presence of appropriate cytokines or ligands, macrophages can polarize into two types: M1 and M2 tumor-associated macrophages (TAMs) [43]. M1 TAMs demonstrate immunostimulatory properties and confer enhanced tumor resistance and cytotoxicity, while M2 TAMs secrete immunosuppressive cytokines, promote angiogenesis, and support tumor progression [43], invasion, and metastasis and overall have been associated with poorer prognosis in lung, breast, prostate, and cervical cancer [44-50]. The role of TAMs in MPM is still largely unknown, however. Interaction with MPM cells appears to shift mature macrophages toward a M2 phenotype, characterized by poor antigen presentation and increased immunosuppressive activity [51]. Upon cocultivation with MPM cells, macrophages released a significant amount of prostaglandin E₂, an arachidonic acid metabolite with considerable anti-inflammatory properties. The presence and production of prostaglandin E₂ contribute to T_{reg} development, promoting an immunosuppressive tumor microenvironment [52, 53]. With this background, the depletion of pro-tumorigenic M2-TAMs by liposome-encapsulated clodronate (CLIP) in a peritoneal mesothelioma model was performed [54]. Mice with established tumors demonstrated a 50% reduction in both the number of tumors and tumor burden when treated with CLIP compared to control treatments, although CLIP treatment of mice bearing established tumors did not prolong survival. The results of this study imply that TAMs contribute to the development and progression of mesothelioma; however, additional investigations are needed to clarify the role of TAMs in MPM.

Dendritic cell vaccination

Dendritic cell (DC) vaccination exploits the ability of DC to present antigens and initiate a cascade of immunologic events leading to the destruction of cells expressing these specific tumor antigens [55, 56]. Hegmans et al. [57] showed that tumor lysate-pulsed DCs can prevent engraftment and halt mesothelioma progression by stimulating tumor-directed CD8⁺ cytotoxicity. The protective effect of DC immunotherapy appeared to be long-lived as tumor re-challenge after 3 months in this cohort did not result in subsequent mesothelioma outgrowth. Given therapeutically, in mice with established peritoneal mesothelioma, DC treatment was associated with decreased velocity of mesothelioma outgrowth and prolonged survival. Furthermore, adoptively transferred CD8⁺ T cells from mice treated with tumor lysate-pulsed DCs retained tumor-specific cytotoxicity when injected into mesothelioma-bearing mice, prolonging survival in mice when given either 1 week before or 2 days after tumor inoculation. Veltman et al. [58] demonstrated that combination treatment with DC immunotherapy and cyclophosphamide potentiated therapy, significantly improving mice survival compared with cyclophosphamide or DC immunotherapy monotherapy. Timing and dose of cyclophosphamide administration have previously been demonstrated to be vital to whether the drug displays immunosuppressive or immunostimulatory effects [59].

Tumor antigen-targeted immunotherapy

Although tumor lysates contain a repertoire of MPM-associated antigens, it is possible that critical MPM antigens are neither shed nor highly expressed. Therefore, studies have sought

to identify MPM antigens as specific targets for immunotherapies. Two such candidate cancer antigens are mesothelin and Wilms' tumor-1 (WT-1) protein. These proteins demonstrate tumor-specific overexpression and the ability to elicit both active and humoral immune responses [60-64]. Mesothelin, a cell-surface cancer-associated antigen with uncertain function, is overexpressed in the vast majority of epithelioid MPM [65]. While it is highly expressed in MPM, normal mesothelin expression is restricted to the mesothelial lining of the peritoneum, pleura, and pericardium. In addition, soluble mesothelin-related protein (SMRP) may be detected in the serum of patients with MPM and may prove a useful biomarker to follow treatment response [66, 67]. Studies in a murine model of mesothelin-expressing epidermoid carcinoma suggest that targeting mesothelin-expressing cells using SS1P, a recombinant immunotoxin consisting of the variable fragment (Fv) of an anti-mesothelin antibody fused to a *Pseudomonas* exotoxin, can cause tumor regression with enhanced anti-tumor efficacy observed when combined with chemo- or radiotherapy [68, 69]. Similarly, MORAb-009, a chimeric mouse-human monoclonal IgG_{1κ} antibody with high affinity for human mesothelin, was found to prevent the adhesion of mesothelin-expressing MPM cells, conferring antibody-dependent cellular cytotoxicity upon cells exhibiting mesothelin positivity [70].

Another approach targeting tumor-associated antigens is the chimeric antigen receptor (CAR). CARs are comprised of a tumor antigen-binding domain linked to an intracellular signaling domain able to activate T cells [71]. CARs unite high-affinity antigen recognition with T-cell signal transduction, two functions that fundamentally arise from separate complexes, the TCR $\alpha\beta$ -heterodimer and the CD3 complex. The extracellular, antigen-binding domain is usually derived from a murine or human monoclonal antibody, but other receptors have also been utilized. It follows that antigen recognition by CARs is not MHC-restricted, as is the case for TCR-mediated antigen recognition. Antigen binding by CARs activates the signaling cascade that induces antigen-specific cytotoxicity, effector cytokine secretion, and T-cell proliferation. A recent investigation utilizing mesothelin-targeted CARs to target mesothelin-expressing MPM (derived from the malignant pleural effusion of a mesothelioma patient) showed that these genetically engineered T cells were able to proliferate, express effector cytokines, and eliminate tumor cells both in vitro and in vivo in a flank MPM model [72]. Engineered T lymphocytes demonstrated anti-tumor efficacy at effector-to-target ratios of approximately 1:10 and 1:40 in vitro and in vivo, respectively. In vivo, both intratumoral administration and intravenous administration were found to be efficacious, with intravenous administration being associated with delayed treatment response, however. In an attempt to improve T-cell trafficking into MPM tumors, T cells bearing mesothelin-targeted CARs were transduced to express the CCL2 chemokine receptor, as CCL2 was found to be highly expressed by MPM tumors [73]. In mice with established flank MPM tumors, mesothelin CAR-transduced T cells bearing CCL2 receptors infiltrated tumors significantly greater than T cells transduced with mesothelin CARs only, improving anti-tumor efficacy.

No pre-clinical investigations are available regarding the targeting of WT-1 in MPM.

Cytokine therapy

Interferon- β —Type I interferons (interferon- α/β) possess direct and indirect anti-viral, anti-proliferative, and immunomodulatory properties and in particular have been implied in the regulation of both CD4⁺ and CD8⁺ T cells during the initial phases of antigen recognition [74]. The direct anti-proliferative effects of interferon- β gene transfer upon MPM cells were demonstrated by Odaka et al. [75]. Recombinant interferon- β administration led to statistically significant improved survival; however, no survival advantage was observed for low- versus high-dose treatment. Single-dose adenovirally

encoded interferon- β gene transfer was also shown to prolong survival in a peritoneal MPM model. Mice treated with interferon- β gene transfer retained long-term immunity and anti-tumor response, as tumor re-challenge approximately 2 months afterward did not cause MPM tumor growth upon flank injection with MPM cells. Tumor killing appeared to be dependent on CD8⁺ T cells as CD8⁺-depleted mice demonstrated tumor growth and survival nearly equivalent to untreated controls. Furthermore, the therapeutic efficacy of interferon- β gene transfer was nearly obviated in SCID mice (which lack B and T cells). Neo-adjuvant adenovirus encoding interferon- β significantly reduced tumor recurrence and resulted in improved 25-day, tumor-free survival in mice [76].

Interleukin-2—The pro-inflammatory cytokine interleukin-2 (IL-2) is produced by activated T cells, resulting in increased proliferation, survival, and development of cytotoxic T lymphocytes [77, 78]. IL-2 administration increased survival in treated mice bearing small (~10 mm³) flank tumors at treatment initiation, while mice bearing large (~30 mm³), established MPM tumors failed to demonstrate improved survival [79]. Importantly, IL-2-responsive mice remained tumor free for greater than 2 months afterward, in addition to resisting subsequent tumor re-challenge 2–6 months following initial tumor inoculation. IL-2-treated small tumors were associated with increased number and cytotoxicity of tumor-infiltrating CD8⁺ T lymphocytes, as well as decreased tumor-associated vasculature; however, treatment failed to increase antigen presentation within tumor-draining lymph nodes when compared with untreated control mice. Histopathologic examination of tumor specimens from mice subjected to CD8⁺ T-cell depletion did not demonstrate a reduction in tumor-associated vasculature, implying a key role of CD8⁺ T cells in IL-2-mediated anti-tumor mechanisms.

Combination of intratumoral IL-2 and anti-CD40 antibody immunotherapy was attempted by Jackaman et al. [79–81] to overcome the limitations of monotherapy and apply treatment to larger tumors. In this study, over 80% of mice exhibited complete tumor regression without subsequent progression. The anti-CD40 treatment primarily recruited B cells, whereas IL-2 treatment increased CD4⁺ and CD8⁺ T-cell infiltration into the MPM tumor microenvironment, as determined by flow cytometry. In addition to CD8⁺ T cells, mice treated with combination therapy were observed to have significantly increased infiltration neutrophils as well. In mice bearing small MPM flank tumors, depletion of both CD4⁺ and CD8⁺ cells did not prevent the efficacy of combination IL-2 and anti-CD40 treatment, implying other effector mechanisms may be important in early tumor eradication. Combination treatment was also effective against large (>25 mm³) tumors that were resistant to IL-2 or anti-CD40 monotherapy. Furthermore, mice treated with combination therapy resisted tumor re-challenge at multiple subsequent time points thereafter. CD4⁺ and CD8⁺ cell depletion in mice bearing large tumors treated with combination therapy nearly obviated the anti-tumor efficacy of the regimen, while CD4⁺-depleted mice retained the efficacy of treatment, again supporting the vital role which CD8⁺ cells play in the elimination of large tumors treated with IL-2 and anti-CD40 treatment. Neutrophil infiltration of large tumors was also shown to be required in order to eradicate tumors, as depletion of neutrophils also prevented tumor regression, implying that both CD8⁺ and neutrophils, together, are required for an effective anti-tumor response.

Interleukin-12—Interleukin-12 (IL-12) is a multifunctional cytokine vital to cell-mediated immunity, playing a key role in driving T_H1 differentiation while promoting T-cell cytotoxicity, proliferation, and interferon- γ production [82]. IL-12 has been shown to produce significant anti-tumor immune responses in pre-clinical studies and is an attractive cytokine for use in MPM immunotherapy [83, 84]. Caminschi et al. [85] investigated the anti-tumor effects of systemic IL-12 administration in a flank MPM model. Synchronous systemic administration of IL-12 during tumor inoculation prevented tumor growth in

approximately 70% of treated mice. Furthermore, half of IL-12-treated mice resisted tumors re-challenge, implying that long-term anti-tumor effects had occurred. Intratumoral IL-12 injections into established tumors also resulted in the reduction in tumor volume and reduced growth; however, this effect was temporary. The IL-12-mediated anti-tumor effects relied on the presence of both CD4⁺ and CD8⁺ T cells as depletion of either resulted in increased tumor growth. A subsequent study by Caminschi et al. [86] assessed the anti-tumor efficacy of IL-12 by transfecting a murine MPM cell line with IL-12 genes, inducing local IL-12 production within the tumor microenvironment. Local production of IL-12 appeared to prevent tumor formation, as IL-12-transfected tumor cells were unable to form tumors in BALB/c mice. IL-12-transfected MPM cells were able to form tumors, however, in athymic nude mice, providing further evidence to the key role which T lymphocytes play in the anti-tumor response.

Cytokine therapy has demonstrated efficacy in improving median survival in murine models. Depletion studies have documented the importance of both CD4⁺ and CD8⁺ lymphocytes in mediating the anti-tumor effects of cytokine therapy. Furthermore, investigations have revealed evidence of cytokine therapy conferring long-term immunity as demonstrated by resistance to tumor re-challenge.

Cytokine/chemokine blockades

A dual strategy aimed at bolstering the anti-tumor immune response while dampening the immunosuppressive forces within the tumor environment may be an effective way to permit unabated anti-tumor immunity. Studies by Fridlender et al. [87] suggest that blocking CCL2 and CCL12—chemoattractants that attract T_{regs} and dampen CD8⁺ T-cell effector functions—augments anti-tumor immune responses by inhibiting T_{regs} trafficking and immunosuppression within the tumor microenvironment. Blocking CCL2/12 alone with intraperitoneally administered monoclonal antibodies had limited efficacy, leading to significantly slowed tumor growth, but not regression. When combined with mesothelin-vaccine immunotherapy, however, CCL2/12 blockade resulted in a significant decrease in both tumor burden (~50% cure rate) and intratumoral T_{regs}, as well as an increase in the number and activity of intratumoral CD8⁺ T cells. Importantly, blockade of CCL2/12, in concert with vaccine immunotherapy, induced ~twofold higher expression of the pro-inflammatory molecules CXCL10, ICAM, IL-10, IL-12, IFN- γ , and TNF- α .

A similar approach has used TGF- β blockade to potentiate immunotherapy [88]. TGF- β is produced by MPM at high levels and is believed to play a pivotal role in generating an immunosuppressive tumor microenvironment via promoting T_{reg}-mediated immunosuppression, impeding tumor infiltration by T cells, and inhibiting anti-tumor T-cell functions [89-91]. TGF- β blockade given for 7 days prior to intratumoral injection of adenovirus expressing IFN- β caused significant reduction in the size of established MPM flank tumors; monotherapy with TGF- β blockade or intratumoral IFN- β gene transfer caused only minimal reduction in tumor burden. Compared with untreated controls, mice receiving TGF- β blockade demonstrated an increased intratumoral CD8⁺ T-cell infiltration and activation and increased expression of pro-inflammatory cytokines, chemokines, and cell adhesion molecules.

TGF- β has also been shown to polarize macrophages toward a pro-tumorigenic M2 phenotype [92] and theorized to polarize neutrophils toward a pro-tumorigenic state N2 phenotype (as opposed to the anti-tumor, pro-inflammatory N1 phenotype); as such, TGF- β blockade's effect on tumor-associated neutrophil (TAN) phenotype was examined in a recent study [93]. TANs derived from flank MPM tumors in mice treated with TGF- β blockade demonstrated an elevated mRNA expression of pro-inflammatory molecules (TNF- α , CCL3, and ICAM-1), as compared to untreated control MPM tumors. Interestingly,

arginase levels were decreased 2- to 5-fold in TANs isolated from mice treated with TGF- β blockade, compared to untreated controls; the immunosuppressive functions of arginase have been shown previously [94]. The aforementioned data led the authors to hypothesize that TGF- β blockade resulted in the polarization of TANs toward the anti-tumor, N1 phenotype. Depletion of TANs in untreated mice (that is, removal of the immunosuppressive, N2 TANs) led to ~twofold increase in CD8⁺ T-cell activation as shown by flow cytometry, as well as a small reduction in tumor growth. Conversely, depletion of pro-inflammatory, N1 TANs in mice treated with TGF- β blockade significantly blunted T-cell activation, allowing for increased tumor growth. The results of this study imply that following TGF- β blockade, TANs that exhibit an anti-tumor phenotype (N1 TANs) are recruited into the MPM tumor microenvironment, leading to elaboration of pro-inflammatory cytokines with subsequent activation of intratumoral CD8⁺ T cells. Although the MPM tumor-neutrophil relationship has not been extensively studied, it warrants further investigation as the pro-inflammatory environment setup by N1 TANs may be an important mechanism for tumor regression.

Immuno-adjuvant therapies

An alternative approach to immunotherapy aims to enhance the intrinsic activity of immune system. In a flank model of MPM, Currie et al. [95] intratumorally injected Toll-like receptor (TLR) ligands to activate DCs and induce T-cell-dependent cytotoxicity in a type I interferon (IFN)-dependent manner. Intratumorally administered TLR7 agonist induced systemic, CD8⁺ T-cell and type-I IFN anti-tumor responses that prevented primary MPM tumor growth but not the growth of distant, untreated tumor foci [96]. The anti-tumor effects were obviated following CD8⁺ cell depletion as well as in nude mice. The relative paucity of intratumoral CD4⁺ T cells, along with the lack of observed systemic CD4⁺ T-cell activation in mice with response, implied that CD4⁺ T-cell assistance may be required for a robust systemic anti-tumor response. Indeed, distant untreated tumor foci were targeted when an agonistic anti-CD40 antibody (mimicking CD4⁺ T-cell assistance) was administered systemically in combination with local TLR7 agonist. The authors hypothesized that systemic anti-CD40 antibody administration, a surrogate for CD4⁺ T helper cells, increased tumor cell sensitivity to cytotoxic T cells or increased the quality of the CD8⁺ T-cell response.

Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is an inhibitory molecule found on T cells, hypothesized to regulate T-cell activation [97]. Anti-CTLA-4 antibodies (ipilimumab and tremelimumab) that block interaction between CTLA-4 and its associated ligands (B7.1 and B7.2) have been observed to promote anti-tumor immune responses in pre-clinical investigations of various solid tumors [98-100]. Recent clinical successes in treating melanoma with ipilimumab (Yervoy) have led to its FDA approval [101, 102]. While the use of CTLA-4 has been investigated in other non-melanoma solid tumors, there have yet to be any notable pre-clinical investigations of its use in MPM models. Interestingly, however, a phase II clinical trial utilizing tremelimumab as second-line therapy for unresectable MPM is in progress in a single Italian institution [Trial MESOT-TREM-2008].

Although the vast majority of these studies were performed in either flank or peritoneal MPM models, ortho-topic MPM models have been previously well described [103-105] and should be utilized in future studies to more accurately mimic the pleural tumor microenvironment.

Human immunotherapy trials

In this section, we discuss the use of cytokine- and cellular-based immunotherapies and analyze their utility in mesothelioma treatment as determined by recent clinical trials (Table 3).

Cytokine-based therapy

As a result of the aforementioned promising pre-clinical results, a recent phase I clinical trial assessing intrapleural interferon- β gene transfer in patients with MPM and malignant pleural effusions was performed [106]. Successful interferon- β gene transfer was detected in seven of ten patients. Variable anti-tumor immune responses were observed in seven out of ten MPM patients, as demonstrated by the activation of natural killer cells and cytotoxic T cells, as well as the generation of antibodies to MPM-associated antigens. An update of this cohort, following repeated intrapleural administration of adenoviral encoded interferon- β , published more recently observed that nine out of ten MPM patients had demonstrated humoral immune responses to MPM-associated antigens following treatment [107]. While the majority succumb to progressive disease, three out of ten MPM patients were alive at 18, 39, and 42 months following treatment, demonstrating either mixed response or stable disease on follow-up PET imaging. Another study by Boutin et al. [108] suggested that intrapleural administration of recombinant interferon- γ may be beneficial to MPM patients with early (stage I) disease; eight patients demonstrated significant histologic response and nine patients yielded partial responses for an overall response rate of 20%.

A phase I-II clinical trial utilizing intrapleural administration of IL-2 in 23 stage I-IIA patients detailed some anti-tumor efficacy and an acceptable side-effect profile, with high intrapleural IL-2 levels observed with low corresponding serum levels [109]. Pleural effusion-derived mononuclear cells displayed significantly higher lymphokine-activated killer (LAK) activity than peripherally obtained mononuclear cells. No correlation between IL-2 dose and LAK activity or clinical response was observed. Additional phase II clinical trials assessing intrapleural administration of IL-2 in MPM have been carried out reporting marginal histopathologic responses and modest improvements in median survival [110-113]. Unfortunately, immunologic parameters following treatment were not detailed in these investigations. The results from clinical trials assessing intrapleural IL-2 efficacy in MPM are conflicting and, although some increase in overall survival appears to be achieved in these studies, the potential of IL-2 to significantly impede disease progression has not been convincingly demonstrated. While phase II intrapleural IL-2 has demonstrated some effectiveness in treating MPM, other routes of administration (intravenous and/or subcutaneous) as first-line treatment have failed to do so [114,115]. In a group of 60 MPM patients who received pre-operative intrapleural IL-2 treatment, a significantly higher number of T_{regs} was observed in surgical specimens, as compared to 33 patients who did not receive IL-2 [116]. Within the IL-2-treated group, high T_{regs}, defined as greater than the median on immunohistochemical assessment, was correlated with both decreased patient survival and increased tumor recurrence on subgroup analysis. Furthermore, pre-operative, intrapleural IL-2 therapy in MPM was associated with a high level of tryptase-positive mast cell infiltration, which correlated with both increased overall survival and time to progression [117]. When taken together, the density of tryptase-positive mast cell and T_{reg} infiltration was able to more accurately predict prognosis than either parameter alone; a high tryptase-positive mast cell and low T_{reg} tumor infiltrate were found to possess the best overall patient survival. The results from these studies must be interpreted with caution however, since IL-2 is known to induce a wide range of immunomodulatory effects, several of which may contribute to the aforementioned survival benefit.

Active immunization

Based on pre-clinical studies demonstrating the efficacy of DC-based immunotherapy [57], Hegmans et al. [118] conducted a small, phase I clinical trial where DCs were pulsed with autologous tumor lysate, matured ex vivo, and then administered intradermally and intravenously to MPM patients following cytoreductive surgery and cisplatin-based chemotherapy. Although DCs immunotherapy was well tolerated and appeared to elicit an immunologic response, no survival benefit was noted. Interpreting this data is challenging, however, due to small sample size ($n = 10$) and inclusion of a highly selected patient population; only patients with the epithelioid subtype of MPM and who had either a partial response or stable disease following multimodality therapy were included.

To specifically target MPM tumors, cancer antigens, such as mesothelin and WT-1, that are highly expressed and thought to be physiologically relevant for the tumor may be targeted. Recently, the NCI cancer panel identified WT-1 as the leading tumor-associated antigen to be targeted [119]. Studies assessing WT-1 as a target for immunotherapy have been carried out in leukemias and have shown clinical efficacy [62, 63, 120] and are the basis for expanding these therapies to MPM. Since MPM expresses WT-1 at high levels [121, 122] and provokes an immune response [63], it is an attractive target for MPM immunomodulatory therapy. A phase I peptide vaccination clinical trial from Memorial Sloan-Kettering Cancer Center for unresectable or relapsed MPM patients is ongoing. In these patients, inoculation with WT-1 peptide elicited WT-1-specific CD4 and CD8 T-cell responses, with minimal toxicity [123].

Immunotoxin therapy

Based on promising pre-clinical results targeting mesothelin with the immunotoxins SS1P and MORAb-009, clinical trials were initiated [65, 124-126]. Phase I clinical trials in patients with advanced MPM, pancreatic, and ovarian cancers, utilizing both bolus and continuous intravenous administration of SS1P while well tolerated, have shown only modest clinical responses [124, 125]. Similarly, a phase I MORAb-009 dose-escalation trial in MPM, pancreatic, and ovarian cancer patients failed to demonstrate any complete or partial responses [126]. Pre-clinical studies, however, have demonstrated significant anti-tumor efficacy using combination of MORAb-009 and chemotherapy treatment [70]; as such, a multicenter phase II clinical trial utilizing cisplatin/pemetrexed with MORAb-009 in MPM patients is currently ongoing [Clinicaltrials.gov Identifier-NCT00738582].

Despite early pre-clinical results utilizing genetically engineered T cells to target MPM-associated tumor antigens, the efficacy of adoptive T-cell therapy has yet to be evaluated in MPM patients.

Conclusion

Lymphocytic infiltration has been demonstrated in multiple solid tumors (melanoma, ovarian and colorectal cancers), with type and density of the immune cell infiltrate correlated with clinical outcomes [19, 20, 127-130]. These investigations lend support and provide rationale for developing immunotherapies that will increase immune cell infiltration within solid tumors. Human clinicopathologic and pre-clinical studies have provided evidence that lymphocytic invasion influences prognosis in MPM as well. In comparison with other solid malignancies, there continues to be mounting evidence that the MPM tumor microenvironment is highly immunosuppressive, with the presence of immunosuppressive cytokines (in particular, TGF- β) and immune cells (T_{regs}, M2 TAMs, and N2 TANs) playing key roles. These observations imply that a better understanding of the immunostimulatory

and immunoinhibitory components of the MPM tumor microenvironment will be helpful in strategizing a successful immunotherapy.

In MPM, studies have implied that the beneficial anti-tumor effects are CD8⁺ lymphocyte-mediated and optimal CD8⁺ lymphocyte efficacy is likely dependent upon CD4⁺ lymphocyte assistance. T_{regs} also play an active role in MPM pathogenesis and blockade may prove to be an effective therapy in the future. However, the role of TAMs, TANs, and the human MPM tumor microenvironment is relatively unexplored and should be the focus of future investigations. Pre-clinical studies utilizing immunotoxins and immunotherapy have shown promising results in murine MPM models, with evidence amassing that adjuvant immunomodulation may be needed for success within the clinical realm. Success with tumor-associated antigen-targeted therapy has been limited to the pre-clinical stage, however. Clinical studies have demonstrated intrapleural cytokine therapies to be safe and well tolerated, but their efficacy is unproven.

While different approaches are available, this review suggests that an efficacious immunotherapy may result from a treatment that will both enhance the anti-tumor activity and dampen the pro-tumor effects of the immune system. Immunotherapy offers a promising therapeutic approach, either alone or in concert with current standard-of-care treatments for MPM. Perhaps more importantly, as pleural MPM is a regionally aggressive malignancy, investigating the utility of regionally administered therapies may prove effective in delivering high potency treatment while minimizing systemic toxicities.

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References

1. Krug LM, Pass HI, Rusch VW, Kindler HL, Sugarbaker DJ, Rosenzweig KE, Flores R, Friedberg JS, Pisters K, Monberg M, Obasaju CK, Vogelzang NJ. Multicenter phase II trial of neoadjuvant pemetrexed plus cisplatin followed by extrapleural pneumonectomy and radiation for malignant pleural mesothelioma. *J Clin Oncol*. 2009; 27:3007–3013. [PubMed: 19364962]
2. Flores RM, Krug LM, Rosenzweig KE, Venkatraman E, Vincent A, Heelan R, Akhurst T, Rusch VW. Induction chemotherapy, extrapleural pneumonectomy, and postoperative high-dose radiotherapy for locally advanced malignant pleural mesothelioma: a phase II trial. *J Thorac Oncol*. 2006; 1:289–295. [PubMed: 17409872]
3. Anraku M, Cunningham KS, Yun Z, Tsao M-S, Zhang L, Keshavjee S, Johnston MR, de Perrot M. Impact of tumor-infiltrating T cells on survival in patients with malignant pleural mesothelioma. *J Thorac Cardiovasc Surg*. 2008; 135:823–829. [PubMed: 18374762]
4. Yamada N, Oizumi S, Kikuchi E, Shinagawa N, Konishi-Sakakibara J, Ishimine A, Aoe K, Gamba K, Kishimoto T, Torigoe T, Nishimura M. CD8⁺ tumor-infiltrating lymphocytes predict favorable prognosis in malignant pleural mesothelioma after resection. *Cancer Immunol Immunother*. 2010; 59:1543–1549. [PubMed: 20567822]
5. Hegmans JP, Hemmes A, Hammad H, Boon L, Hoogsteden HC, Lambrecht BN. Mesothelioma environment comprises cytokines and T-regulatory cells that suppress immune responses. *Eur Respir J*. 2006; 27:1086–1095. [PubMed: 16540497]
6. Davis JM. The histopathology and ultrastructure of pleural mesotheliomas produced in the rat by injections of crocidolite asbestos. *Br J Exp Pathol*. 1979; 60:642–652. [PubMed: 540105]

7. Moalli PA, MacDonald JL, Goodglick LA, Kane AB. Acute injury and regeneration of the mesothelium in response to asbestos fibers. *Am J Pathol.* 1987; 128:426–445. [PubMed: 2820232]
8. Shin ML, Firminger HI. Acute and chronic effects of intraperitoneal injection of two types of asbestos in rats with a study of the histopathogenesis and ultrastructure of resulting mesotheliomas. *Am J Pathol.* 1973; 70:291–313. [PubMed: 4689512]
9. Wagner JC, Berry G. Mesotheliomas in rats following inoculation with asbestos. *Br J Cancer.* 1969; 23:567–581. [PubMed: 5360333]
10. Mossman BT, Churg A. Mechanisms in the pathogenesis of asbestosis and silicosis. *Am J Respir Crit Care Med.* 1998; 157:1666–1680. [PubMed: 9603153]
11. Hansen K, Mossman BT. Generation of superoxide ($O_2^{\cdot -}$) from alveolar macrophages exposed to asbestiform and nonfibrous particles. *Cancer Res.* 1987; 47:1681–1686. [PubMed: 3028612]
12. Dostert C, Pétrilli V, Van Bruggen R, Steele C, Mossman BT, Tschopp J. Innate immune activation through Nalp3 inflammasome sensing of asbestos and silica. *Science.* 2008; 320:674–677. [PubMed: 18403674]
13. Tanaka S, Choe N, Hemenway DR, Zhu S, Matalon S, Kagan E. Asbestos inhalation induces reactive nitrogen species and nitrotyrosine formation in the lungs and pleura of the rat. *J Clin Investig.* 1998; 102:445–454. [PubMed: 9664087]
14. Choe N, Tanaka S, Xia W, Hemenway DR, Roggli VL, Kagan E. Pleural macrophage recruitment and activation in asbestos-induced pleural injury. *Environ Health Perspect.* 1997; 105(Suppl 5): 1257–1260. [PubMed: 9400734]
15. Wang Y, Faux SP, Hallden G, Kirn DH, Houghton CE, Lemoine NR, Patrick G. Interleukin-1beta and tumour necrosis factor-alpha promote the transformation of human immortalised mesothelial cells by erionite. *Int J Oncol.* 2004; 25:173–178. [PubMed: 15202003]
16. Donaldson K, Murphy F, Duffin R, Poland C. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. *Part Fibre Toxicol.* 2010; 7:5. [PubMed: 20307263]
17. Poland CA, Duffin R, Kinloch I, Maynard A, Wallace WAH, Seaton A, Stone V, Brown S, MacNee W, Donaldson K. Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. *Nat Nano.* 2008; 3:423–428.
18. Takagi A, Hirose A, Nishimura T, Fukumori N, Ogata A, Ohashi N, Kitajima S, Kanno J. Induction of mesothelioma in p53+/-mouse by intraperitoneal application of multi-wall carbon nanotube. *J Toxicol Sci.* 2008; 33:105–116. [PubMed: 18303189]
19. Pages F, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molitor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med.* 2005; 353:2654–2666. [PubMed: 16371631]
20. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN, Rubin SC, Coukos G. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N Engl J Med.* 2003; 348:203–213. [PubMed: 12529460]
21. Suzuki K, Kadota K, Sima CS, Sadelain M, Rusch VW, Travis WD, Adusumilli PS. Chronic inflammation in tumor stroma is an independent predictor of prolonged survival in epithelioid malignant pleural mesothelioma patients. *Cancer Immunol Immunother.* Jul 19.2011 [Epub ahead of print].
22. Amini RM, Aaltonen K, Nevanlinna H, Carvalho R, Salonen L, Heikkilä P, Blomqvist C. Mast cells and eosinophils in invasive breast carcinoma. *BMC Cancer.* 2007; 7:165. [PubMed: 17727696]
23. Chan JK, Magistris A, Loizzi V, Lin F, Rutgers J, Osann K, DiSaia PJ, Samoszuk M. Mast cell density, angiogenesis, blood clotting, and prognosis in women with advanced ovarian cancer. *Gynecol Oncol.* 2005; 99:20–25. [PubMed: 16055178]
24. Ibaraki T, Muramatsu M, Takai S, Jin D, Maruyama H, Orino T, Katsumata T, Miyazaki M. The relationship of tryptase and chymase-positive mast cells to angiogenesis in stage I non-small cell lung cancer. *Eur J Cardiothorac Surg.* 2005; 28:617–621. [PubMed: 16125954]

25. Rajput AB, Turbin DA, Cheang MC, Voduc DK, Leung S, Gelmon KA, Gilks CB, Huntsman DG. Stromal mast cells in invasive breast cancer are a marker of favourable prognosis: a study of 4,444 cases. *Breast Cancer Res Treat.* 2008; 107:249–257. [PubMed: 17431762]
26. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. *J Clin Oncol.* 2005; 23:8959–8967. [PubMed: 16219934]
27. Cho H, Hur HW, Kim SW, Kim SH, Kim JH, Kim YT, Lee K. Pre-treatment neutrophil to lymphocyte ratio is elevated in epithelial ovarian cancer and predicts survival after treatment. *Cancer Immunol Immunother.* 2009; 58:15–23. [PubMed: 18414853]
28. Sarraf KM, Belcher E, Raevsky E, Nicholson AG, Goldstraw P, Lim E. Neutrophil/lymphocyte ratio and its association with survival after complete resection in non-small cell lung cancer. *J Thorac Cardiovasc Surg.* 2009; 137:425–428. [PubMed: 19185164]
29. Kao SC, Pavlakakis N, Harvie R, Vardy JL, Boyer MJ, van Zandwijk N, Clarke SJ. High blood neutrophil-to-lymphocyte ratio is an indicator of poor prognosis in malignant mesothelioma patients undergoing systemic therapy. *Clin Cancer Res.* 2010; 16:5805–5813. [PubMed: 20956618]
30. Robinson BW, Robinson C, Lake RA. Localised spontaneous regression in mesothelioma—possible immunological mechanism. *Lung Cancer.* 2001; 32:197–201. [PubMed: 11325491]
31. Sakaguchi S, Toda M, Asano M, Itoh M, Morse SS, Sakaguchi N. T cell-mediated maintenance of natural self-tolerance: its breakdown as a possible cause of various autoimmune diseases. *J Autoimmun.* 1996; 9:211–220. [PubMed: 8738965]
32. Woo EY, Chu CS, Goletz TJ, Schlienger K, Yeh H, Coukos G, Rubin SC, Kaiser LR, June CH. Regulatory CD4⁺ CD25⁺ T cells in tumors from patients with early-stage non-small cell lung cancer and late-stage ovarian cancer. *Cancer Res.* 2001; 61:4766–4772. [PubMed: 11406550]
33. Curiel TJ, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med.* 2004; 10:942–949. [PubMed: 15322536]
34. Sasada T, Kimura M, Yoshida Y, Kanai M, Takabayashi A. CD4⁺ CD25⁺ regulatory T cells in patients with gastrointestinal malignancies. *Cancer.* 2003; 98:1089–1099. [PubMed: 12942579]
35. Gao Q, Qiu S-J, Fan J, Zhou J, Wang X-Y, Xiao Y-S, Xu Y, Li Y-W, Tang Z-Y. Intratumoral balance of regulatory and cytotoxic T cells is associated with prognosis of hepatocellular carcinoma after resection. *J Clin Oncol.* 2007; 25:2586–2593. [PubMed: 17577038]
36. Rudge G, Barrett SP, Scott B, van Driel IR. Infiltration of a mesothelioma by IFN-gamma-producing cells and tumor rejection after depletion of regulatory T cells. *J Immunol.* 2007; 178:4089–4096. [PubMed: 17371963]
37. Needham DJ, Lee JX, Beilharz MW. Intra-tumoural regulatory T cells: a potential new target in cancer immunotherapy. *Biochem Biophys Res Commun.* 2006; 343:684–691. [PubMed: 16563349]
38. Anraku M, Tagawa T, Wu L, Yun Z, Keshavjee S, Zhang L, Johnston MR, de Perrot M. Synergistic antitumor effects of regulatory T cell blockade combined with pemetrexed in murine malignant mesothelioma. *J Immunol.* 2010; 185:956–966. [PubMed: 20548032]
39. van der Most RG, Currie AJ, Mahendran S, Prosser A, Darabi A, Robinson BW, Nowak AK, Lake RA. Tumor eradication after cyclophosphamide depends on concurrent depletion of regulatory T cells: a role for cycling TNFR2-expressing effector-suppressor T cells in limiting effective chemotherapy. *Cancer Immunol Immunother.* 2009; 58:1219–1228. [PubMed: 19052741]
40. van der Most RG, Currie AJ, Cleaver AL, Salmons J, Nowak AK, Mahendran S, Larma I, Prosser A, Robinson BW, Smyth MJ, Scalzo AA, Degli-Esposti MA, Lake RA. Cyclophosphamide chemotherapy sensitizes tumor cells to TRAIL-dependent CD8 T cell-mediated immune attack resulting in suppression of tumor growth. *PLoS One.* 2009; 4:e6982. [PubMed: 19746156]
41. Wu L, Yun Z, Tagawa T, Rey-McIntyre K, Anraku M, de Perrot M. Tumor cell repopulation between cycles of chemotherapy is inhibited by regulatory T-cell depletion in a murine mesothelioma model. *J Thorac Oncol.* 2011 Publish Ahead of Print.

42. Jackaman C, Cornwall S, Lew AM, Zhan Y, Robinson BW, Nelson DJ. Local effector failure in mesothelioma is not mediated by CD4⁺ CD25⁺ T-regulator cells. *Eur Respir J*. 2009; 34:162–175. [PubMed: 19251786]
43. Sica A, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A. Macrophage polarization in tumour progression. *Semin Cancer Biol*. 2008; 18:349–355. [PubMed: 18467122]
44. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol*. 2002; 196:254–265. [PubMed: 11857487]
45. Koukourakis MI, Giatromanolaki A, Kakolyris S, O'Byrne KJ, Apostolikas N, Skarlatos J, Gatter KC, Harris AL. Different patterns of stromal and cancer cell thymidine phosphorylase reactivity in non-small-cell lung cancer: impact on tumour neoangiogenesis and survival. *Br J Cancer*. 1998; 77:1696–1703. [PubMed: 9635852]
46. Lissbrant IF, Stattin P, Wikstrom P, Damber JE, Egevad L, Bergh A. Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol*. 2000; 17:445–451. [PubMed: 10938382]
47. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer*. 2004; 4:71–78. [PubMed: 14708027]
48. Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. 2010; 141:39–51. [PubMed: 20371344]
49. Salvesen HB, Akslen LA. Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. *Int J Cancer*. 1999; 84:538–543. [PubMed: 10502735]
50. Volodko N, Reiner A, Rudas M, Jakesz R. Tumour-associated macrophages in breast cancer and their prognostic correlations. *The Breast*. 1998; 7:99–105.
51. Izzi V, Chiurciu V, D'Aquilio F, Palumbo C, Tresoldi I, Modesti A, Baldini PM. Differential effects of malignant mesothelioma cells on THP-1 monocytes and macrophages. *Int J Oncol*. 2009; 34:543–550. [PubMed: 19148491]
52. Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G, Reckamp K, Dohadwala M, Sharma S, Dubinett SM. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4⁺ T cells. *J Immunol*. 2005; 175:1483–1490. [PubMed: 16034085]
53. Sharma S, Yang S-C, Zhu L, Reckamp K, Gardner B, Baratelli F, Huang M, Batra RK, Dubinett SM. Tumor Cyclooxygenase-2/Prostaglandin E2-dependent promotion of FOXP3 expression and CD4⁺ CD25⁺ T regulatory cell activities in lung cancer. *Cancer Res*. 2005; 65:5211–5220. [PubMed: 15958566]
54. Miselis NR, Wu ZJ, Van Rooijen N, Kane AB. Targeting tumor-associated macrophages in an orthotopic murine model of diffuse malignant mesothelioma. *Mol Cancer Ther*. 2008; 7:788–799. [PubMed: 18375821]
55. Steinman RM. Dendritic cells in vivo: a key target for a new vaccine science. *Immunity*. 2008; 29:319–324. [PubMed: 18799140]
56. Steinman RM, Banchereau J. Taking dendritic cells into medicine. *Nature*. 2007; 449:419–426. [PubMed: 17898760]
57. Hegmans JP, Hemmes A, Aerts JG, Hoogsteden HC, Lambrecht BN. Immunotherapy of murine malignant mesothelioma using tumor lysate-pulsed dendritic cells. *Am J Respir Crit Care Med*. 2005; 171:1168–1177. [PubMed: 15764728]
58. Veltman JD, Lambers ME, van Nimwegen M, de Jong S, Hendriks RW, Hoogsteden HC, Aerts JG, Hegmans JP. Low-dose cyclophosphamide synergizes with dendritic cell-based immunotherapy in antitumor activity. *J Biomed Biotechnol*. 2010; 2010:798467. [PubMed: 20508851]
59. Wada S, Yoshimura K, Hipkiss EL, Harris TJ, Yen H-R, Goldberg MV, Grosso JF, Getnet D, Demarzo AM, Netto GJ, Anders R, Pardoll DM, Drake CG. Cyclophosphamide augments antitumor immunity: studies in an autochthonous prostate cancer model. *Cancer Res*. 2009; 69:4309–4318. [PubMed: 19435909]

60. Ho M, Hassan R, Zhang J, Wang QC, Onda M, Bera T, Pastan I. Humoral immune response to mesothelin in mesothelioma and ovarian cancer patients. *Clin Cancer Res.* 2005; 11:3814–3820. [PubMed: 15897581]
61. Johnston FM, Tan MC, Tan BR Jr, Porembka MR, Brunt EM, Linehan DC, Simon PO Jr, Plambeck-Suess S, Eberlein TJ, Hellstrom KE, Hellstrom I, Hawkins WG, Goedegebuure P. Circulating mesothelin protein and cellular antimesothelin immunity in patients with pancreatic cancer. *Clin Cancer Res.* 2009; 15:6511–6518. [PubMed: 19843662]
62. Keilholz U, Letsch A, Busse A, Asemissen AM, Bauer S, Blau IW, Hofmann WK, Uharek L, Thiel E, Scheibenbogen C. A clinical and immunologic phase 2 trial of Wilms tumor gene product 1 (WT1) peptide vaccination in patients with AML and MDS. *Blood.* 2009; 113:6541–6548. [PubMed: 19389880]
63. May RJ, Dao T, Pinilla-Ibarz J, Korontsvit T, Zakhaleva V, Zhang RH, Maslak P, Scheinberg DA. Peptide epitopes from the Wilms' tumor 1 oncoprotein stimulate CD4⁺ and CD8⁺ T cells that recognize and kill human malignant mesothelioma tumor cells. *Clin Cancer Res.* 2007; 13:4547–4555. [PubMed: 17671141]
64. Rezvani K, Brenchley JM, Price DA, Kilical Y, Gostick E, Sewell AK, Li J, Mielke S, Douek DC, Barrett AJ. T-cell responses directed against multiple HLA-A*0201-restricted epitopes derived from Wilms' tumor 1 protein in patients with leukemia and healthy donors: identification, quantification, and characterization. *Clin Cancer Res.* 2005; 11:8799–8807. [PubMed: 16361568]
65. Hassan R, Ho M. Mesothelin targeted cancer immunotherapy. *Eur J Cancer.* 2008; 44:46–53. [PubMed: 17945478]
66. Wheatley-Price P, Yang B, Patsios D, Patel D, Ma C, Xu W, Leighl N, Feld R, Cho BCJ, O'Sullivan B, Roberts H, Tsao MS, Tammemagi M, Anraku M, Chen Z, de Perrot M, Liu G. Soluble mesothelin-related peptide and osteopontin as markers of response in malignant mesothelioma. *J Clin Oncol.* 2010; 28:3316–3322. [PubMed: 20498407]
67. Robinson BWS, Creaney J, Lake R, Nowak A, Musk AW, de Klerk N, Winzell P, Hellstrom KE, Hellstrom I. Mesothelin-family proteins and diagnosis of mesothelioma. *Lancet.* 2003; 362:1612–1616. [PubMed: 14630441]
68. Hassan R, Williams-Gould J, Steinberg SM, Liewehr DJ, Yokokawa J, Tsang KY, Surawski RJ, Scott T, Camphausen K. Tumor-directed radiation and the immunotoxin SS1P in the treatment of mesothelin-expressing tumor xenografts. *Clin Cancer Res.* 2006; 12:4983–4988. [PubMed: 16914588]
69. Zhang Y, Xiang L, Hassan R, Paik CH, Carrasquillo JA, Jang BS, Le N, Ho M, Pastan I. Synergistic antitumor activity of taxol and immunotoxin SS1P in tumor-bearing mice. *Clin Cancer Res.* 2006; 12:4695–4701. [PubMed: 16899620]
70. Hassan R, Ebel W, Routhier EL, Patel R, Kline JB, Zhang J, Chao Q, Jacob S, Turchin H, Gibbs L, Phillips MD, Mudali S, Iacobuzio-Donahue C, Jaffee EM, Moreno M, Pastan I, Sass PM, Nicolaides NC, Grasso L. Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. *Cancer Immun.* 2007; 7:20. [PubMed: 18088084]
71. Sadelain M, Rivière I, Brentjens R. Targeting tumours with genetically enhanced T lymphocytes. *Nat Rev Cancer.* 2003; 3:35–45. [PubMed: 12509765]
72. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhai M, Suhoski MM, Varela-Rohena A, Haines KM, Heitjan DF, Albelda SM, Carroll RG, Riley JL, Pastan I, June CH. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. *Proc Natl Acad Sci USA.* 2009; 106:3360–3365. [PubMed: 19211796]
73. Moon EK, Carpenito C, Sun J, Wang L-CS, Kapoor V, Predina JD, Powell DJ, Riley J, June CH, Albelda SM. Expression of a functional CCR2 receptor enhances tumor localization and eradication by human T cells expressing a mesothelin-specific chimeric antibody receptor. *Clin Cancer Res.* 2011; 17(14):4719–4730. [PubMed: 21610146]
74. Huber JP, David Farrar J. Regulation of effector and memory T-cell functions by type I interferon. *Immunology.* 2011; 132:466–474. [PubMed: 21320124]
75. Odaka M, Serman DH, Wiewrodt R, Zhang Y, Kiefer M, Amin KM, Gao G-P, Wilson JM, Barsoum J, Kaiser LR, Albelda SM. Eradication of intraperitoneal and distant tumor by adenovirus-mediated interferon- γ Gene therapy is attributable to induction of systemic immunity. *Cancer Res.* 2001; 61:6201–6212. [PubMed: 11507073]

76. Kruklitis RJ, Singhal S, Delong P, Kapoor V, Sterman DH, Kaiser LR, Albelda SM. Immuno-gene therapy with interferon-beta before surgical debulking delays recurrence and improves survival in a murine model of malignant mesothelioma. *J Thorac Cardiovasc Surg.* 2004; 127:123–130. [PubMed: 14752422]
77. Smith KA. Interleukin-2: inception, impact, and implications. *Science.* 1988; 240:1169–1176. [PubMed: 3131876]
78. Kern DE, Gillis S, Okada M, Henney CS. The role of interleukin-2 (IL-2) in the differentiation of cytotoxic T cells: the effect of monoclonal anti-IL-2 antibody and absorption with IL-2 dependent T cell lines. *J Immunol.* 1981; 127:1323–1328. [PubMed: 6456304]
79. Jackaman C, Bundell CS, Kinnear BF, Smith AM, Filion P, van Hagen D, Robinson BW, Nelson DJ. IL-2 intratumoral immunotherapy enhances CD8⁺ T cells that mediate destruction of tumor cells and tumor-associated vasculature: a novel mechanism for IL-2. *J Immunol.* 2003; 171:5051–5063. [PubMed: 14607902]
80. Jackaman C, Lew AM, Zhan Y, Allan JE, Koloska B, Graham PT, Robinson BW, Nelson DJ. Deliberately provoking local inflammation drives tumors to become their own protective vaccine site. *Int Immunol.* 2008; 20:1467–1479. [PubMed: 18824504]
81. van Mierlo GJ, den Boer AT, Medema JP, van der Voort EI, Franssen MF, Offringa R, Melief CJ, Toes RE. CD40 stimulation leads to effective therapy of CD40(–) tumors through induction of strong systemic cytotoxic T lymphocyte immunity. *Proc Natl Acad Sci USA.* 2002; 99:5561–5566. [PubMed: 11929985]
82. Del Vecchio M, Bajetta E, Canova S, Lotze MT, Wesa A, Parmiani G, Anichini A. Interleukin-12: biological properties and clinical application. *Clin Cancer Res.* 2007; 13:4677–4685. [PubMed: 17699845]
83. Brunda MJ, Luistro L, Warriar RR, Wright RB, Hubbard BR, Murphy M, Wolf SF, Gately MK. Antitumor and anti-metastatic activity of interleukin 12 against murine tumors. *J Exp Med.* 1993; 178:1223–1230. [PubMed: 8104230]
84. Zou JP, Yamamoto N, Fujii T, Takenaka H, Kobayashi M, Herrmann SH, Wolf SF, Fujiwara H, Hamaoka T. Systemic administration of rIL-12 induces complete tumor regression and protective immunity: response is correlated with a striking reversal of suppressed IFN-gamma production by anti-tumor T cells. *Int Immunol.* 1995; 7:1135–1145. [PubMed: 8527411]
85. Caminschi I, Venetsanakis E, Leong CC, Garlepp MJ, Scott B, Robinson BW. Interleukin-12 induces an effective anti-tumor response in malignant mesothelioma. *Am J Respir Cell Mol Biol.* 1998; 19:738–746. [PubMed: 9806738]
86. Caminschi I, Venetsanakis E, Leong CC, Garlepp MJ, Robinson BW, Scott B. Cytokine gene therapy of mesothelioma. Immune and antitumor effects of transfected interleukin-12. *Am J Respir Cell Mol Biol.* 1999; 21:347–356. [PubMed: 10460752]
87. Fridlender ZG, Buchlis G, Kapoor V, Cheng G, Sun J, Singhal S, Crisanti MC, Wang LC, Heitjan D, Snyder LA, Albelda SM. CCL2 blockade augments cancer immunotherapy. *Cancer Res.* 2010; 70:109–118. [PubMed: 20028856]
88. Kim S, Buchlis G, Fridlender ZG, Sun J, Kapoor V, Cheng G, Haas A, Cheung HK, Zhang X, Corbley M, Kaiser LR, Ling L, Albelda SM. Systemic blockade of transforming growth factor-beta signaling augments the efficacy of immunogene therapy. *Cancer Res.* 2008; 68:10247–10256. [PubMed: 19074893]
89. Marie JC, Letterio JJ, Gavin M, Rudensky AY. TGF-β1 maintains suppressor function and Foxp3 expression in CD4⁺ CD25⁺ regulatory T cells. *J Exp Med.* 2005; 201:1061–1067. [PubMed: 15809351]
90. Thomas DA, Massagué J. TGF-[beta] directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. *Cancer Cell.* 2005; 8:369–380. [PubMed: 16286245]
91. Ahmadzadeh M, Rosenberg SA. TGF-β1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. *J Immunol.* 2005; 174:5215–5223. [PubMed: 15843517]
92. Allavena P, Sica A, Garlanda C, Mantovani A. The Yin-Yang of tumor-associated macrophages in neoplastic progression and immune surveillance. *Immunol Rev.* 2008; 222:155–161. [PubMed: 18364000]

93. Fridlender ZG, Sun J, Kim S, Kapoor V, Cheng G, Ling L, Worthen GS, Albelda SM. Polarization of tumor-associated neutrophil phenotype by TGF-beta: "N1" versus "N2" TAN. *Cancer Cell*. 2009; 16:183–194. [PubMed: 19732719]
94. Rodriguez PC, Ochoa AC. Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. *Immunol Rev*. 2008; 222:180–191. [PubMed: 18364002]
95. Currie AJ, van der Most RG, Broomfield SA, Prosser AC, Tovey MG, Robinson BW. Targeting the effector site with IFN-alpha-beta-inducing TLR ligands reactivates tumor-resident CD8 T cell responses to eradicate established solid tumors. *J Immunol*. 2008; 180:1535–1544. [PubMed: 18209049]
96. Broomfield SA, van der Most RG, Prosser AC, Mahendran S, Tovey MG, Smyth MJ, Robinson BW, Currie AJ. Locally administered TLR7 agonists drive systemic antitumor immune responses that are enhanced by anti-CD40 immunotherapy. *J Immunol*. 2009; 182:5217–5224. [PubMed: 19380767]
97. Egen JG, Kuhns MS, Allison JP. CTLA-4: new insights into its biological function and use in tumor immunotherapy. *Nat Immunol*. 2002; 3:611–618. [PubMed: 12087419]
98. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996; 271:1734–1736. [PubMed: 8596936]
99. Shrikant P, Khoruts A, Mescher MF. CTLA-4 blockade reverses CD8⁺ T cell tolerance to tumor by a CD4⁺ T cell- and IL-2-dependent mechanism. *Immunity*. 1999; 11:483–493. [PubMed: 10549630]
100. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. *J Exp Med*. 1999; 190:355–366. [PubMed: 10430624]
101. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, Akerley W, van den Eertwegh AJ, Lutzky J, Lorigan P, Vaubel JM, Linette GP, Hogg D, Ottensmeier CH, Lebbe C, Peschel C, Quirt I, Clark JI, Wolchok JD, Weber JS, Tian J, Yellin MJ, Nichol GM, Hoos A, Urba WJ. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–723. [PubMed: 20525992]
102. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH Jr, Gascon P, Lotem M, Harnankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011; 364:2517–2526. [PubMed: 21639810]
103. Adusumilli PS, Eisenberg DP, Stiles BM, Chung S, Chan M-K, Rusch VW, Fong Y. Intraoperative localization of lymph node metastases with a replication-competent herpes simplex virus. *J Thorac Cardiovasc Surg*. 2006; 132:1179.e1–1188.e1. [PubMed: 17059941]
104. Adusumilli PS, Stiles BM, Chan M-K, Mullerad M, Eisenberg DP, Ben-Porat L, Huq R, Rusch VW, Fong Y. Imaging and therapy of malignant pleural mesothelioma using replication-competent herpes simplex viruses. *J Gene Med*. 2006; 8:603–615. [PubMed: 16475242]
105. Adusumilli PS, Stiles BM, Chan MK, Eisenberg DP, Yu Z, Stanziale SF, Huq R, Wong RJ, Rusch VW, Fong Y. Real-time diagnostic imaging of tumors and metastases by use of a replication-competent herpes vector to facilitate minimally invasive oncological surgery. *FASEB J*. 2006; 20:726–728. [PubMed: 16467372]
106. Serman DH, Recio A, Carroll RG, Gillespie CT, Haas A, Vachani A, Kapoor V, Sun J, Hodinka R, Brown JL, Corbly MJ, Parr M, Ho M, Pastan I, Machuzak M, Benedict W, Zhang XQ, Lord EM, Litzky LA, Heitjan DF, June CH, Kaiser LR, Vonderheide RH, Albelda SM, Kanther M. A phase I clinical trial of single-dose intrapleural IFN-beta gene transfer for malignant pleural mesothelioma and metastatic pleural effusions: high rate of antitumor immune responses. *Clin Cancer Res*. 2007; 13:4456–4466. [PubMed: 17671130]
107. Serman DH, Recio A, Haas AR, Vachani A, Katz SI, Gillespie CT, Cheng G, Sun J, Moon E, Pereira L, Wang X, Heitjan DF, Litzky L, June CH, Vonderheide RH, Carroll RG, Albelda SM. A phase I trial of repeated intrapleural adenoviral-mediated interferon-beta gene transfer for

- mesothelioma and meta-static pleural effusions. *Mol Ther*. 2010; 18:852–860. [PubMed: 20068553]
108. Boutin C, Nussbaum E, Monnet I, Bignon J, Vanderschueren R, Guerin JC, Menard O, Mignot P, Dabouis G, Douillard JY. Intrapleural treatment with recombinant gamma-interferon in early stage malignant pleural mesothelioma. *Cancer*. 1994; 74:2460–2467. [PubMed: 7923001]
 109. Goey SH, Eggermont AM, Punt CJ, Slingerland R, Gratama JW, Oosterom R, Oskam R, Bolhuis RL, Stoter G. Intrapleural administration of interleukin 2 in pleural mesothelioma: a phase I-II study. *Br J Cancer*. 1995; 72:1283–1288. [PubMed: 7577483]
 110. Astoul P, Picat-Joossen D, Viallat JR, Boutin C. Intrapleural administration of interleukin-2 for the treatment of patients with malignant pleural mesothelioma: a phase II study. *Cancer*. 1998; 83:2099–2104. [PubMed: 9827714]
 111. Castagneto B, Zai S, Mutti L, Lazzaro A, Ridolfi R, Piccolini E, Ardizzoni A, Fumagalli L, Valsuani G, Botta M. Palliative and therapeutic activity of IL-2 immunotherapy in unresectable malignant pleural mesothelioma with pleural effusion: results of a phase II study on 31 consecutive patients. *Lung Cancer*. 2001; 31:303–310. [PubMed: 11165411]
 112. Lucchi M, Chella A, Melfi F, Dini P, Tibaldi C, Fontanini G, Mussi A. Four-modality therapy in malignant pleural mesothelioma: a phase II study. *J Thorac Oncol*. 2007; 2:237–242. [PubMed: 17410047]
 113. Lucchi M, Chella A, Melfi F, Dini P, Ambrogi M, Fino L, Fontanini G, Mussi A. A phase II study of intrapleural immuno-chemotherapy, pleurectomy/decortication, radiotherapy, systemic chemotherapy and long-term sub-cutaneous IL-2 in stage II-III malignant pleural mesothelioma. *Eur J Cardiothorac Surg*. 2007; 31:529–533. discussion 533-4. [PubMed: 17188885]
 114. Bretti S, Berruti A, Dogliotti L, Castagneto B, Bertulli R, Spadaro P, Toscano G, Astorre P, Verusio C, Lionetto R, Bruzzi P, Santoro A. Combined epirubicin and interleukin-2 regimen in the treatment of malignant mesothelioma: a multicenter phase II study of the Italian group on rare tumors. *Tumori*. 1998; 84:558–561. [PubMed: 9862516]
 115. Mulatero CW, Penson RT, Papamichael D, Gower NH, Evans M, Rudd RM. A phase II study of combined intravenous and subcutaneous interleukin-2 in malignant pleural mesothelioma. *Lung Cancer*. 2001; 31:67–72. [PubMed: 11162868]
 116. Ali G, Boldrini L, Lucchi M, Picchi A, Dell'Omodarme M, Prati MC, Mussi A, Corsi V, Fontanini G. Treatment with interleukin-2 in malignant pleural mesothelioma: immunological and angiogenetic assessment and prognostic impact. *Br J Cancer*. 2009; 101:1869–1875. [PubMed: 19935800]
 117. Ali G, Boldrini L, Lucchi M, Mussi A, Corsi V, Fontanini G. Tryptase mast cells in malignant pleural mesothelioma as an independent favorable prognostic factor. *J Thorac Oncol*. 2009; 4:348–354. [PubMed: 19155997]
 118. Hegmans JP, Veltman JD, Lambers ME, de Vries IJ, Figdor CG, Hendriks RW, Hoogsteden HC, Lambrecht BN, Aerts JG. Consolidative dendritic cell-based immunotherapy elicits cytotoxicity against malignant mesothelioma. *Am J Respir Crit Care Med*. 2010; 181:1383–1390. [PubMed: 20167848]
 119. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, Mellman I, Prindiville SA, Viner JL, Weiner LM, Matrisian LM. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res*. 2009; 15:5323–5337. [PubMed: 19723653]
 120. Xue SA, Gao L, Thomas S, Hart DP, Xue JZ, Gillmore R, Voss RH, Morris E, Stauss HJ. Development of a Wilms' tumor antigen-specific T-cell receptor for clinical trials: engineered patient's T cells can eliminate autologous leukemia blasts in NOD/SCID mice. *Haematologica*. 2010; 95:126–134. [PubMed: 19679884]
 121. Amin KM, Litzky LA, Smythe WR, Mooney AM, Morris JM, Mews DJ, Pass HI, Kari C, Rodeck U, Rauscher FJ III, et al. Wilms' tumor 1 susceptibility (WT1) gene products are selectively expressed in malignant mesothelioma. *Am J Pathol*. 1995; 146:344–356. [PubMed: 7856747]
 122. Oates J, Edwards C. HBME-1, MOC-31, WT1 and calretinin: an assessment of recently described markers for mesothelioma and adenocarcinoma. *Histopathology*. 2000; 36:341–347. [PubMed: 10759948]

123. Krug LM, Dao T, Brown AB, Maslak P, Travis W, Bekele S, Korontsvit T, Zakhaleva V, Wolchok J, Yuan J, Li H, Tyson L, Scheinberg DA. WT1 peptide vaccinations induce CD4 and CD8 T cell immune responses in patients with mesothelioma and non-small cell lung cancer. *Cancer Immunol Immunother*. 2010; 59:1467–1479.
124. Hassan R, Bullock S, Premkumar A, Kreitman RJ, Kindler H, Willingham MC, Pastan I. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. Infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. *Clin Cancer Res*. 2007; 13:5144–5149. [PubMed: 17785569]
125. Kreitman RJ, Hassan R, FitzGerald DJ, Pastan I. Phase I trial of continuous infusion anti-mesothelin recombinant immunotoxin SS1P. *Clin Cancer Res*. 2009; 15:5274–5279. [PubMed: 19671873]
126. Hassan R, Cohen SJ, Phillips M, Pastan I, Sharon E, Kelly RJ, Schweizer C, Weil S, Laheru D. Phase I clinical trial of the chimeric anti-mesothelin monoclonal antibody MORAb-009 in patients with mesothelin-expressing cancers. *Clin Cancer Res*. 2010; 16:6132–6138. [PubMed: 21037025]
127. Pages F, Galon J, Fridman WH. The essential role of the in situ immune reaction in human colorectal cancer. *J Leukoc Biol*. 2008; 84:981–987. [PubMed: 18559950]
128. Pages F, Kirilovsky A, Mlecnik B, Asslaber M, Tosolini M, Bindea G, Lagorce C, Wind P, Marliot F, Bruneval P, Zatloukal K, Trajanoski Z, Berger A, Fridman WH, Galon J. In situ cytotoxic and memory T cells predict outcome in patients with early-stage colorectal cancer. *J Clin Oncol*. 2009; 27:5944–5951. [PubMed: 19858404]
129. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pages C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoue F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pages F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science*. 2006; 313:1960–1964. [PubMed: 17008531]
130. Clemente CG, Mihm MC Jr, Bufalino R, Zurrida S, Collini P, Cascinelli N. Prognostic value of tumor infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer*. 1996; 77:1303–1310. [PubMed: 8608507]
131. Broomfield SA, et al. Partial, but not complete, tumor-debulking surgery promotes protective antitumor memory when combined with chemotherapy and adjuvant immunotherapy. *Cancer Res*. 2005; 65(17):7580–7584. [PubMed: 16140921]

Fig. 1.

When challenged with either long asbestos fibers (**a**) or long carbon nanotubes (**b**), macrophages fail to satisfactorily phagocytose the encountered particles, as the particles are longer than macrophages can accommodate. Repeated phagocytotic efforts by macrophages fail to clear the long fibers (“frustrated phagocytosis”), resulting in continued generation of reactive oxygen species and secretion of pro-inflammatory cytokines, leading to prolonged inflammation. (Reprinted by permission from Macmillan Publishers Ltd: Nature Nanotechnology, [17].)

Fig. 2.

Immune interactions and immunomodulation in MPM. $CD4^+/CD8^+$ T cells and N1 neutrophils have anti-tumor associations while regulatory T cells (T_{reg}), N2 neutrophils, and M2 macrophages have pro-tumor associations. Immunomodulation is designed to potentiate the anti-tumor cells, and dendritic cell therapy, TLR agonist, and IFN- β increase CD8 response, while anti-CD40 antibody mimics $CD4^+$ assistance; IL-2 and IL-12 increase $CD4^+$ and $CD8^+$ responses. Therapies can also impair the pro-tumor cells. TGF- β polarizes neutrophils and macrophages to pro-tumor N2 and M2 phenotypes, respectively, as well as promoting T_{reg} . TGF- β blockade results in increased N1 neutrophils and $CD8^+$ cells. Anti-CD25 antibody and cyclophosphamide can deplete T_{reg} , while anti-CCL2/12 antibody may impair T_{reg} trafficking. In addition, therapies can target a specific tumor antigen—SS1P, MORAb-009, and mesothelin CAR target mesothelin, while WT-1 peptide vaccine augments the immune response against WT-1-expressing MPM

Table 1

Human clinicopathological studies in MPM

Immune cell	# patients	Stage	Histology	Observations/conclusion	Survival advantage	Author/journal
Peripheral blood NLR	173	Unavailable for entire cohort	Epithelioid—110 (64%) Biphasic—26 (15%) Sarcomatoid—12 (7%) “Others”—11 (6%) Unspecified—14 (8%)	NLR found to be an independent predictor of survival after multivariate analysis Median OS—16.7 versus 6.6 months	<i>NLR</i> <5 and ≥5: 1-year OS for—60% versus 26%	Kao [29]
CD8 ⁺	44	Stage I—3 (7%) Stage II—17 (39%) Stage III—21 (48%) Stage IV—3 (7%)	Epithelioid—26 (59%) Biphasic—14 (32%) Sarcomatoid—4 (9%)	High CD8 ⁺ associated with improved prognosis in patients who underwent EPP	2-year OS 53% versus 26%*	Yamada [4]
T _{reg}	32	Stage II—7 (22%) Stage III—23 (72%) Stage IV—2 (6%) Stage IB—1 (1%)	Epithelioid—25 (78%) Biphasic—6 (19%) Sarcomatoid—1 (3%)	High CD8 ⁺ associated with better prognosis in patients undergoing EPP	3-year OS 83% versus 28%	Anraku [3]
T _{reg}	93	Stage IB—1 (1%)	Epithelioid—71 (76%)	High regulatory T cells (Foxp3 ⁺) associated with poorer OS and time to progression following <i>IL-2 treatment</i>	Approx 24-month increase in median survival for low versus high T _{reg} (in <i>IL-2</i> -treated patients)	Ali [116]
Mast cells	60	Stage II—31 (33%) Stage III—61 (66%) Stage IB—1 (2%)	Biphasic—12 (13%) Sarcomatoid—10 (11%) Epithelioid—46 (77%)	High tryptase-positive mast cells associated with improved OS and time to progression following <i>intrapleural IL-2</i>	Median Survival—21 versus 12 months Median time to progression—18 versus	Ali [117]

Immune cell	# patients	Stage	Histology	Observations/conclusion	Survival advantage	Author/journal
		Stage II—14 (25%)	Biphasic—9 (15%)	induction therapy	9 months	
		Stage III—42 (73%)	Sarcomatoid—5 (8%)			

NLR neutrophil-to-lymphocyte ratio, *Treg* regulatory T cell, *OS* overall survival, *EPP* extrapleural pneumonectomy

* values extrapolated from reported Kaplan–Meier survival curves

Table 2

Pre-clinical immunotherapy for malignant pleural mesothelioma

Therapeutic target	Therapeutic agent	Translational potential	Murine model	Observations	Author/study
T _{reg}	anti-CD25 Ab; pemetrexed	Yes	NOD/SCID; intrathoracic	↑ survival with combination therapy ↓ tumor-infiltrating T _{regs} ↑ IL-2 production ↑ dendritic cell maturation ↑ CD3 CD8 IFN-γ tumor-infiltrating T cells	Anraku [38]
	CPA prior to DC immunotherapy	Yes	BALB/c; peritoneal	Addition of CPA to DC immunotherapy improved median and overall survival	Veltman [58]
	anti-CD25 Ab or CPA	Yes	BALB/c; flank	Genetic ablation eradicated tumors only when T _{regs} were concurrently depleted	van der Most [39]
	CPA treatment	Yes	BALB/c; flank	CPA kills tumor cells by apoptosis and has a CD8 ⁺ and NK cell-dependent anti-tumor effect	van der Most [40]
	anti-CD25 Ab	Yes	BALB/c; flank	↑ IFN-γ-producing T cells found in tumor tissue ↑ increased rejection and restricted growth of implanted tumors	Rudge [36]
TAM	anti-CD25 Ab	Yes	C57Bl/6; flank	Intratumoral T _{reg} depletion significantly decreased tumor growth	Needham [37]
	anti-CD25 Ab	Yes	BALB/c; peritoneal	↑ survival following T _{reg} depletion	Hegmans [5]
DC	CLIP		C57Bl/6; peritoneal	↓ tumor growth and invasion with TAM depletion	Miselis [54]
Tumor antigen	Pulsed DC Immunotherapy	Yes	BALB/c; peritoneal	Pulsed DCs prevented mesothelioma outgrowth (high tumor burden and delayed administration prevented effect)	Hegmans [57]
	Anti-mesothelin CAR-transduced T cells		NOD/SCID; flank	Anti-mesothelin CAR-engineered T cells reduced tumor burden	Carpenito [72]
TLR	Anti-mesothelin immunotoxin SS IP; radiation	Yes	Athymic nude; flank	SS IP addition significantly decreased tumor growth	Hassan [68]
	Intratumoral imiquimod and systemic CD40 agonist	Yes	BALB/c; flank	Combination therapy significantly enhanced local and distal anti-tumor responses	Broomfield [96]
CCL2	Intratumoral TLR agonist injection	Yes	BALB/c; flank	Significantly delayed tumor progression and caused complete tumor resolution in some mice	Currie [95]
	anti-CCL2 Ab and intratumoral adenoviral injection		C57Bl/6; flank	↓ tumor volume (cured ~ 50% of tumors) ↓ intratumoral regulatory T cells ↓ intratumoral CD8 ⁺ cells	Fridlender [87]

Therapeutic target	Therapeutic agent	Translational potential	Murine model	Observations	Author/study
TGF- β	CCL2R transduction of mesothelin CAR T cells		NOD/SCID/ γ -chain KO	$\uparrow$ T-cell tumor infiltration and anti-tumor efficacy	Moon [73]
	TGF- β inhibitor		BALB/c; flank	TGF- β blockade increased the recruitment and activation of antitumor neutrophils	Fridlander [93]
	TGF- β inhibitor and adenoviral vaccine immunotherapy		BALB/c; flank	$\uparrow$ immunostimulatory cytokines $\uparrow$ intratumoral CD8 $^{+}$ cells $\uparrow$ activated (CD25 $^{+}$) intratumoral CD8 $^{+}$ T cells	Kim [88]
IFN- β	Adenovirus encoded IFN- β	Yes	Peritoneal; Flank BALB/c SCID SCID/Bg	$\uparrow$ overall survival following treatment $\uparrow$ tumor growth following tumor re-challenge	Odaka [75]
IL-2	Adenovirus encoding IFN- β (pre-debulking)	Yes	BALB/c; flank	$\uparrow$ tumor-free survival $\downarrow$ tumor growth following tumor re-challenge	Krukutis [76]
	Intratumoral IL-2 and anti-CD 40 ab	Yes	Flank BALB/c C57Bl/6 BALB/c— Foxn1 $^{nu/nu}$	Resulted in resolution of locally treated and untreated distal tumors	Jackaman [80]
	Intratumoral IL-2	Yes	Flank BALB/c C57Bl/6	Mice with small tumors $\uparrow$ tumor regression Remained tumor-free for >2 months Survived tumor re-challenge	Jackaman [79]
IL-12	IL-12 gene transfer		Flank BALB/c Athymic nude	Mice with large tumors failed to respond IL-12-transfected MPM cells did not form tumor in BALB/c mice	Caminschi [86]
Multimodality	Systemic or intratumoral IL-12		BALB/c; flank	Systemic IL-12 at time of tumor inoculation prevented tumor growth Intratumoral IL-12 temporarily reduced tumor	Caminschi [85]
	Surgical debulking followed by gemcitabine and anti-CD40 ab		BALB/c; flank	Complete resection followed by combination chemotherapy/immunotherapy led to high rate of cure (>80%)	Broomfield [131]

CAR chimeric antigen receptor, CCL2R CCL2 receptor, CLIP liposome-encapsulated clodronate, CPA cyclophosphamide, DC dendritic cell, IFN interferon, TAM tumor-associated macrophage, TLR Toll-like receptor, Treg regulatory T cells

Table 3

Clinical immunotherapy trials for malignant pleural mesothelioma

Therapeutic agent	Route of administration	Phase	# patients	Histology	Immunologic response	Clinical response	Safety/toxicity	Author/study
Pulsed DC vaccination	IV, SQ	I	10	Epithelioid—10 (100%)	anti-KLH IgG and IgM detected	Median survival (after diagnosis)—19 months	Flu-like symptoms—8 (80%) Injection site reaction—8 (80%)	Hegmans [118]
WT1 peptide vaccination	SQ	I	12	Epithelioid—9 (100% of MPM)	WT-1-specific CD4 ⁺ and CD8 ⁺ cell responses	Median survival (after 1st vaccination)—14 months	Injection site reaction—7 (58%)	Krug [123]
Recombinant immunotoxin SSIP (anti-mesothelin dsFv-PE38)	IV	I	24	Not specified		Partial response—1/24 Stable disease—23/24 Progressive Disease—11/24 No difference between continuous and bolus infusion	Edema—71% Hypoalbuminemia—62% Fatigue—62% Weight gain—54% Nausea—46% Fever—46%	Kreitman [125]
	IV	I	34	Not specified		Stable disease or partial response seen in 14/19 mesothelioma patients	Hypoalbuminemia—21 (62%) Fatigue—19 (56%) Fever—9 (26%) Edema—9 (26%) Abdominal Pain—8 (24%) Pleural effusion—7 (21%) Vascular leak syndrome—7 (21%) Hypoxia—4 (12%) Hypotension—3 (9%) Pleuritis—3 (9%)	Hassan [124]
Chimeric Anti-mesothelin Monoclonal Antibody (MC)RAb-009	IV	I	24	Epithelioid—13 (100% of MPM)		No complete or partial responses noted. 11 subjects with stable	Abdominal Pain—1 Anemia—1 DVT/PE—1 Dizziness—1 Allergic reaction—1 Infusion reaction—4	Hassan [126]

Therapeutic agent	Route of administration	Phase	# patients	Histology	Immunologic response	Clinical response	Safety/toxicity	Author/study
IFN- β gene transfer	Intrapleural	I	10	Not specified		disease	Flushing—2 Fatigue—2 Hypotension—1 Headache—1 Elevated LFTs—1 Nausea—1 Serum Sickness—1 Vomiting—1	Sterman [106]
IL-2	Pre-operative intrapleural, post-operative SQ	II	49	Epithelioid—39 (80%) Biphasic—6 (12%) Sarcomatoid—4 (8%)		4 (40%) showed disease stability or regression by PET or CT at Day 60	Transient lymphopenia—2 (20%) Hypoxia—1 (10%) Elevated LFTs—1 (10%)	Lucchi [113]
	IV, SQ	II	29	Epithelioid—20 (62%) Sarcomatoid—1 (3%) biphasic—5 (7%) Unclassified—3 (28%)		Median survival—26 months 31 months—stage II 21 months—stage III	Fever* Nausea/ vomiting	Mulatero [115]
	Intrapleural	II	31	Epithelioid—22 (71%) Sarcomatoid—4 (13%) Biphasic—3 (10%) Unclassified—2 (6%)		Median overall survival Complete Responders—39 months Partial Responders—21.5 months Stable Disease—20 months Progressive Disease—9 months	Fever—15 (52%) Nausea/vomiting—10 (34%) Cutaneous manifestations—2 (7%)	Castagneto [111]
	Intrapleural	II	22	Epithelioid—19 (86%) Sarcomatoid—1 (5%) Biphasic—2 (9%)		Median survival of responders versus non-responders: 28 months versus 8 months	Fever—25 (81%) Cardiac Failure—1 (3%) Gastrointestinal—3 (10%) Neuropathy—2 (6%) Weight Gain—7 (22%) Eosinophilia—15 (48%)	Astoul [110]

Therapeutic agent	Route of administration	Phase	# patients	Histology	Immunologic response	Clinical response	Safety/toxicity	Author/ study
IFN- γ	Intrapleural	I-II	23	Epithelioid—23 (100%)	Increased intrapleural LAK activity	Median survival— 15.6 months	Cutaneous manifestation —2 (9%) Elevated Cr—3 (14%) LFT elevation—2 (9%) Eosinophilia—6 (27%)	Goez [109]
	Intrapleural	I	89	Epithelioid—71 (80%)		Mean duration of remission: Biphasic—18 (20%)	Fever—10 (48%) Empyema—5 (24%) Infection—7 (33%) Fever—89 (100%)	Boutin [108]
						17 months—patients with complete response 19 months—patients with partial response Response significantly higher in Stage I than Stage II disease (45% vs. 6%)	Severe Laboratory abnormalities —9 (10%) Empyema—7 (8%) Pneumothorax —1 (1%) Pneumonia— 1 (1%) Sepsis—1 (1%) Anaphylaxis —1 (1%) Seizure—1 (1%)	

DC dendritic cell, SQ subcutaneous, IV intravenous, IFN interferon, IL-2 interleukin 2, LAK lymphokine-activated killer, CTL cytotoxic T lymphocyte, CHF congestive heart failure, Cr creatinine; MPM malignant pleural mesothelioma

* patient numbers not given