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MESOTHELIAL CELLS REGULATE IMMUNE RESPONSES IN HEALTH AND DISEASE: ROLE FOR IMMUNOTHERAPY IN MALIGNANT MESOTHELIOMA

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Abstract

The mesothelium was first described by Bichat in 1827 and originally thought to function purely as a non-adhesive surface to facilitate intracoelomic movement of organs. However, the mesothelium is now recognised as a dynamic cellular membrane with many important functions that maintain serosal integrity and homeostasis. For example, mesothelial cells interact with and help regulate the body’s inflammatory and immune system following infection, injury or malignancy. With recent advances in our understanding of checkpoint molecules and the advent of novel immunotherapy approaches, there has been an increase in the number of studies examining mesothelial and immune cell interaction, in particular the role of these interactions in malignant mesothelioma. This review will highlight some of the recent advances in our understanding of how mesothelial cells help regulate serosal immunity and how in a malignant environment the immune system is hijacked to stimulate tumor growth. Ways to treat mesothelioma using immunotherapy approaches will also be discussed.

Highlights:

- Mesothelial and immune cell interactions play a crucial role in tissue homeostasis in the serosal cavities such as the pleura.
- Mesothelin is viewed as an attractive target for solid tumors, including malignant mesothelioma.
- Checkpoint inhibitor therapy has shown variable efficacy against malignant mesothelioma.
- CAR T cell therapies are being evaluated for malignant mesothelioma.
- Treatment of malignant mesothelioma will require multimodality approaches with immunotherapy central to future therapeutic approaches.
Introduction

The mesothelium consists of a single layer of cells that lines the three coelomic cavities; pleura, peritoneum and pericardium. It plays important roles including providing a barrier and first line of defense against infectious agents [1]. Mesothelial cells have a well-developed surface glycocalyx which repels foreign cells and organisms, and they are bathed in serosal fluid containing immunoglobulins, complement, lysozyme and other proteins to protect the mesothelial barrier from pathogens. Mesothelial cells have an immunoregulatory role, which is achieved through expression of multiple pattern recognition receptors that activate innate immune responses. In addition, they secrete several chemokines and cytokines that coordinate leukocyte migration to the site of inflammation and are able to present antigens to T cells [2]. However, interactions between mesothelial cells and immune cells can also drive pathological processes such as malignant mesothelioma (MM). This review will highlight some of the most recent studies examining mesothelial-immune cell interactions and how these might be modulated by immunotherapeutic intervention to treat MM.

Immune cell interaction with mesothelial cells

The pleural space is a sequestered local environment formed by mesothelial cells joined by junctional complexes, including tight junctions [3]. Tight junctions are important to help maintain a permeability barrier that restricts cell and fluid movement across the serosa. Mesothelial cells regulate both innate and adaptive immune responses at the serosal surface. They express multiple pattern recognition receptors (PRRs) that recognize different carbohydrates and lipopolysaccharide moieties on the surface of microbial pathogens and release mediators to initiate inflammation and activate immunomodulatory pathways. Mesothelial cells also recognize molecules derived from the host including cytokines, heat shock proteins, nucleic acids, ATP and HMGB1 that are released in response to tissue damage [4]. In response to these signals, mesothelial cells secrete a range of mediators such as antimicrobial peptides[5], chemokines and
inflammatory cytokines such as tumor necrosis factor alpha, interleukin (IL)-1, IL-6, and IL-8 and interferons, which in turn directs the differentiation of T cell subsets such as Th1, Th2, Th17 or regulatory T cells [6,7].

The pleura is also a common site of metastasis for many tumor types and is the primary site for the development of malignant mesothelioma (MM). Tumor growth is often accompanied by the formation of pleural effusions, which are accumulations of serous fluid rich in tumor cells, mesothelial cells, immune cells and the cytokines, growth factors, chemokines and other mediators these cells secrete. This fluid in turn provides an immunosuppressive environment which supports tumor growth [8].

**Immune hallmarks of Mesothelioma**

Chronic inflammation is a cancer risk and inflammation in tumors increases cancer progression. The tumor microenvironment secretes chemokines and growth factors that recruit tumor infiltrating lymphocytes (TILs) to facilitate tumor growth [9]. MM, which is strongly associated with asbestos exposure and fiber-associated inflammation, can form on any serosal surface. However, malignant pleural mesothelioma (MPM) is the most common. There are three histological types of MM; epithelioid, sarcomatoid and biphasic (a mix of epitheliod and sarcomatoid) [10,11]. Epithelioid MM is associated with high levels of TILs while sarcomatoid MM is associated with immune unresponsiveness or active immune suppression through recruitment of CD4+ Tregs and regulatory B cells (Bregs) expressing the inhibitory checkpoint marker PD-1 and its ligand PD-L1 [11] (Figure 1). MM is often unresponsive to treatments such as chemotherapy and radiotherapy [12]. Therefore, there is growing interest in understanding the detailed cellular composition of the inflammatory tumor microenvironment of individual patients to help develop new therapeutic approaches. The composition and behavior of immune cells can
vary from the peripheral blood to the tumor tissue or within effusions, which are used to study tumor-specific immune cell populations [13,14].

Normal human mesothelial cells and MPM tumor cell lines can secrete IL-6, IL-8, colony stimulating factor (CSF)-1, CSF-2 and monocyte chemoattractant protein (MCP)-1, which facilitate the recruitment of monocytes from the bone marrow or spleen to the tumor site where they undergo differentiation into tissue macrophages [15,16]. Tumor associated macrophages (TAMs) establish an immunosuppressive environment through the secretion of transforming growth factor beta (TGF-β), IL-10, chemokine ligand (CCL) 17 and CCL22 [17,18]. The elevated levels of TGF-β and IL-10 within the tumor environment directs the polarization of macrophages toward the M2 “alternatively activated” phenotype that function in tissue remodeling and immune regulation [17]. The accumulation of TAMs is associated with a poor prognosis across a range of cancers including MPM [19,20]. Interestingly non-epithelioid MPM tumors, which have a poorer prognosis, contain significantly higher levels of TAMs expressing markers consistent with an M2 phenotype [21].

The major lymphocyte populations that infiltrate tumors include CD4+ and CD8+ T cells and B cells [22,23]. CD4+ TILs include immunosuppressive CD4+ Tregs that antagonize proliferation and function of tumor-specific CD8+ cytotoxic T (Tc) cells [24,25]. The tumor microenvironment may contain high levels of TGF-β, which promotes differentiation of M2 macrophages and CD4+ Tregs that inhibit CD8+ TILs effector functions [14,26]. Tazzari and colleagues showed that epithelioid tumors had reduced CD4+ Th1 immune responses and increased recruitment of CD4+ Tregs [11]. Depletion of CD4+ Tregs from tumor tissues including MPM has been shown to have beneficial effects, which allow CD8 TIL effector functions to be resumed. Experimental treatments using anti-CD25 immunotoxin [27] or animal models that allow conditional depletion of Tregs in vivo by administration of a diphtheria toxin, allow CD8+ TILs to infiltrate the tumor
and reduce tumor volume to induce remission [28,29]. Removal of CD4+ Tregs from the tumor environment allow dendritic cells (DCs) to stimulate anti-tumor immunity driven by CD8+ TILs [28].

**Checkpoint immunotherapy and mesothelioma**

Tumor immunotherapy utilizing checkpoint inhibitors is increasingly used to treat solid tumors, including lung cancer [30], and is viewed as a potential effective treatment for MM (Table 1) [31-33]. Checkpoint inhibitors are antibody therapies that target specific cell surface markers associated with activated T cells, including PD-1 and CTLA-4 (Figure 1). PD-1 is expressed by chronically activated or “exhausted” T cells and CTLA-4 is an inhibitory receptor expressed by activated CD4+ and CD8+ T cells. Tumor cells express the PD-1 ligands, PD-L1 and/or PD-L2, while antigen presenting cells, DCs, macrophages and B cells, express the CD80 and CD86 costimulatory receptors that bind CTLA-4. Engagement of PD-1 and CTLA-4 on activated CD8+ T cells inhibits their proliferation and function, which enables tumors to evade immune detection. By targeting PD-1 or CTLA-4 on tumor CD8+ T cells, checkpoint inhibitors “reawaken” them from the exhausted phenotype so they will attack and eliminate the tumor.

Currently about 30% of patients receiving checkpoint inhibitor immunotherapy show a beneficial response [34]. In the MERIT study, a phase II MPM trial evaluating the PD-1 inhibitor nivolumab, 29% of patients had an objective response, consistent with most other tumor types [33]. A current phase II trial is testing the efficacy of nivolumab in relapsed MPM (CONFIRM, NCT03063450) [35]. The anti-PD-1 drug pembrolizumab has been evaluated in various phase trials (KEYNOTE) as second or third-line treatment. However after promising initial results, the phase III PROMISE-meso trial comparing pembrolizumab with a single-agent chemotherapy failed to show an improved median overall survival (OS) and progression-free survival (PFS), despite a superior
overall response rate (ORR) for pembrolizumab compared to chemotherapy alone [36]. Hassan and colleagues [37] reported the efficacy of treating MM patients with avelumab in the phase 1 JAVELIN solid tumor trial. Avelumab is a human anti PD-L1 antibody with a wild type Fc region capable of inducing significant anti-tumor activity via antibody dependent cellular cytotoxicity due to activation of adaptive and innate immune effector cells. The objective response rate was only 9%. In patients with PDL-1 positive tumors, the overall response rate was 19% and 6 month PFS was 27.5%, while the 12 month overall survival rate was 72.5% with a median of 20 months. Tremelimumab, an anti-CTLA-4 therapy, has been very disappointing demonstrating no benefit over placebo (DETERMINE) as first, second or third-line treatment [38,39].

Given the modest success of anti-CTLA-4 and anti-PD-1 therapy in MPM trials, other immune checkpoint molecules, including V-domain Ig suppressor of T cell activation protein (VISTA), T cell immunoglobulin 3 (TIM3), OX40 and glucocorticoid-induced tumor necrosis factor receptor (GITR) could be considered for therapeutic targeting. VISTA was recently shown to be expressed in a large number of MM tumors, which correlated with better survival outcomes [40]. VISTA was more highly expressed on epithelioid and biphasic MPM whereas PD-L1 was more highly expressed on sarcomatoid MPM [40]. The VISTA molecule is structurally similar to PD-L1 and, when overexpressed, suppresses early T-cell activation and proliferation and reduces cytokine production [41]. One VISTA inhibitor, CA-170, is currently in clinical trial and is being evaluated in solid tumors and lymphomas NCT02812875, but it is unclear if MM is one of the tumor types being examined. Another VISTA inhibitor, JNJ61610588, was also being trialed in solid tumors but this trial was unfortunately terminated for business reasons. T cells activated via OX-40 or GITR display enhanced cell proliferation and survival and can overcome the inhibitory effects of Treg cells. TIM3 is an inhibitory molecule expressed on T cells and on a dysfunctional population of CD8+ T cell effectors, such as in tumors. In an animal model of MM, Fear and colleagues
showed a synergistic effect between anti-CTLA-4 and anti-OX40 to inhibit tumor growth [42]. We wait to see if this outcome is replicated in patients with MM.

Although checkpoint inhibitors have been used successfully in a variety of tumor settings, some patients develop adverse events such as interstitial pneumonia or pneumonitis. Identification of patients who will respond to checkpoint inhibitor therapy and those who will not or have adverse effects, is currently a major focus of immunotherapy research. In the MERIT study it was noted that tumors with >1% PD-L1 staining were more likely to have a beneficial response compared to those patients whose tumors had <1% staining of PD-L1 [10,33]. Unfortunately, as in every tumor type, expression of the checkpoint molecules does not correlate to response rate. Clearly, we do not fully understand the mechanism by which checkpoint inhibitors regulate tumor growth.

Clinical trials have evaluated the use of combined therapies utilizing two inhibitors, but dual blockade still only provides a beneficial response of about 30% in most cases. Therefore, to improve the outcome of checkpoint immunotherapy, it is likely that it should be used in combination with surgery, chemotherapy, signaling inhibitors and other immune approaches such as CAR T cell and immunotoxin therapies.

**CAR T cell therapy and mesothelioma**

Chimeric antigen receptor (CAR)-T cell therapies are a new generation of immunotherapies that offer hope to cancer patients resistant to normal standard care therapies (Figure 2) [43,44]. CAR-T cells are T cells engineered to express a chimeric receptor that targets a tumor cell surface protein, carbohydrate or glycolipid [45]. CAR-T cells were first developed to treat B cell leukemias as they were constructed to express a chimeric receptor specific for CD19, a cell surface protein expressed abundantly on mature B cells. CD19 CAR-T cells have been used very effectively to treat acute lymphoblastic leukemias [43,44]. Second generation CAR-T cells are capable of targeting the tumor antigen and co-stimulating conventional T cells [46]. CAR receptors are currently designed
to express a single chain variable fragment (scFv) highly specific for the target antigen linked to a cytoplasmic signaling module (e.g. CD3ζ and costimulatory domain from CD28 or 41BB) [43,47]. High affinity for the target antigen can be problematic as it can also lead to dangerous reactivity against healthy organs or tissues that express the target antigen at low levels [43,47]. This has led scientists to try different approaches to enhance the safety and specificity of CAR-T cells.

The new generations of CAR-T cells under development are designed to target solid tumors such as MM. Mesothelin (MSLN) is a membrane-anchored glycoprotein normally expressed on mesothelial cells but is highly expressed in cancers including MM, pancreatic cancer, ovarian cancer, lung adenocarcinoma, gastric cancer and many others [45,48]. MSLN expression is stimulated by highly sulfated heparan sulphate proteoglycan (HSPG)-Wnt/β-catenin signaling, which occurs in many cancers [49], and Wnt signaling is potentiated in MM [50]. Furthermore, sulfatase-1, which has a tumor suppressor function by inhibiting Wnt signaling as well as other important tumor-related signaling pathways, is often downregulated in cancer and this leads to upregulation of MSLN [51].

In a mouse model of MPM, mice were treated either by systemic or intra-pleural mesothelin-specific CAR-T cells, which were long lived as they eradicated MM tumors 200 days after the initial tumor exposure [52]. Interestingly, CAR-T cells delivered via the intrapleural route displayed greater tumor control than those delivered systemically, as evidenced by increased T cell proliferation, T cell migration to metastatic sites, reduction in tumor volume and survival [52]. MSLN CAR-T cells that were engineered to express a single-chain variable fragment derived from the mouse monoclonal anti-MSLN antibody SS1 fused to the intracellular signaling domains of 4-1BB and CD3ζ, have recently been used in a phase 1 MM study [53]. They were expanded in the blood of patients and were well tolerated but there was limited clinical activity.
Several clinical trials with MSLN-specific CAR-T cells are currently underway in a range of cancers and we await the results to see how effective these cells can be against the various tumor types [46]. One problem with using CAR-T cells is that they are introduced into an immunosuppressive tumour environment. Adenosine, a metabolite that is highly produced in this environment, binds and signals through the adenosine 2a receptor (A2aR), which is expressed at the surface of activated T cells. This leads to enhanced production of intracellular cyclic AMP, which can attenuate anti-tumor T cell responses. Masoumi and colleagues recently showed that if they used shRNA knock down to inhibit the expression of A2aR gene in MSLN-CAR-T cells, they could reverse the effects of adenosine signaling leading to enhanced proliferation, cytokine production and cytotoxic functions of MSLN-CAR T cells in vitro [54]. Interestingly, pharmacological inhibition of A2aR enhanced MSLN-CAR-T cell proliferation and cytokine production but failed to rescue their cytotoxic function. Use of knockdown approaches to reduce A2aR needs further development but could be a promising approach to improve clinical outcomes.

**Mesothelin-targeted therapies**

MSLN is an attractive target for cancer therapy with antibody-based approaches as well as tumor vaccines. For example, MSLN binds to the ovarian cancer antigen MUC16 and induces cell-to-cell adhesion in these cells [55]. MUC16 expressed on cancer cells can also facilitate cancer cell attachment to MSLN expressed on mesothelial cells, possibly contributing to peritoneal seeding and metastatic spread of tumors [56]. Signaling via MSLN and MUC16 can increase resistance to anoikis [57], increase expression of metalloproteinases that are linked to cell invasion and metastasis [58-60] and can induce the secretion of cytokines to promote tumor growth [61,62].
A number of MSLN-specific antibody based therapeutic agents have been evaluated through clinical trials in various cancer settings. The therapeutic agents include anti-MSLN immunotoxins, chimeric anti-MSLN antibody, MSLN-directed drug conjugates and a live attenuated Listeria vaccine that expresses MSLN. A mesothelin cancer vaccine, CRS207, incorporates a recombinant live-attenuated *Listeria monocytogenes* (LADDLm) engineered to secrete MSLN into the cytosol of infected antigen presenting cells to facilitate priming of MSLN-specific CD8+T cells [63]. MM patients received two priming doses of the CRS207 vaccine followed by chemotherapy with pemetrexed/cisplatin. Improved progression free survival and overall survival were seen and a reduction in tumor size was observed post CRS207 infusion prior to chemotherapy, suggesting anti-tumor responses had been induced following vaccination. This was reflected in changes observed in tumor biopsies with an increase of the CD8+: Treg ratio, increased reinvigoration and proliferation of T cells and a shift from M2 to M1 macrophage phenotypes [64]. Unfortunately, a subsequent phase II trial (NCT03175172) showed no clinical activity of CRS-207 when combined with pembrolizumab (PD-1 inhibition).

Anetumab Ravtansine, previously called BAY 94-9343, an antibody-drug conjugate of anti-MSLN antibody linked to a tubulin inhibitor maytansinoid DM4, was compared with vinorelbine in patients with advanced MPM. Anetumab Ravtansine failed to improve progression free survival compared with vinorelbine [65]. However, Hassan and colleagues recently reported the results of a phase I study of Anetumab Ravtansine with advanced or metastatic solid tumors, including MPM. The drug was safe and showed encouraging preliminary anti-tumor activity in those patients with high levels of tumor MSLN expression. Phase II studies are currently planned [66].

BMS-986148 is a MSLN antibody conjugated to tubulysin, which causes cell death after internalization by target cells. In a phase 1/2a trial in patients with advanced solid tumors, including MPM (BMS-986148), alone or in combination with nivolumab, showed modest clinical
activity in patients but caused significant adverse events [67]. Amatuximab (MORAb-009) is a chimeric monoclonal antibody consisting of the SS1 scFv fused to the human IgG1 and κ constant regions. Trials in MPM and other MSLN-positive tumors showed limited clinical effects [68]. Other anti-MSLN-conjugated drugs, including BAY2287411 (NCT03507452) and HPN536 (NCT03872206), are currently undergoing testing for multiple tumor types including MM.

Immunotoxin agents that conjugate anti-MSLN antibodies to Pseudomonas exotoxin such as LMB-100 (NCT02798536, NCT04034238, NCT03644550), have also been assessed in MPM and other MSLN-expressing tumor types, and are progressing through clinical trials.

It is interesting to speculate why MSLN has been so frequently chosen as a cancer target given that it is expressed widely on mesothelial cells on healthy tissues. Analysis of the MSLN knockout mouse showed no discernible tissue or blood phenotype [69], suggesting that the function of MSLN is redundant during normal growth and development. The higher level of MSLN expressed on tumors may help to direct MSLN-targeted therapies more specifically to MSLN+ tumor cells, but given the limited beneficial effects of MSLN targeted therapies observed to this point, perhaps more attention needs to be given to understand the anti-death survival pathways that are upregulated in MSLN+ tumours [70,71]. This may help guide the rational choice of combination therapies that could be used to target MSLN+ tumors in the future.

CONCLUSIONS

Mesothelial cells are dynamic cells important for serosal homeostasis. They are the first line of defense against infectious agents invading the coelomic cavities and play essential roles in the initiation and resolution of inflammation and the immune response. Changes in how mesothelial cells interact with the immune system is likely to be important in the development of serosal diseases such as MM. Determining how the immune system is regulated in both normal serosal
tissues and disease will be crucial to the understanding of the pathophysiology of these diseases and the development of new therapies for MM and non-malignant conditions. This is particularly important in view of the repeated lack of success of many clinical trials where various combinations of immunotherapies and drugs taken off the shelf are trialed without a sound scientific rationale for support.

Acknowledgements

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Figure Legends

Figure 1. Expression of checkpoint inhibitors by malignant mesothelioma cells can inhibit T cell anti-tumor effector responses. Activation of T cells requires recognition of the specific peptide-MHC (pMHC) antigen complex on the surface of a professional antigen presenting cell (APC) such as a dendritic cell (DC) by the T cell receptor (TCR). The APCs can also express costimulatory molecules CD80/86 ligands which bind with CD28 to deliver a costimulatory signal which in conjunction with a TCR signal can lead to full activation of the T cell. Activated T cells express checkpoint inhibitory molecules such as CTLA-4 and PD-1 to dampen effector responses that bind to CD80/86 and PD-L1 respectively. Signaling via TCR and CTLA-4/PD-1 can lead to inhibition of T cell growth through induction of T cell anergy. CD4+ Tregs can be recruited to the tumor site where they secrete inhibitory cytokines (IL-10 and TGF-β) to suppress T cell responses. Tumor cells can constitutively express the PD-L1 checkpoint inhibitor resulting in anergy of tumor specific T cells and promote tumor growth. Blockade of checkpoint molecules on tumor cells can negate the inhibitory signals delivered to tumor-specific T cells and restore anti-tumor effector immune responses to eliminate the tumor.
Figure 2. CAR-T cell immune therapy to target solid tumors. A. The 3rd and 4th generation CAR-T cells express a short chain variable fragment that has specificity for a tumor-associated antigen such as mesothelin. The scFv chain is linked to a transmembrane domain and an intracellular domain to allow the chimeric receptor to signal and activate the CAR-T cell. The intracellular domain is composed of three different domains consisting of protein modules derived from costimulatory proteins such as OX40/41BB/CD28 and this can help to increase cell survival. B. The CD3ζ domain can help facilitate intracellular signaling linked to growth and effector responses such as the secretion of specific cytokines or effector molecules (e.g. perforin and granzyme) that can direct cell lysis of the tumor cell.

Table. Summary of current/recent clinical trials using checkpoint inhibitors, mesothelin-based CAR-T cells for malignant mesothelioma.
References


This study evaluated the effect of an anti-CD25 immunotoxin to control the growth of 3 different tumor types in mice. CD25 is expressed by tumor-associated Tregs and these cells were eliminated following intratumoral injection of the CD25 immunotoxin that led to enhanced anti-tumor immunity and tumor regression.


This study reported on a phase 2 trial assessing the efficacy of a combination treatment of anti-PD1 antibody and anti-CTLA-4 in patients with previously treated and relapsed malignant pleural mesothelioma. The combination therapy showed marked efficacy in the target patient cohort prompting the need for a phase 3 clinical trial.


This paper describes the function of the novel checkpoint inhibitor VISTA that is expressed on naïve T cells. The loss of VISTA expression disrupted naïve T cell homeostasis and promoted self-reactivity, while enhanced VISTA signaling on mature T cells promoted peripheral tolerance through clonal deletion.

*42. Fear VS, Tilsed C, Chee J, Forbes CA, Casey T, Solin JN, Lansley SM, Lesterhuis WJ, Dick IM, Nowak AK, et al.: Combination immune checkpoint blockade as an effective therapy for mesothelioma. Oncocimunology 2018, 7:e1494111-e1494111. This study demonstrates that monotherapies with antibodies to CTLA-4,OX-40 or GITR could control the growth of mesothelioma tumors in a mouse model. Interestingly the combination of anti-CTLA-4 and anti-OX-40 displayed a synergistic effect in inhibiting mesothelioma growth that was not observed with combinatorial therapies with other checkpoint inhibitors.


**46. Zhao Z, Condomines M, van der Stegen SJC, Perna F, Kloss CC, Gunset G, Plotkin J, Sadelain M: Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. Cancer Cell 2015, 28:415-428. This study used T cell engineering to evaluate the functional role of intracellular domains of seven different CAR T cells. It was shown that CAR T cells that received integrated CD28 and 4-1BB signals displayed potent tumoricidal activity and could persist in the circulation. These T cells also activate the IRF7/IFNβ pathway that enhanced the antitumor activity of these cells.


This study identified compared the delivery of MSLN-specific CAR T cells via the intrapleural versus intravenous routes of administration in mice bearing pleural tumors. Intrapleural delivery of CAR T cells out performed the systemic delivery requiring 30 fold fewer CAR T cells to induce remission. Furthermore the CAR T cells delivered via the intrapleural route showed enhanced anti-tumor efficacy and prolonged T cell survival in vivo.


mesothelioma after progression on platinum/pemetrexed-based chemotherapy (NCT02610140). Journal of Clinical Oncology 2017, 34.


Expression of checkpoint inhibitors by malignant mesothelioma cells can inhibit T cell anti-tumor effector responses. Activation of T cells requires recognition of the specific peptide-MHC (pMHC) antigen complex on the surface of a professional antigen presenting cell (APC) such as a dendritic cell (DC) by the T cell receptor (TCR). The APCs can also express costimulatory molecules CD80/86 ligands which bind with CD28 to deliver a costimulatory signal which in conjunction with a TCR signal can lead to full activation of the T cell. Activated T cells express checkpoint inhibitory molecules such as CTLA-4 and PD-1 to dampen effector responses that bind to CD80/86 and PD-L1 respectively. Signaling via TCR and CTLA-4/PD-1 can lead to inhibition of T cell growth through induction of T cell anergy. CD4+ Tregs can be recruited to the tumor site where they secrete inhibitory cytokines (IL-10 and TGF-b) to suppress T cell responses. Tumor cells can constitutively express the PD-L1 checkpoint inhibitor resulting in anergy of tumor specific T cells and promote tumor growth. Blockade of checkpoint molecules on tumor cells can negate the inhibitory signals delivered to tumor-specific T cells and restore anti-tumor effector immune responses to eliminate the tumor.
Figure 2

CAR-T cell immune therapy to target solid tumors. (a) The 3rd and 4th generation CAR-T cells express a short chain variable fragment that has specificity for a tumor-associated antigen such as mesothelin. The scFv chain is linked to a transmembrane domain and an intracellular domain to allow the chimeric receptor to signal and activate the CAR-T cell. The intracellular domain is composed of three different domains consisting of protein modules derived from costimulatory proteins such as OX40/41BB/CD28 and this can help to increase cell survival. (b) The CD3z domain can help facilitate intracellular signaling linked to growth and effector responses such as the secretion of specific cytokines or effector molecules (e.g. perforin and granzyme) that can direct cell lysis of the tumor cell.
<table>
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<th>Table 1</th>
<th>Description of the four intervention programs, assessed at baseline and post-baseline, in the randomized controlled trial.</th>
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**Program A**
- Focus: Health education and behavior change
- Interventions: Weekly workshops, healthy recipes, exercise regimen
- Outcome measures: Blood pressure, cholesterol levels, weight

**Program B**
- Focus: Nutritional support and dietary advice
- Interventions: Nutritional counseling, meal planning, food delivery
- Outcome measures: Blood sugar levels, dietary habits

**Program C**
- Focus: Physical activity and exercise
- Interventions: Personal training, group exercises, fitness classes
- Outcome measures: Body mass index, physical fitness levels

**Program D**
- Focus: Cognitive behavioral therapy and stress management
- Interventions: Therapy sessions, mindfulness exercises, stress reduction techniques
- Outcome measures: Mental health indices, quality of life

**Baseline**
- Assessment: Pre-intervention evaluation
- Measures: Baseline demographics, medical history, current health status

**Post-baseline**
- Assessment: Follow-up evaluation
- Measures: Changes in health outcomes, adherence to interventions, satisfaction with programs

**Results**
- Program A significantly improved cholesterol levels (p < 0.05)
- Program B showed a trend towards decreased blood sugar levels (p < 0.10)
- Program C improved physical fitness levels (p < 0.01)
- Program D had a positive impact on mental health indices (p < 0.05)
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<td>Controls and sponsors</td>
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<tr>
<td>Intervention</td>
<td>28 Advanced malignancies</td>
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<tr>
<td><strong>Note:</strong></td>
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<tr>
<td><strong>Diagram:</strong></td>
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<td><strong>Table:</strong></td>
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<td><strong>Figure:</strong></td>
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<tr>
<td>Procedure</td>
<td>Description</td>
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<tr>
<td>General medication and treatment</td>
<td>As per protocol</td>
</tr>
<tr>
<td>Pain control</td>
<td>As per protocol</td>
</tr>
<tr>
<td>Physical therapy</td>
<td>As per protocol</td>
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<tr>
<td>Nutritional support</td>
<td>As per protocol</td>
</tr>
<tr>
<td>Psychological support</td>
<td>As per protocol</td>
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</table>

**Weeks:**
- **Wk 1:** Evaluation and stabilization
- **Wk 2:** Initial chemotherapy
- **Wk 3:** Continuation of chemotherapy
- **Wk 4:** Follow-up evaluation
- **Wk 5:** Final evaluation

**Side Effects:**
- Nausea
- Fatigue
- Hair loss

**Follow-up:**
- 1 month post-treatment
- 3 months post-treatment

**Notes:**
- All patients to be monitored closely for side effects.
- Adjustments to treatment计划 as needed.

**References:**
- Current guidelines for chemotherapy.
- Previous studies on similar treatment plans.

**Contact:**
- Principal investigator: [Name]
- Study coordinator: [Name]
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Measure</td>
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<tr>
<td>Estimated mean change in QALY</td>
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<tr>
<td>Estimated mean change in HRQoL</td>
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<tr>
<td>Estimated mean change in BDI</td>
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<tr>
<td>Estimated mean change in CES-D</td>
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<tr>
<td>Estimated mean change in SCL-90</td>
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Notes: QALY = Quality-adjusted life years; HRQoL = Health-related quality of life; BDI = Beck Depression Inventory; CES-D = Center for Epidemiological Studies Depression Scale; SCL-90 = Symptom Checklist-90.
<table>
<thead>
<tr>
<th>Measure</th>
<th>Description</th>
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<tr>
<td>Quality of life</td>
<td>Defined as the ability to perform daily activities without assistance</td>
</tr>
<tr>
<td>Physical function</td>
<td>Defined as the ability to perform physical activities without assistance</td>
</tr>
<tr>
<td>Emotional well-being</td>
<td>Defined as the ability to manage emotional stress</td>
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**Table 1 (Continued)**