Making cold malignant pleural effusions hot: driving novel immunotherapies

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**ABSTRACT**

Malignant pleural effusions, arising from either primary mesotheliomas or secondary malignancies, heralds advanced disease and poor prognosis. Current treatments, including therapeutic thoracocentesis and tube thoracostomy, are largely palliative. The immunosuppressive environment within the pleural cavity includes myeloid derived suppressor cells, T-regulatory cells, and dysfunctional T cells. The advent of effective immunotherapy with checkpoint inhibitors and adoptive cell therapies for lung cancer and other malignancies suggests a renewed examination of local and systemic therapies for this malady. Prior strategies reporting remarkable success, including instillation of the cytokine interleukin-2, perhaps coupled with checkpoint inhibitors, should be further evaluated in the modern era.

**Introduction**

Advanced thoracic malignancies are commonly associated with malignant pleural effusions (MPE), which are defined as significant accumulations of fluid exudate, containing tumor tissue or cells, within the pleural cavity. The incidence of MPE is over 200,000 cases/year in the US, of which non-small cell lung cancer (NSCLC) (36.0%), breast carcinoma (26%), and lymphoma (13.0%) are the most common etiologies. MPEs are also found in over 90% of patients with malignant pleural mesothelioma (MM), an equally devastating disease on the rise in developing countries. MPEs may present as the first sign of malignancy in 15–40% of asymptomatic patients but are commonly indicative of advanced cancer and poor prognosis. The median survival of patients with MPE from NSCLC is less than five months, and not only is MPE an independent predictor of lower overall survival due to advanced disease, but the effects of diminished breathing capacity can limit a patient’s ability to tolerate systemic treatment. Uncontrolled MPE can result in hypoxia and ultimately be a primary cause of death. Despite improvements in cancer therapies, patients with MPE are largely only palliated to provide symptomatic relief. There is currently no effective definitive treatment for metastatic pleural disease, but a multimodal therapeutic approach is commonly offered to patients with early MM.

Proposed pathophysiological mechanisms include fluid development following primary parietal pleura invasion, limiting the normal function of the rich lymphatic drainage present within the parietal pleura. Other mechanisms include visceral pleural invasion enabling fluid egress via the pulmonary vasculature followed by secondary parietal pleural dissemination, as found in patients with advanced lung malignancy. In other diseases, such as lymphoma or breast cancer, lymphatic dissemination or direct tumor invasion of the chest wall, diaphragm, or lung are possible. Tumor viability and immune resistance is dependent on the cancer’s adherence to the mesothelium, allowing immune evasion, while enabling nutritional access and growth stimuli of the neo-tumor environment. In early MM, tumor nodules may be evident in the parietal and visceral pleura at the same time. Pleural fluid formation results from disrupted lymphatic drainage, increased capillary and pleura permeability, and increased fluid production.

Research on the pathophysiology of MPE has also shed light on the immune landscape of the malignant pleural space and has helped to convey critical clinical and prognostic information. MPE harbors immunogenic exosomes, immune cells, and immune factors, but are functionally ‘cold’ with progression of tumor. This review defines the role of the innate and adaptive immune system in primary and secondary MPE. We highlight treatments that could transform MPE to be functionally ‘hot’ and drive effective immunotherapy.

**Current treatments for malignant pleural effusion**

The current standards for MPE treatment are largely palliative: drainage via thoracocentesis, tube thoracostomy with or without pleurodesis, pleureperitoneal shunt, tunneled pleural catheter, or other less common procedures (Table 1).
Management choice is guided by the patient’s prognosis, preference, functional status, rate of pleural effusion accumulation and resolution, failed therapeutic options, and the surgical team’s experience. To date, there are no established criteria for selecting from the available therapeutic options. The decision to undergo pleurodesis is often based upon an anticipated survival of longer than three to four months. Despite the potential therapeutic benefits, pleurodesis failure remains a major drawback. A meta-analysis of 62 randomized trials involving over 3,000 patients compared and ranked agents based on pleurodesis efficacy. Talc poudrage was identified as the superior method when compared to bleomycin, meipacine, or iodine installation. There was no evidence of survival benefit associated with any of the individual types of pleurodesis. Failure of lung expansion remains a contraindication for chemical pleurodesis and the introduction of the intrapleural catheter has served as an initial suitable remedy for lung entrapment.

Intrapleural catheters (IPC) have more recently become the primary means for managing MPEs because of their technical simplicity and cost effectiveness. A retrospective study assessing the financial benefits of outpatient management for patients with MPE versus inpatient management was conducted. Outpatient management was in fact, more cost effective. A retrospective study assessing the financial benefits of outpatient management for patients with MPE versus inpatient management was conducted. Outpatient management was in fact, more cost effective.

### Table 1. Existing MPE therapeutic options.

| Therapy                        | Advantages                                                                 | Disadvantages                                                                 | Mortality (30 days) | Morbidity | Success Rate |
|--------------------------------|---------------------------------------------------------------------------|-------------------------------------------------------------------------------|---------------------|-----------|--------------|
| Thoracentesis                  | Outpatient procedure, limited anesthesia is required; technical simplicity; drain large volume of fluid (approximately 1.5 liters without risk of reexpansion pulmonary edema) | Recurrent pleural effusion; 96% failure rate in 30 days                       | 37%                 | <1%       | 4%           | 14,15          |
| General Chemical Pleurodesis   | Minimal insertion of tubes and decreased risk of frequent thoracostomy     | Associated pain and fever; prolonged hospitalization (median time: 4 days); pleurodesis failure | 32%                 | 6–33%     | 68–85%       | 1,16-19         |
| Talc Pleurodesis               | Minimal drainage following instillation; superior agent in comparison to bleomycin, doxycycline, and tetracycline; comparable to chemical pleurodesis, regarding: quality of life and symptomatic relief | Risk of ARDS ranging between 1–9%; pleurodesis failure; pain is a common post-operative complaint | 2%                  | 9–38%     | 98%          | 20–22          |
| Indwelling Pleural Catheter    | Indicated for lung entrapment syndrome and failed pleurodesis; technical simplicity; outpatient management; drainage guided by symptoms (patients have more autonomy) | Risk of infection is higher than chemical pleurodesis; increased risk for catheter-tract metastases in patients with mesothelioma | x                   | 10%       | 48–58%       | 23–26          |
| Indwelling Pleural Catheter and Talc Pleurodesis | Outpatient management: Pain, empyema, hydropneumothorax are known adverse effects; remains under study. | x | 9% | 43–92% | 25,27,28 |
| Pleuro-peritoneal Shunt         | Use in refractory MPE or trapped lung; post-operative morbidity is low       | Infectious risk due to infection of the peritoneal cavity with infected pleural fluid; shunt occlusion (12–25%), tumor seeding into the peritoneal cavity. | 21%                 | 14%       | 95%          | 29–32          |
| Thoracoscopy and Pleurodesis    | Video-assisted thoracoscopic surgery allows surgeon to assess pleura, diaphragm and pericardium for tumor implants; perform concurrent procedures (mediastinal lymphadenectomy, pleurectomy, etc.); visualize pleural effusion; shorter interval for chest drainage in comparison to chest tube thoracostomy | Patient has to tolerate single lung ventilation; post-operative complications (3%-25%); Prolonged hospitalization (7–10 days) | 2.8%                | 2.8%      | 90%          | 26,30,33       |
| Pleurectomy with Chemotherapy   | Indicated in refractory MPE and mesothelioma                                | Invasive; 12% mortality risk; prolonged hospitalization; offered based on patient selection per hospital and surgical experience; not standard of care | 4–12%               | 10–19%    | x            | 14,34,35       |
| Chemotherapy                   | Intrapleural chemotherapy (IC) can treat the underlying malignancy and pleural effusion and has been used in mesothelioma; chemosensitive malignancies with associated MPE, may respond to chemotherapy | IC maybe inferior to existing chemical pleurodesis; patient may not tolerate systemic chemotherapy given functional and physiologic status | 50% at 1 year       | 7–40%     | 30–70%       | 36–38          |
| Radiotherapy, alone            | Reduce risk of needle tract metastasis; radiation targeted at underlying malignancy may treat associated MPE; used in multi-modal treatment approach for mesothelioma | Radiation pneumonitis; limited studies on efficacy for MPE and secondary malignant pleural effusions | 17% at 1 year       | x         | x            | 39,40          |
| Immunotherapy                  | Most current studies involve mesothelioma; immune checkpoint inhibition appears very promising strategy in MM; IL-2 installation could be reconsidered for local therapy | Toxicity; limited studies regarding efficacy | x                   | 7–90%     | 10–20%       | 41,42          |
Malignant pleural effusions are an inflammatory condition within the chest containing immunity (lymphocytes) can be utilized as prognostic indicators of Immunotherapy for patients with malignant pleural effusions site. Several Alongside the The survival benefit catheters are commonly used, but other types of IPCs have been studied and compared. PleurX™ catheters are commonly used, but other types of IPCs (Aspira) have shown comparable efficacy, safety, and complication rates. Novel catheter designs, allowing for frequent installation of chemotherapeutic and immunologic agents, continuous infusion of therapeutic agents, and withdrawal of excessive fluid should be considered. These strategies would also allow regular sampling of pleural fluid including tumor and host cells, enabling timely assessment of immunotherapies and their application.

There is also a role for the surgical treatment of patients with primary MPE. Extrapleural pneumonectomy (EPP) and pleurectomy/decortication (P/D) can achieve macroscopic complete resection in early stage MM. Improved quality of life and safety has been demonstrated in selected patients undergoing P/D for MM, but the procedure has a 6–12% risk of operative mortality. The survival benefit of EPP remains widely debated, with some trials suggesting added morbidity without survival benefit.

Despite advances in approaches to palliative care for MPE, local treatment continues to lag. Intrapleural chemotherapy (i.e. doxorubicin, methotrexate, 5-fluorouracil, etc.) has been slowly adopted as an adjunctive treatment for MPE. The impact of intrapleural chemotherapy with cisplatin and cytarabine has been studied in the presence of MPE secondary to NSCLC, and treatment responses have been associated with decidedly mixed outcomes. The Lung Cancer Study Group (LCSG 861) showed that IPC with cisplatin and cytarabine has a relatively low response rate (49%). Park’s group demonstrated a more favorable response rate (97.3%) and durability of benefit (12 month median duration of response). Intrapleural doce-taxel was studied in a phase I clinical trial and was proven to be safe with low toxicity and with reasonable radiographic control. Currently, intrapleural chemotherapy is not used as a monotherapy but is integrated into other treatment strategies for secondary MPE. Chemotherapy response is estimated at 15% following single agent use in MM. Application of combined chemotherapy agents (cisplatin and pemetrexed) improves both quality of life and survival (9.3 – 13.3 month median survival).

While radiotherapy has minimal efficacy as a single agent in MM, there may be a survival benefit when used as an adjunct following surgery, with median survivals of 33.8 months for stage I and II but limited to 10 months for stage III and IV tumors.

Given the existing limitations in treating MPE, new therapeutic options, especially application of modern immunotherapy, may enhance development of palliative and potentially curative therapies (Figure 1).

**Innate immunity within the MPE**

Innate immune cells are recruited to the sites of tumor cells undergoing unscheduled cell death associated with release of damage associated molecular pattern molecules (DAMPs), Initial recruitment and activation of neutrophils, macrophages, mast cells, dendritic cells (DCs), and natural killer (NK) cells, follows elaboration of chemokines and cytokines in response to signals of tissue injury. Cancer cells and their products are distinguished from healthy tissues through pattern recognition receptors (PRRs) on the surface and within.

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**Suppressive Immune Pathways**

**Innate Pathways**
- TLR-2 Immunosuppression
- Mast Cell TSAB1 & IL-1β Production
- Tumor Associated M2 Macrophages
- ↓ NK Cell Cytotoxicity
- Proangiogenic NK Cells

**Adaptive Pathways**
- ↓ CD8+/NKT Infiltration
- Th2 Differentiation
- sCD40 Limiting B cell & APC Function
- cNK Cell Exhaustion, Receptor Homogeneity

**Negative Prognostic Markers**
- ↑ NLR Ratio
- ↑ SII
- ↑ LENT
- ↑ PROMISE
- ↑ Tumor Mutational Load

**Clinical Approaches**

Previously Tested
- IL-2
- DC Vaccination
- OK-33/ Staph aureus SSaag T Lymphocyte Infusion
- IFN-α2b

Novel Testing
- Checkpoint Blockade (CTLA-4, PD-1, PD-L1)
- Adoptive Cell Therapy
  - TIL
  - CAR-T (MSLN, MUC1, EGFR)

Next Generation
- Intrapleural CpG or STING agonists
- c-Kit+ Mast Cell Inhibition
- M2 Macrophage to M1 Repolarization
- IL-2/IL-15 Stimulated NK cell Adoptive Transfer
- Oncolytic Herpes or Vaccinia Virus
- LAG3/IM3STIGT Checkpoint Blockade
- Combination CAR-T and Checkpoint Blockade
- Combination IL-2 Intrapleural and Checkpoint Blockade

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**Figure 1. Immunotherapy for patients with malignant pleural effusions.** Malignant pleural effusions are an inflammatory condition within the chest containing immunologically active but most often exhausted cells associated with both bulk tumor and tumor cells in suspension. Thus, they are a functionally ‘cold’ site. Several suppressive innate and adaptive pathways have been identified. MPE pathophysiology is closely correlated with the upregulation of these inflammatory pathways. Systemic measures associated with acute inflammation (neutrophils) and chronic inflammation – immunity (lymphocytes) can be utilized as prognostic indicators of disease outcome and for disease stratification. Previously tested clinical approaches, including IL-2 therapy and DC vaccination hold much promise, and should be further investigated in the context of trials evaluating checkpoint inhibition and adoptive cell therapy. Next generation immunotherapies may enable personalized treatments, leverage improved cell-based therapies, and modulate the local suppressive tumor microenvironment. Local therapies could be more effectively deployed.
the cytoplasm of innate immune cells. PRRs recognize pathogen-associated molecular pattern molecules (PAMPs) and DAMPs. These signals initiate the inflammatory and subsequent immune response to tumor. Frequently members of the Toll-like receptor (TLR) family, which recognizes conserved microbial and endogenous motifs, constitute the primary means for response to so-called ‘danger’ or ‘stranger’ signals. Dysregulated inflammatory innate immune networks support MPE development and tumor cell immune evasion (Figure 2).

**Pleural mesothelial cells** (PMCs) reside within the parietal and visceral pleura and are essential to pleural homeostasis. Dysregulation of PMC responses leads to activation and formation of MPE. Pleural cavity injury during infection, trauma, or malignancy is initially recognized by PMCs and results in a response-specific inflammatory cascade. In addition to their negatively charged surface glycoconjugates that limit errant cells, PMCs express TLRs 1–9 and mediate inflammation via release of platelet derived growth factor (PDGF), interleukin-8 (IL-8), monocyte chemotactic peptide (MCP-1), and nitric oxide. Sialidases on malignant cells remove the defensive sialomucin complex layer on PMCs. Release of vascular endothelial growth factor (VEGF) increases permeability of PMCs, allowing for leakage of high molecular weight proteins, promoting migration of cells into the pleural space. A retrospective analysis of 21 patients with NSCLC associated MPE treated with bevacizumab, an anti-VEGF antibody, and chemotherapy demonstrated a remarkable 71.4% response rate of MPE to antibody treatment.

In an *in vivo* model of MPE, enhanced TLR-2 expression on recruited CD14+ inflammatory macrophages, Ly6G+ neutrophils, CD4+CD8+ T cells, and CD19+ B cells accelerated the development of MPE and death in mice bearing MPE by suppressing Th9 and Th17 cell differentiation, while promoting Th2 differentiation. A clinical study evaluating TLR-2 expression following treatment of MPE with talc pleurodesis demonstrated decreased granulocyte TLR-2 expression directly and 24 hours following talc pleurodesis compared to pretreatment levels. Accordingly, soluble levels of TLR-2 were significantly increased following pleurodesis. Interestingly, patients retrospectively sorted into a lower prognostic scoring group (higher thorascore, larger pretreatment pleural fluid, and recurrence of MPE) had lowered levels of soluble TLR-2 following treatment compared to the prognostically favorable group. TLR-2 is a critical PRR at the interface of microbial and sterile inflammation in MPE. Questions remain concerning the mechanisms of its upregulation in the pleural space.

Inflammatory Mediator Release

| PMCs | TLRs 1–9 | Sialomucin Complex Destruction |
|------|---------|-------------------------------|
| PDGF | IL-8    | Nitric Oxide                  |
| MCP-1|         |                               |

**Figure 2.** *Innate immune signaling pathways in malignant pleural effusions.* The altered tumor microenvironment enables MPE formation by enhancing angiogenesis, promoting vascular permeability, driving tumor growth, unscheduled cell death, and releasing damage associated molecular pattern molecules (DAMPs). Natural Killer (NK) cells exhibit a proangiogenic phenotype, with reduced cytotoxicity, increasing endothelial cell recruitment with production of IL-8 and vascular endothelial growth factor (VEGF). Pleural Mesothelial Cells (PMCS) respond to pleural cavity DAMPs and other ligands via toll-like receptor (TLR) 1–9 signaling, promoting recruitment of inflammatory cells with release of platelet derived growth factor (PDGF), IL-8, nitric oxide, and monocyte chemotactic protein 1 (MCP-1). Tumor cell production of VEGF and sialidase promote vascular permeability with destruction of the protective extracellular PMC sialomucin complex. Leukotriene B4 (LB4) and epithelial neutrophil-activating peptide-78 (ENA-78) increase neutrophil recruitment to the pleural space and neutropenia is sustained with CD47 mediated inhibition of apoptosis. Accompanying neutrophil and macrophage (M0) TLR2 upregulation support MPE formation with a skewed Th2 response. Tumor associated macrophages (TAMs), favoring M2 polarization protect tumor cells from apoptosis and promote angiogenesis, immune evasion, and tumor growth with a milieu of proangiogenic chemokines, cytokines, DAMPs, and growth factors. TAMs also produce chemokine ligand 18 (CCL18), which promotes T regulatory cell (T-reg) differentiation and limits dendritic cell (DC) maturation. DCs facilitate immunosuppression via B7-H3 T cell coinhibition and upregulation of RFD1, CD86, HLA-DR, DC40, and CD1a expression. Tumor cells avoid ingestion by expressing CD47, a ‘don’t eat me’ signal and produce CC12 and osteopontin, inducing mast cell recruitment and c-KIT activation respectively. Increased mast cells are found to be associated with MPE formation with tumor growth promoting IL-1β and tryptase alpha/beta-1 (TSAB1) mediated enhanced vascular permeability.
MPE. Unlike TLR-2, TLR-4 expression appears to be immunoprotective in the formation of MPE. TLR-4+/− mice with MPE had augmented Th1 differentiation, via enhanced STAT1 signaling, and suppressed STAT3 dependent Th17 cells, accelerating the death of mice with MPE.73 The role of other PRRs, such as NOD-like receptors, RIG-I like receptors, AIM-2 like receptors, and C type lectin receptors (CLRs) remains to be investigated in MPE. CLRs have been identified as a molecular switch of the inflammatory response to tuberculosis associated pleural fluid, suggesting a potential role in the setting of MPE.74

**Mast Cells** are typically activated during allergic responses, and are among the first cells to infiltrate the tumor microenvironment and promote tumor progression via inflammatory and tumor angiogenesis signaling.75 Thought relatively sparse, mast cells are surprisingly elevated in MPE compared to benign effusions and are critical to MPE development.76 Pleural adenocarcinomas mobilize mast cells into the pleural space during MPE development through elaboration of CC family chemokine 12 (CCL12).76 In addition to its vasoactive components, tumor originating osteopontin, encoded by the secreted phosphoprotein 1 (SPP1) gene, promotes c-KIT+ mast cell activation and degranulation, leading to MPE formation with release of tryptase alpha/beta-1 (TSAB1) and IL-1β, causing vascular permeability and NF-κB mediated tumor growth respectively.76 Treatment with the clinically available imatinib mesylate, a mast cell c-KIT inhibitor, hampered mast cell pleural accumulation, vascular leakiness, and limited effusion development in murine models of MPE.76 Mast cells and their identified intermediary signaling molecules, CCL2, SPP1, TPSAB1, and IL-1β should be further investigated for more targeted approaches to MPE treatments.

**Macrophages** are phagocytic, antigen presenting cells (APCs) that serve as a bridge between innate and adaptive immunity.77 The polarized macrophage model describes macrophage activation in response to differing environmental and inflammatory triggers. M1 polarization promotes macrophages capable of producing proinflammatory cytokines (IFN-γ, TNF-α, IL-1α, IL-1β, IL-6) and cytotoxic reactive oxygen and nitrogen species (ROS, NRS) while M2 polarization directs an immunoregulatory and wound healing response that promotes Th2 responses critical for the development of cancer.78–80 Macrophages constitute over half of all the cells found in the pleural space. In the setting of MPE, they modulate T cell proliferation and differentiation with release of IL-1β, TNF-α, and IL-8.81-82 Tumor associated macrophages (TAMs) have decreased cytotoxicity and promote tumor cell growth and immune evasion.83 In MPE, TAMs protect cancer cells from apoptosis,84 ingest those that are apoptotic, and promote angiogenesis with release of proangiogenic chemokines (CXCL1, CXCL2, CXCL8), cytokines (TNF-α, IL-1α, IL-1 β, IL-6), DAMPs (high mobility group box 1 (HMGB1)), and growth factors (TGF-α, VEGF, PDGF, angiopoietins).85 Uregulation of MM CD47, a ‘don’t eat me signal’ that inhibits macrophage phagocytosis, promotes tumor cell immune evasion.86,87 In the respiratory tract, surfactant protein-A (SPA) is another important DAMP that is upregulated in human NSCLC MPE compared to non-malignant pleural effusion. Elevations in SPA positively correlate with increases in M2 polarized macrophages with TLR-2 and TLR-4 expression.88 Decreased CD163+ TAMs independently predict better NSCLC MPE progression free survival (PFS). Increased levels of M2 polarized TAMs correlate with poor prognosis.89 Interestingly, treatment with *Pseudomonas aeruginosa*-mannose sensitive hemagglutinin (PA-MSHA) for lung cancer MPE helped to re-educate M2 macrophages into an M1 phenotype *in vitro*.90 This pathway was TLR-4 mediated as treatment with a TLR-4 blocking antibody reversed M1 polarization. Inhibition of TAMs or repolarization of M2 macrophages are compelling therapeutic strategies for MPE.

**Neutrophils** are the body’s most abundant immune cell and are the major mediators of inflammation, the seventh hallmark of cancer.90,91 Leukotriene B4 and epithelial neutrophil-activating peptide-78, potent inflammatory chemoattractants, are present in exudative pleural effusions, and actively contribute to neutrophil recruitment to the pleural space.92,93 Flow cytometric analysis of patient NSCLC tumor specimens showed a robust immune response (50% of cellular content within tumor were CD45+), with neutrophils (20%) comprising the most abundant immune cell subset. The same study showed significant correlations between increased neutrophil counts and decreased lymphocytes, indicative of neutrophils potential to suppress lymphocytes within the tumor environment.94 NSCLC neutrophilia may be explained by increased neutrophil CD47 expression, which is associated with a delay in neutrophil apoptosis and phagocytic clearance.95 Neutrophil elastase is an inflammatory serine protease and mediates neutrophil induced proliferation of lung cancer cells *in vitro* in a COX-2 dependent fashion.96 Moreover, depletion of neutrophils with anti-Ly6g antibodies decreased tumor formation in a urethane model of murine lung cancer.97 Neutrophils are also a major source of IL-1β, which promotes lung cancer tumorigenesis and is indicative of poor survival in NSCLC patients.98,99 Neutrophils isolated from NSCLC patients with chronic obstructive pulmonary disease produce more APRIL (A proliferation-inducing ligand), an inflammatory regulator that promotes NSCLC growth and development.96,100 Neutrophil Extracellular Trap (NET) formation, a cell death mechanism that releases intracellular DNA, histone, elastase, and granule proteins, promotes lung cancer cell adhesion *in vitro* and increases in vivo micrometastases.101–103 Neutrophils and circulating neutrophil microRNAs serve as biomarkers for detection of NSCLC and are an independent negative predictor of survival in patients with advanced disease.104–106 Mechanistic studies on the role of neutrophils in MPE are warranted given their immunosuppressive role in NSCLC and adverse prognostic value in MPE.107

**Dendritic cells** (DCs) are professional antigen presenting cells (APCs) that cross-present antigens, induce adaptive immune responses, and regulate the immune system by modulating T and B lymphocyte antigen specific responses via their major histocompatibility (MHC) class I and II receptors and ability to produce IL-12 family cytokines.108 A meta-analysis evaluating the significance of immune cells in NSCLC showed that increased tumor conventional dendritic cells (cDCs) were associated with improved overall survival (hazard ratio 0.55; 95% confidence interval 0.44–0.68).109 Increased stromal cDCs were also prognostic for increased disease-specific survival (hazard ratio 0.62; 0.47–0.83).109
However, DCs also have an immunosuppressive role. B7-H3, a potent T cell coinhibitory molecule, is expressed on NSCLC tumor-derived DCs and significantly reduced T cell proliferation during in vitro coculture experiments. Interestingly, soluble B7-H3 is upregulated in NSCLC associated with advanced tumor staging, indicating the need for follow-up studies. CC chemokine ligand 18 (CCL18), implicated in immature DC and lymphocyte trafficking during homeostasis and inflammation, is present at a high level in NSCLC MPE and inhibits DC maturation, subsequently stimulating CD25⁺, FOXP₃⁺ regulatory T cell (T-reg) differentiation. Accumulation of DCs in MPE (15.2%) is significantly greater than that found in benign pleural effusions (7.1%), demonstrating the chronic inflammatory nature of MPE. In both firmly adherent or loosely adherent mononuclear cells isolated from bronchogenic carcinoma MPEs, >80% of cells are HLA-DR⁺ immunosuppressive RFD1⁺ DCs. DCs isolated from lung cancer MPE patients expressed higher levels of CD86, HLA-DR, CD40, CD1a, and but lower CD14 compared to DCs from benign effusions. DCs from MPE increased stimulation of allogeneic lymphocyte proliferation and subsequent IFN-γ production compared with control DCs. These findings contrast with tumor derived DCs isolated from NSCLC tissues, which exhibit a semi-mature phenotype with poor APC function. Given the preliminary success of DC vaccination trials in MM associated MPE, further study is warranted.

Natural Killer (NK) cells play four major roles in tumor biology and immunosurveillance: 1) promoting lysis of genomically unstable and stressed tumor cells; 2) producing cytokines (IL-8, IL-10, VEGF, HMGB1, and IFN-γ) to shape the inflammatory and proangiogenic response; 3) driving DC maturation in secondary lymphoid sites; and 4) promoting and initiating autophagy. NK cells from human MPE exhibit poor cytotoxicity, having impaired degranulation and reduced perforin release. In the malignant pleural environment, NK cells exhibit proangiogenic function, supporting capillary-like structures from recruited endothelial cells and produce angiogenic and vascular permeability inducing factors, including VEGF. Interestingly, CD56dim and CD56bright NK cells isolated from human MPE cultured with IL-2 for 72 hours had potent antitumor cytolytic activity. The same group found that IL-15 activated pleural effusion NK cells exhibited increased in vitro cytotoxicity and in vivo tumor clearance in mice. Interestingly, an earlier study showed that NK cells from MPE lung cancer patients stimulated with IL-2 and IL-12 produced more IFN-γ and IL-10 than blood mononuclear cells. These findings suggest that IL-2 and IL-15 activated NK cells from pleural fluid, normally discarded during thoracoscopy, represent a potential source of effector cells that could be activated productively with cytokine instillation into the pleural space as part of an immunotherapeutic regimen, perhaps coupled with checkpoint inhibitors delivered systemically. This would also allow for sequential monitoring of changes in the nonsclerosed pleural space.

Systemic inflammatory indicators in MPE patients

Differentiating MPE from benign effusions can be difficult, although identification of malignant cells by pleural fluid cytology or pleural tissue histology defines the condition. Inflammatory measures, including neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) are easily obtained hematological parameters that can aid in MPE diagnosis and, more importantly, disease prognosis (Table 2).

Increased NLR is a poor predictor of overall survival (OS) in patients with NSCLC and most other malignancies. This finding is presumably related to the exuberant release of DAMPs recruiting neutrophils but limiting lymphocyte expansion and survival. NLR assessment alone is not always an effective diagnostic metric. A study specifically assessing NLR in the MPE fluid (mNLR), demonstrated that mNLR values could not reliably distinguish malignant and benign pleural effusions. A subsequent study investigating NLR as a prognostic indicator in lung cancer patients with MPE, showed that blood NLR (NLR), in combination with the effusion NLR score was an independent predictor of OS. Moreover, a combination of platelet and lymphocyte to monocyte ratio (COP-LMR) is an independent predictor of shorter OS in stage IV NSCLC and MPE. Neither NLR nor the COP-LMR alone completely reflect the overall host inflammatory and hematopoietic response.

The systemic immune-inflammation index (SII), based on peripheral lymphocyte (L), neutrophil (N), and platelet (P) counts (SII = P x N/L), was first described in the context of hepatocellular carcinoma. SII’s predicative capability was shown to be greater than other conventional parameters such as tumor staging, tumor differentiation, and tumor number. SII is a powerful prognostic indicator, which when elevated, confers a poor outcome in patients with various cancers. SII is an independent prognostic indicator of poor outcomes for patients with stage III NSCLC and is a superior prognostic indicator to other inflammation-based indices, including NLR and PLR. Its low cost, easy determination, and high reproducibility from a simple complete blood count and differential make SII a promising tool for individualized lung cancer treatment strategies. The efficacy of SII as a prognostic indicator in patients with MPE is yet to be determined.

Developed in 2014, the LENT prognostic score, which incorporates pleural fluid lactate dehydrogenase, Eastern Cooperative Oncology Group performance score (ECOG PS), NLR, and tumor type, is another predictor of progression free survival (PFS) that is superior to ECOG PS in MPE. The LENT underestimates survival in patients having MPE secondary to lung adenocarcinoma. A more robust prognostic indicator was developed earlier in 2018. The eight variable PROMISE score, comparing hemoglobin level, C-reactive protein, white blood cell count, ECOG PS, cancer type, pleural fluid tissue inhibitor of metalloproteinases 1 (TIMP1) concentration, and previous chemotherapy or radiotherapy, is the first prospectively validated prognostic model for MPE that accurately estimates 3-month mortality that will aid in the development of more personalized
Table 2. Prognostic indicators in lung cancer amenable for use in MPE.

| Index and Definition | Patient Population | Prognostic Cutoff Value | Outcome Group | Ref |
|----------------------|--------------------|-------------------------|---------------|-----|
| NLR                  | 382 (NSCLC)        | Low: NLR < 1.5          | 5 yr OS:      | 257 |
|                      |                    | Intermediate: 1.5 ≤ NLR < 3.5 |              |     |
|                      |                    | High: NLR ≥ 3.5         |               |     |
|                      |                    | Low: NLR ≤ 4x10^9       | Median Survival: | 258 |
|                      |                    | High: NLR > 4x10^9      |               |     |
| NLR                  | 88 (advanced NSCLC) | Low: NLR < 4x10^9       |               |     |
|                      |                    | High: NLR > 4x10^9      |               |     |
| NLR                  | 109 (advanced NSCLC)| Low: NLR ≤ 4x10^9       |               | 259 |
|                      |                    | High: NLR ≥ 5           |               |     |
| SII                  | 183 Low            | 660 × 10^9              |               | 108 |
|                      | 149 High           |                         |               |     |
| (NSCLC)              |                    |                         |               |     |
| SII                  | 140 Low: 71 male   | 395.4 × 10^9            | Male 5 yr % OS: | 109 |
|                      | 69 female          |                         | Low (53.4)    |     |
|                      | 270 High: 196 male |                         | High (35.4)   |     |
|                      | 74 female (NSCLC)  |                         | p<0.0008      |     |
| SII                  | 214 Low            | 471.2 × 10^9            | Low 83.61%    | 260 |
|                      | 127 High           |                         | High 60.39%   |     |
| (NSCLC)              |                    |                         | p<0.0001      |     |
| SII                  | 381 (NSCLC)        | Low SII < 471.2 × 10^9  | 5 yr OS (all p < 0.001): | 261 |
|                      |                    | High SII ≥ 471.2 × 10^9 | Low SII (83.61) |     |
| NLR                  |                    | Low ALI ≥ 37.66        | Low ALI (60.39) |     |
|                      |                    | High ALI < 37.66       | Low ALI (57.61) |     |
| PLR                  |                    | Low NLR < 5:1          | High ALI (84.20) |     |
| PNI                  |                    | High NLR ≥ 5:1         | Low NLR (76.75) |     |
|                      |                    | PLR 0 < 150:1          | High NLR (40.18) |     |
|                      |                    | PLR 1 = 150 – 300      | PLR 0 (80.92)  |     |
|                      |                    | PLR 2 > 300:1          | PLR 1-2 (62.89) |     |
|                      |                    | PNI 0 ≥ 45             | PNI 0 (67.40)  |     |
|                      |                    | PNI 1 < 45             | PNI 1 (79.01)  |     |
|                      |                    |                         |               |     |
| LENT                 | 43 Low-risk        | Low-risk LENT score:   | Median survival (days) | 110 |
|                      | 129 Moderate-risk  | IQR 228–549            | Low-risk: 319  |     |
|                      | 31 High-risk       | Moderate-risk LENT score: | HR (95% CI): not specified |     |
| (MPE)                |                    | IQR 47–467             | Moderate-risk: 130 |     |
|                      |                    | High-risk LENT score:  | HR (95% CI): 1.49 |     |
|                      |                    | IQR 22–77              | High-risk: 44  |     |
|                      |                    | High-risk score: ≥ 5   | HR (95% CI): 5.97 |     |
| LENT                 | 36 High-risk       | Moderate-risk score:   | Median survival: | 111 |
|                      | 34 Moderate-risk   | ≥ 5                    | High-risk: 190.5 days Moderate-risk: 346 days |     |
| (lung adenocarcinoma | (lung adenocarcinoma|                        |                |     |
| presenting with MPE) |         |                         |                |     |
|                      |                    | Moderate-risk score: 2- |                |     |
|                      |                    | 4                      |                |     |
| PROMISE              | 162 (stage IV cancer with MPE) | Categories | 3 month survival C statistic value | 133 |
|                      |                    | A: 0%-24% risk         | Internal validation: 0.78 |     |
|                      |                    | B: 25%-40% risk        | External validation: 0.89 |     |
|                      |                    | C: 50%-74% risk        | p<0.05         |     |
|                      |                    | D:75%-100% risk        |                |     |

Key: Systemic Immune-Inflammation Index (SII), Neutrophil to Lymphocyte Ratio (NLR), Non-Small Cell Lung Cancer (NSCLC), Malignant Pleural Effusion (MPE), Interquartile Range (IQR), Complete Response (CR), Partial Response (PR), Stable Disease (SD), Progressive Disease (PD), Overall Survival (OS), Disease Control Rate (DCR), Advanced Lung Cancer Inflammation Index (ALI), Prognostic Nutritional Index (PNI), Platelet to Lymphocyte Ratio (PLR)

treatment plans and enable stratification in randomized studies. Unfortunately, the PROMISE study was unable to identify markers of successful pleurodesis treatment, indicating the need to further specify prognostic criteria to improve clinical decision making in the setting of MPE.
Adaptive immunity within MPE

The adaptive immune system comprises both antigen-specific antibody and cell-mediated responses. B cells produce various immunoglobulins, act as APCs, and support T-reg cell differentiation and cytokine liberation. Upon recognition of cognate antigen and activation of costimulatory receptors, CD8+ T lymphocytes can directly recognize and destroy tumor cells or virally infected cells. Upon stimulation and exposure to varying microenvironmental signals, CD4+ helper T cells sustain and regulate adaptive responses. T-regs and naïve B cells in the MPE environment support tumor cell proliferation and immune evasion (Figure 3).

T cell populations vary between healthy and malignant pleural fluid, both in phenotype and function. Healthy subjects display mainly effector-memory phenotypes within both CD4+ and CD8+ T cell subsets with a low CD4+/CD8+ ratio (0.59) in pleural fluid. Patients with MPE, however, show a high CD4+/CD8+ ratio (>2.2) in the pleural fluid with an increased percentage of central memory CD4+ T cells and decreased CD8+ effector-memory T cells, indicative of potential immune escape by tumor cells. Despite the stoichiometric changes in T cell population in MPE, no differences are observed in relative abundance of T lymphocytes based on receptor expression (TCRα/β or TCRγ/δ) compared to benign effusions. However, another group did find that TCRγ chain downregulation was associated with T cell apoptosis in MPE and related to the abundance of high monocyte populations in effusions.

Despite increased levels of circulating natural killer T (NKT) cells, NKT recruitment to the pleural cavity is decreased in MPE patients. It is hypothesized that chemotactic tumor signaling may disrupt local effector T cell recruitment which contributes to immune response evasion. Another notion suggests that rapid apoptosis through activation-induced cell death of effector CD8+ T cells, leads to the accumulation of memory cells and a depletion of effector cells. Impaired T cell cytotoxicity, as measured by production of IFN-γ and granzyme B, has been attributed to TAM production of TGF-β, and presents a valuable target for MPE cell therapy.

T regulatory cells are elevated in MPE with CD4+ CD25+ cells expressing high mRNA levels of FOXP3, CTLA-4, and CD28 compared to those found in benign effusions. Decreased expression of the micro-RNA 141, causing increased production of CXCL1, recruits T-regs into the MPE pleural environment through CXCR2 and CCL22 chemokine signaling. Moreover, increased MPE T-regs correlates with a decrease in the overall percentage of lymphocytes and an increase in expression of CD4+/CD4+CD25+ T cells, which are present at the highest

Figure 3. Adaptive immune signaling pathways in malignant pleural effusions. The immunosuppressive MPE environment modulates the ‘adaptome’, B, NKT, αβ T cell and γδ T lymphocyte biology to promote tumor growth and immune evasion. Tumor cells secrete chemokine ligand 1 (CXCL1) and DAMPs, promoting further cell proliferation and T-regulatory (T-reg) cell recruitment. T-regs promote an immunosuppressive environment, inhibiting Th1, Th2, Th9, and Th17 responses. Moreover, T lymphocytes display a high CD4+/CD8+ ratio with an increased percentage of central memory CD4+ T cells and decreased CD8+ effector-memory T cells. T cell programmed cell death protein 1 (PD-1), T cell immunoglobulin and mucin domain 3 (TIM-3), and lymphocyte-activation gene 3 (LAG-3) immune checkpoint expression inhibit lymphocyte activity. Tumor Associated Macrophage (TAM) transforming growth factor beta (TGF-β) production decreases effector T cell cytotoxicity with reduced production of interferon gamma (IFN-γ) and granzyme B. These changes are accompanied with decreased NKT recruitment to the effusion site. Increased soluble CD40 (sCD40) levels inhibit B cell function by competing for CD154 (CD40 ligand) on T lymphocytes. Despite decreased B cell density, increased expression of CD80, CD86, MHC II, CD44, CD69, and programmed death ligand 1 (PD-L1) promote Th2 responses that can also support MPE formation.
frequency in patients with the most advanced clinical stage of lung cancer.\textsuperscript{156,158,159} CD39\textsuperscript{170} T-reg cells have also been implicated in inhibiting the generation and differentiation of Th17 cells, through a TGF-β1 latency associated peptide mechanism.\textsuperscript{160} While Th1 differentiation has traditionally thought to promote antitumor responses, IFN-γ deficient mice, devoid of Th1 and rich in Th17 cells, were protected from MPE development, while IL-17A deficient mice, rich in Th1 and devoid of Th17 cells, had enhanced pleural tumor cell proliferation and vascular leakiness.\textsuperscript{161} These studies showcase the special local MPE environment and the need for additional studies to understand the interplay between tumor induced inflammatory cascades and lymphocyte activity.

**B Cells** are decreased in number in MPEs compared to peripheral blood, however, MPE B cells express higher levels of CD80, CD86, MHC-II, CD44, CD69, and PD-L1 molecules.\textsuperscript{162} Tumor cells in this environment have abundant expression of MHC class I molecules but lack the B7-1 costimulatory molecule, important for B and T cell activation. In murine models, B\textsuperscript{−/−} animals had decreased Th1 and increased Th17 responses, which increased survival time and decreased effusion volume.\textsuperscript{162} Adoptive transfer of activated naïve B cells reverses this trend, increasing Th1 and inhibiting Th17 expansion by targeting the PD-1/PD-L1 pathway.\textsuperscript{162} Coculture of naïve B cells with CD4\textsuperscript{+} Th1 or Th17 conditions resulted in increased Th1 cell expansion, but decreased Th17 expansion; however, treatment with anti-PD-L1 mAb reversed Th17 expansion.\textsuperscript{162} Thus, by regulating Th1/Th17 cell responses and skewing antitumor cellular immunity, naïve B cells support MPE formation and are an attractive therapeutic target.\textsuperscript{162} The costimulatory protein, CD40, is expressed on B lymphocytes and DCs. The CD40 ligand, CD154, is expressed by T lymphocytes following initial T cell receptor cross-linking as well as being expressed on platelets.\textsuperscript{163} Soluble CD40 (sCD40) levels are increased in MPE and are indicative of poor prognosis.\textsuperscript{164} High concentrations of sCD40 inhibit B cell function by competing for CD154 on T lymphocytes, limiting T cell help and promoting another means of immune evasion.\textsuperscript{164} B cell B7-H4 expression is also elevated and is associated with poor prognosis for patients with metastatic pleural adenocarcinoma.\textsuperscript{165} Interestingly, anti-B7-H4 mAb effectively suppressed pleural effusion formation in a mouse model of MPE.\textsuperscript{165}

**Completed MPE clinical trials promoting adaptive immune responses**

Adaptive immunotherapies that involve the selection, activation, and expansion of T cells to elicit a tumor specific response with immunological memory hold much promise in treating MPE. Though many MPEs share similar characteristics, the primary site of disease appears to influence the composition of the effusion as demonstrated when comparing MM and breast/lung cancer patients.\textsuperscript{166} In addition to T cells, the pleural space harbors other immunomodulatory elements that vary within patients, suggesting a personalized approach to restoring a functional ‘hot’ immune tumor microenvironment, amenable to immunotherapy. In general, ‘hot’ immune infiltrated tumors are those within which intimate contact between T cells can be observed in the tumor. Dysfunctional T cells found within effusions are dispersed, like tumor cells, in the pleural fluid.\textsuperscript{167} Local administration of agents such as TLR-9 agonists (CpG\_s), stimulator of interferon genes (STING) agonists, or installation of oncolytic viruses would seem to be logical approaches and need to be evaluated. Sequential studies of the pleural fluid should enable detailed mechanistic studies in this setting.

**Interleukin 2 therapy**

In 1993, a phase I trial of intrapleural recombinant IL-2 infusion examined 22 MPE patients with various cancers.\textsuperscript{168} This trial aimed to exploit IL-2 efficacy in treating MPE patients with disease stemming from MM (15), adenocarcinomas (6), or squamous cell carcinoma (1). The treatment resulted in 1 complete response (CR) and 9 partial responses (PR) with acceptable side effects. A subsequent study also assessed IL-2 intrapleural treatment, promoting stimulation of lymphokine activated killer (LAK) cell activity. In the treated patient population, a remarkable 30 of 33 patients responded to treatment; with 18 CR, 12 PR, and 3 NR.\textsuperscript{169} IL-2 administration has been shown to function by reversing the exhaustion phenotype of CD8\textsuperscript{+} T cells found within MPEs.\textsuperscript{170} This loss of effector function is proposed to be due to prolonged exposure and stimulation by nominal tumor antigens.\textsuperscript{171} A trial exploring this exhaustion phenotype showed initially low levels of granzyme B, IFN-γ, and CD8\textsuperscript{+} T cell proliferation paired with high PD-1 expression, that were then reversed with the IL-2 therapy.\textsuperscript{170} Toxicity was dose-dependent, ranging from fever to transient abnormal renal function. IL-2 has been approved for treatment of MPE in China since 1998; a recent meta-analysis of 18 IL-2 MPE trials found that despite increases in treatment related fever, thoracic injection of IL-2 plus cisplatin had higher objective response rates (4.1x greater), disease control rates (7.86x greater), quality of life (2.75x greater), and lower non-response rates than cisplatin alone.\textsuperscript{172} Moreover, IL-2 treatment reduced T cell expression of PD-1, while reversing exhaustion phenotypes: increasing proliferation and expression of granzyme B and IFN-γ.\textsuperscript{170} Following the introduction of IL-2 as a primary treatment regimen for patients with melanoma and renal cancer, as well as the exploration of its use in treating MPE patients, the field has since turned to other novel immunotherapeutic options for MPEs (Table 3). Although approved in China for therapy of MPE, investigators in Europe and the US were less experienced in the use of locally applied ‘microdose’ IL-2, associated with limited and acceptable outpatient toxicity. IL-2 should be reexamined in this setting, perhaps as part of a rationally designed combination therapy with systemic checkpoint inhibitors or novel cellular therapies.

**Dendritic cell therapies**

One of the extensively tested immunotherapy strategies that harnesses the specificity and memory of the immune system uses DCs to present tumor associated antigens to elicit
Table 3. Previous MPE immunotherapy clinical trials.

| Treatment | Design | Patients | Response # (%) | Adverse Events | Ref |
|-----------|--------|----------|----------------|---------------|-----|
| IL-2      | Phase 1 trial of continuous intrapleural rIL-2 infusion | n = 22 adenov-6, meso-15, squam-1 | CR 1 (5), PR 9 (41) | Accepted tolerance with some side effects; fever/chills, 2 meso died | 168 |
| IL-2 and LAK | Lymphokine activated killer cells used with IL-2 to treat effusions from lung cancer | n = 33 | CR 18 (55), PR 12 (36), NR 3 (9) | No serious side effects | 169 |
| IL-2      | Intrapleural and follow up subcutaneous administration of IL-2 providing palliation of pleural effusion and on primary tumor | n = 31 | ORR 7 (22), SD 10 (32), PD 14 (24) | Manageable toxicity | 173 |
| IL-2      | IL-2 therapy reverses the exhaustion phenotype of MPE CD8+ T cells. Initially low Granzyme B, IFN-γ, and proliferation with high PD-1 expression is reversed to reduce IL-2 expression and increase the others in MPE. Carcino-embryonic antigen reduced by IL-2. | n = 35 (lung) and 12 non-MPE | IL-2 treatment reduced the expression of PD-1, increased the expression of Granzyme B and IFN-γ and enhanced the proliferation of CD8+ T cells in MPE | Dose-dependent severity ranging from fever to abnormal renal function | 170 |
| OK432     | OK432-activated ex vivo and monocyte-derived dendritic cells in combination with low-dose OK432 | n = 5 | Decreased effusion production in all pts. | No severe AEs | 174 |
| IFN-α2b   | Comparing bleomycin (chemotherapy) to IFN-α2b (immunotherapy). Effusion drained and then given either treatment. Second dose of bleomycin administered for nonresponsive patients. Treatment groups were randomly assigned. | n = 160 bleomycin, 77 IFN-α2b | 30 Day Response Bleomycin: 70 (84.3) IFN-α2b: 48 (62.3) | None listed | 175 |
| IFN-β     | Phase 1 ranging single-dose intrapleural IFN-β gene transfer by adenoviral vector through indwelling pleural catheter. Evaluates toxicity, gene transfer, and immune, and tumor responses. | n = 10, 7 MPM, 3 MPE | Gene transfer 7 (70), Antitumor immune response 7 (70), SD 4 (40), PR 4 (40) | Well tolerated, transient lymphopenia most common. Max tolerated 9E11 viral particles No severe AEs | 176 |
| DCs       | Open label pilot study treating MPE patients with DCs derived from autologous CD34+ stem cells stimulated with IL-4, GM-CSF, TNF-α, GM-CSF, and TNF-α; without any prior therapy. | n = 26 | CR 1 (3.8), PR 13 (50), SD 10 (38.5), PD 5 (19.2) | Well tolerated without systemic toxicity, except for transient fatigue and low-grade fever on the day of the DC injection. No grade 3 or higher AEs. | 120,121 |
| DCs and cyclophosphamide | Pilot study using cyclophosphamide and autologous DCs pulsed with MM tumor lysate prior to chemotherapy and P/D. | n = 10 | CR 1 (10), SD 4 (40), PD 2 (20), N/A 3 (30), 8/10 Radiographic improvement Median OS 26 Months | Well tolerated | 119 |
| αCD25 + IL-2 + OK432 | Low-dose anti-CD25 antibody to target T-reg cells. Low concentration of basiliximab augments) production in combination with IL-2. Can also be followed by administration of OK432. Foxp3 expression of ELs (effusion lymphocytes) not definitively changed. Aims to evaluate efficacy of basiliximab followed by OK432 administration (day 0 or 1). | n = 12 | CR 2 (16.7), PR 5 (41.7) | Safe and well tolerated | 177 |
| CIK Cell Therapy | DC and CIK (cytokine-induced killer) cells treat MPE | n = 16 and 15 control | CR 8 (50), PR 5 (31.3), NR 3 (18.8), PD 4 (23.5) | Grade III or below, most commonly fever in both groups Well tolerated without DLTs | 178 |
| Viral Therapy | Phase 1 dose escalation of GMCI strategy with vector for thymidine-kinase gene followed by valacyclovir with chemotherapy, celecoxib added to reduce CRS. | n = 19, 17 evaluable | CR 8 (50), PR 5 (31.3), PD 4 (23.5) | Grade III or below, most commonly fever in both groups Well tolerated without DLTs | 178 |

Key: Interleukin-2 (IL-2), Recombinant IL-2 (rIL-2), Lymphocyte Activated Killer Cell (LAK), Complete Response (CR), Partial Response (PR), No Response (NR), Progressive Disease (PD), Adverse Events (AEs), Pleurectomy with Decortication (P/D) Interferon Gamma (IFN-γ), GMCI (gene-mediated cytotoxic immune), Cytokine Release Syndrome (CRS), DLTs (Disease Limiting Toxicities).

responses. Treatment of 26 MPE patients with DCs derived from autologous peripheral CD34+ stem cells with IL-4, GM-CSF, and TNF-α stimulation resulted in no severe side effects, a 54% overall response rate, and improvement of the Karnofsky performance score (KPS) in 15 patients. In another 10 patient MM pilot study using autologous DCs pulsed with tumor cell lysate, intradermal or intravenous vaccination following cytoxeroptive chemotherapy was well tolerated and resulted in antigen specific DC proliferation and stimulation of granzyme B-associated antitumor T cell activity. While six patients progressed, three attained a PR, and one had stable disease, with a median OS of 19 months. In a follow-up pilot study by the same group, 10 patients with MM were treated with DCs pulsed with autologous tumor lysate combined with T-reg targeting cyclophosphamide following conventional chemotherapy and or...
bleomycin chemotherapy. Again, investigation in modern control. Targeting another especially in patients with lung cancer. The addition of celecoxib decreased suggests that utilizing 

activated lympho-

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activated T cells in MPE patients. The levels were No severe the surface of T cells that interact with their corresponding 

A trial of 14 NSCLC MPE patients with poor pre-treatment status (KPS = 40), received SSAg infusions up to two times a week until the effusion resolved while assessing toxicities. CR and PR were observed in 11 and 3 patients, respectively, without significant adverse effects. The median recurrence was five months and the median survival was 7.9 months compared to a 2.5-month median survival for 18 talc poudrage-treated patients. 9 of 14 SSAg treated patients survived more than six months, while no talc treated patients survived that long. Again, investigation in modern local therapies including installation of CpG or STING agonists seems warranted.

Nonspecific immunostimulants

A number of early studies examined nonspecific immunosti-

ments. For example, infusion of ex vivo activated lymphocytes, monocyte derived DCs and OK432 (lyophilized Streptococcus pyogenes) decreased cancer cell counts in MPE’s in all five patients within a pilot study. No severe adverse events were observed and increased IFN-γ levels were found in three of five patients. Additionally, intrapleural injection of the immunostimulant, Staphylococcus aureus superantigen (SSAg) was hypothesized to stimulate T cells and resolve MPE. A trial of 14 NSCLC MPE patients with 177

CD4+ T-reg populations, resulted in radiographic disease control in eight patients, and encouraging OS gains. Seven of the 10 patients survived more than 24 months. This highly selected patient population had documented stable disease or a partial response to prior chemotherapy. This promising finding warrants larger-scale clinical trials. 

Interferon therapy

In addition to nonspecific immunostimulants, the cytokine IFN-α2b increases the cytotoxic activity of NK and T cells, when produced by infected cells, and directly inhibits tumor proliferation. However, comparing IFN-α2b immunotherapy to standard bleomycin chemotherapy demonstrated that bleomycin was more effective in MPE patients at 30 days. Out of 83 bleomycin and 77 IFN-α2b patients, 84.3% and 62.3%, respectively, responded. This trial demonstrated no survival advantage but did offer palliation and effusion control. Targeting another type I interferon known to inhibit tumor growth and boost the immune system, in a phase I trial evaluating a single dose intrapleural IFN-β gene transfer using an adenoviral vector (Ad.IFN-β) showed no increases in pleural cell infiltrate, but was well tolerated and did elicit antitumor immune responses (7/10 patients). Another targeted treatment involved low-dose anti-CD25 (basilix-

imab) antibody to target Tregs and preserve CD4+CD25dim activated T cells in MPE patients. The treatment was given in combination with IL-2 or followed by OK432. 7 of 12 patients treated with OK432 and basiliximab responded, two of which were CR with acceptable adverse events. 

Other therapies

Trials of combined therapies showed that combination of autologous DC and cytokine-induced killer (CIK) cells to treat effusions had comparably mild side effects but only modest efficacy. An anecdotal report of intrapleural admin-

istration of tumor infiltrating lymphocyte (TIL) infusion com-
pared to traditional cisplatin therapy demonstrated a claimed but very modest greater response rate (33.3% vs 28.57%) and disease control (71.43% vs 66.67%) than MPE patients given cisplatin. Certainly, local and systemic TIL therapy is an area worthy of further consideration. Another combination therapy, intrapleural gene-mediated cytotoxic therapy (GMCI) utilizing a thymidine-kinase gene expressed by an adenovirus-based vector was followed with anti-herpetic pro-
drug valacyclovir and chemotherapy in a phase I trial of patients with MPE. The addition of celecoxib decreased cytokine release syndrome as experienced by some patients. Of the 17 evaluable patients, treatment was safe and well tolerated with encouraging preliminary treatment responses: PR 4, SD 9, PD 4, with 3 patients alive 23–33 months after GMCI. The current approval of oncolytic herpes virus therapy for patients with melanoma, suggests that utilizing herpes or vaccinia virus, might be considered useful in the future treatment of patients with MPE. Local therapies such as these may enable dissemination of memory T cells, capable of controlling disease systemically.

Current antibody and cell therapies

Great strides continue to be taken in improving the efficacy of immunotherapy, including application of check-point inhibitors and Chimeric Antigen Receptor (CAR)-T cells. The use of adoptive therapy with TIL or alternative sources of T cells including the use of lymph nodes or peripheral blood-derived tumor reactive cells is currently under-developed.

Checkpoint inhibition

The immune checkpoint proteins are co-receptors expressed on the surface of T cells that interact with their corresponding ligands on APCs, which in turn effect T cell activation and subsequently may limit cancer cell elimination following T cell recognition. Checkpoint antibody inhibitors prevent receptor and ligand binding and interaction, disrupting immunosuppressive signaling; their administration has resulted in improved survival outcomes for patients with solid tumors, especially in patients with lung cancer (Table 4). Interestingly, almost no information about MPE and checkpoint treatment success (or failure) is currently available.

CTLA-4 antibody

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), an inhibitory receptor that down-modulates the initial stages of T cell activation, was the first clinically validated checkpoint pathway target. When CTLA-4 is mobilized in T cells from cytosolic stores, it binds its counter-receptors CD80 and CD86 on APCs, mediating direct inhibitory effects on the
MHC–TCR pathway and decreasing T cell effector function. Anti-CTLA-4 monoclonal antibodies prevent this binding, amplifying T cell responses against tumors. In a Phase III study of advanced NSCLC, administration of anti-CTLA-4 mAb ipilimumab (Yervoy) with first-line chemotherapy, such as paclitaxel or carboplatin, did not prolong OS (median OS 13.4 vs 12.4 months) compared with chemotherapy alone.

**Programmed Death receptor-1 (PD-1)**

Programmed cell death protein 1 (PD-1) is a key immune-checkpoint receptor expressed by activated T, B, and NK cells that mediates immunosuppression. When PD-1 binds to its ligands PD-L1 (B7-H1) and PD-L2 (B7-DC), expressed by tumor and stromal cells, it moves the T cell receptor out of the so-called central supramolecular activation cluster (CSMAC) with a target, reducing T cell survival, and inhibiting T cell proliferation and production of IFN-γ, TNF-α, and IL-2. Monoclonal antibodies that target PD-1, such as pembrolizumab (Keytruda) and nivolumab (Opdivo), are human IgG4 PD-1 immune-checkpoint–inhibitor antibodies that disrupt PD-1–mediated signaling and have a demonstrated ability to reverse suppression of T cell function and restore antitumor immunity.

**Table 4. Checkpoint inhibitor clinical trials of NSCLC and MM.**

| Immunotheapy          | Patient Population | Response # (%)                          | Ref |
|-----------------------|--------------------|-----------------------------------------|-----|
| BMS-936559 (anti-PD-L1) | 49 NSCLC           | ORR: 5 (10.2)                           | 190 |
| Chemotherapy + Ipiilumab (anti-CTLA-4) | 338 NSCLC (Stage IV or recurrent) | Median OS: 13.4 months | 191 |
| BMS-936558 (anti-PD-1) | 76 NSCLC           | Median PFS: 5.6 months                  | 192 |
| Pembrolizumab (anti-PD-1) | 690 NSCLC: 29 squamous | ORR: 69 (45)                           | 194 |
| Pembrolizumab (anti-PD-1) | 154 NSCLC: 125 non-squamous (18 Brain metastasis) | PFS: median 10.3 months (62.1% at 6 months) | 195 |
| Pembrolizumab (anti-PD-1) | 52 NSCLC: 13 squamous | ORR: 2 (20)                             | 196 |
| Pembrolizumab (anti-PD-1) | 135 NSCLC (non-squamous) (9 CNS metastasis) | ORR: 27 (20)                           | 197 |
| Nivolumab (anti-PD-1) | 52 NSCLC: 13 squamous | ORR: 97 (19.4)                          | 198 |
| Nivolumab (anti-PD-1) | 52 NSCLC: 13 squamous | ORR: 2 (15) squamous                    | 199 |
| Nivolumab (anti-PD-1) | 495 NSCLC           | ORR: 97 (19.4)                           | 200 |
| Nivolumab and Nivolumab + Ipiilumab | 125 MPM: 63 given Nivo | ORR: 11 (17.5)                          | 201 |
| Pembrolizumab (anti-PD-1) | 292 NSCLC (non-squamous) | ORR: 11 (17.5)                          | 202 |
| Pembrolizumab (anti-PD-1) | 473 NSCLC           | ORR: 11 (17.5)                           | 203 |

Key: Programmed Cell Death Protein-1 (PD-1), Programmed Death Receptor Ligand (PD-L1), Objective Response Rate (ORR), Stable Disease (SD), Progression Free Survival (PFS), Partial Response (PR), Complete Response (CR), Disease Control Rate (DCR), Stable Disease (SD), Progressive Disease (PD).
patients who had PD-L1 expression on at least 50% of tumor cells treated with pembrolizumab had a significantly higher PFS and response rate than those treated with docetaxel, leading to US Food and Drug Administration approval of Pembrolizumab as first-line therapy for patients with NSCLC with high PD-L1 expression. In patients with advanced NSCLC, the response rate (20% vs 9%), PFS (3.5 vs 2.8 months), OS (9.2 vs 6.0 months) are longer with nivolumab treatment than with docetaxel. Although 37% of patients experienced Grade 3–4 treatment related AEs, combination therapy of nivolumab and ipilimumab demonstrated encouraging clinical responses in NSCLC (47% confirmed objective response) and survival (median PFS 8.1 months) that should be tested against anti-PD-1 mono-therapy in future trials. Interestingly, in NSCLC and across all cancer types, patients with greater tumor mutational load, the total number of coding mutations per megabase of the tumor genome, correlated with greater response to PD-1 therapy, suggesting additional biomarkers for treatment stratification, moving forward.

**PD-L1 antibody**

Atezolizumab (Tecentriq) is a humanised engineered IgG1 monoclonal antibody that targets PD-L1, inhibiting both PD-L1/PD-1 and PD-L1/B7-1 binding, which might further enhance immune responses to cancer cells. In a randomized, open-label, phase III trial (OAK Study) evaluating Atezolizumab in patients with previously treated NSCLC, PD-L1 inhibition increased median overall survival compared to docetaxel (13.8 vs 9.6 months) with fewer grade III or IV AEs (15 vs 43%). Similarly, the phase III placebo controlled PACIFIC trial demonstrated that durvalumab (anti-PD-L1) is an efficacious consolidation therapy (median PFS 16.8 vs 5.6 months) in patients with stage III NSCLC who did not have disease progression after two or more cycles of platinum-based chemoradiotherapy, which resulted in the approval of durvalumab by the US Food and Drug Administration for use as a maintenance therapy following the completion of platinum-based chemoradiation in unresectable lung cancer.

A few studies are also testing the combination effects of checkpoint inhibitors with anti-human vascular endothelial growth factor (anti-VEGF) receptors. Preliminary results from the ongoing CheckMate 012 phase I trial evaluating the efficacy and safety of switching to nivolumab combined with bevacizumab (anti-VEGF) as maintenance treatment, in a cohort of advanced NSCLC patients with no disease progresssion to first-line platinum-based chemotherapy reported median PFS (37.1 weeks) comparable to approved agents. Another clinical trial currently evaluating the safety and preliminary efficacy of pembrolizumab plus ramucirumab (anti-VEGFR-2) in patients with locally advanced and unresectable or metastatic NSCLC found no unexpected safety signals.

**Additional checkpoint targets**

A number of novel T cell and NK cells checkpoint targets are being tested in the clinic, including antibodies to LAG3, TIM3, TIGIT, KIR2DL1-3, NKG2A, and others. Lymphocyte-activating gene 3 (LAG-3), for example, is expressed in cytotoxic stores and on NK cells, DCs, B cells, and TIL. LAG-3 encodes a protein that binds a nonholomorphic region of MHC class II with greater affinity than CD4 and novel target fibroblast activating protein 1, and inhibits T cell proliferation, activation, and homeostasis. TIL from NSCLC patients express LAG-3, positively correlating with PD-1/PD-L1 expression, consistent with a poor prognosis. T cell immunoglobulin domain and mucin domain-3 (TIM-3) is another immune checkpoint receptor that is expressed on IFN-γ producing CD4+ Th1 and CD8+ T cells and has an inhibitory role in T cell responses. Interaction between TIM-3 and its ligands, including HMGB1/DNA, phosphatidylserine, and galectin-9, inhibits Th1 and Th17 responses and induces peripheral tolerance. TIM-3 is expressed on TIL of NSCLC patients and correlates with poor clinicopathological parameters such as nodal metastasis and advanced cancer stages.

The current efficacy of checkpoint inhibition in MPE remains to be seen. There have been several clinical trials of anti-PD-1 in lung cancer patients, but its effect on MPE are not specifically reported. PD-L1 is upregulated in tumor cells and normal MRC-5 lung fibroblasts, and PD-L1 blockade restores in vitro CD8+ T-cell granzyme B expression. Interestingly, rather than being exhausted, CD8+ TIL in MPE are not completely differentiated and are negatively regulated by PD-L1. Abundant expression of immune checkpoints, PD-1, PD-L1, LAG-3, and TIM-3 have been identified in MPE resident immune and tumor cells, suggesting additional targets in novel therapeutic interventions.

**Modern adoptive cell therapy**

Adaptive cell transfer (ACT) is a promising approach to immunotherapy that most often utilizes a patient’s own immune cells to treat their cancer. Chimeric antigen receptor (CAR) T cell therapy is an ACT with the most advanced clinical development. TIL within a tumor microenvironment often become exhausted, anergic, and nonfunctional. CAR-T cell receptors are specifically engineered to activate in response to tumor specific antigens (TSA) expressed on the cell surface, but most often target common structures on cells, including CD19 expressed on most B-cells. CARs are synthetic receptors most frequently grafted with antibody specificities onto TCR signaling domains. First generation CAR-T cells bound to TSAs with a single chain variable fragment (scFv) fused to the CD3ζ domain demonstrated in vitro cytotoxicity but limited in vivo capabilities due to CD3ζ’s inability to prolong activation of resting T cells. More recently, second and third generation CAR-T cells that contain the scFv with multiple costimulatory signaling domains (CD28 and 4-1BB) in addition to the CD3ζ chain have demonstrated in vivo antitumor efficacy with increased cytokine production and T cell proliferation and persistence. CAR-T cell therapy has had substantial clinical success in treating hematological patients with malignancies including acute lymphoblastic leukemia, chronic lymphocytic leukemia, and acute myeloid leukemia.
leukemia and non-Hodgkin lymphomas. CAR-T cell therapy is under active investigation for the treatment of solid tumors including NSCLC and MM, as outlined in Table 5. None of these specifically target MPE’s although local application is an attractive approach.

Finding suitable TSAs for CAR-T cell therapy is a substantial challenge. Candidate target antigens currently being investigated in clinical trials for lung cancer and MM include overexpressed tumor-associated antigens (TTAs) [carcinoembryonic antigen (CEA), ganglioside (GD2), glypican-3 (GPC3), human epithelial growth factor receptor 2 (HER2), mesothelin (MSLN), epidermal growth factor (EGFR), prostate stem cell antigen (PSCA)], and receptor tyrosine-kinase-like orphan receptor (RO1); abnormal glycosylation proteins [transmembrane glycoprotein mucin 1 (MUC1)]; immunomodulatory antigens [NK2D ligands including MICA/MICB and ULBP1-3, PD-L1, CD80/CD86]; and stromal elements associated with the tumor microenvironment [fibroblast activation protein (FAP) and VEGF Receptor 2].

EGFR and HER2 are receptor tyrosine kinases that are amplified or mutated in a variety of cancers, including over 15% of NSCLC patients in western nations and 45% of NSCLC patients in Asian countries. Second generation EGFR-CAR T cells (CD3’CD8’ T cells) demonstrated high proliferative capacity as well as specific and potent cytotoxicity against NSCLC cells in vitro and in vivo. A Phase I study (NCT01869166) at the Chinese PLA Hospital testing escalating doses of EGFR-CAR-T cell infusions in 11 patients with advanced relapsed NSCLC was well tolerated with pathological eradication of EGFR positive tumor cells and two patients obtaining PR and five patients demonstrating stable disease. Another Phase I/II study (NCT02713984) in the Southwest Hospital of China is testing HER2-CAR-T cells in various refractory malignancies including NSCLC. EGFR mutations can be detected in malignant pleural fluid but have lowered response rates to EGFR tyrosine kinase inhibitors than solid tumors.

MSLN is a cell surface glycoprotein overexpressed in epithelial cancers, including MM and NSCLC that has been associated with poor OS and PFS. Several Phase I studies evaluating systemic and intrapleural administration of MSLN-CAR-T cells are currently underway (NCT01583686, NCT02414269, NCT02580747, NCT02159716, NCT01355965). An initial seven patient MM study at the University of Pennsylvania using transiently expressed second generation MSLN-CAR-T cells containing the CD3ζ and 4-1BB signaling domains showed no “on target, off target” cytotoxicities. Early results from these studies indicate that MSLN-CAR-T cells migrated to primary and metastatic tumor sites and elicited anti-tumor responses (NCT01355965). A follow-up study using escalating doses of MSLN-CAR-T cells and cyclophosphamide, as a lymphodepletion agent, was well tolerated and is nearing completion (NCT02159716).

MUC1 is a transmembrane glycoprotein that is aberrantly glycosylated in many cancers, including NSCLC. MUC1 and PSCA CAR-T cells showed independent and synergistic antitumor efficacy in a patient-derived xenograft model of NSCLC. The First People’s Hospital in Hefei, China is completing a Phase I/II study of MUC1-CAR T cells for patients with MUC1+ advanced refractory NSCLC (NCT02587689). The same group is also completing a similar study using MUC1-CAR-pNK cells, placing the CAR construct in placental derived NK cells, placing the patient against NSCLC cells.

Table 5. Current CAR-T cell clinical trials for thoracic malignancies.

| Target Antigen | Indication | Phase | Location | Status | NCT# |
|----------------|------------|-------|----------|--------|------|
| CEA            | Lung, Colorectal, Gastric, Breast, Pancreatic | I     | China    | Recruiting | NCT02349724 |
| EGFR           | Cholangiocarcinoma, Colorectal, NSCLC, Ovarian, Pancreatic, Renal | I/II  | China    | Recruiting | NCT01869166 |
| FAP            | Mesothelioma | I     | Zurich   | Recruiting | NCT01722149 |
| GD2            | Solid tumors | I/II  | China    | Recruiting | NCT02992210 |
| GPC3           | Hepatocellular Carcinoma, Squamous Cell Lung | I     | China    | Recruiting | NCT03198546 |
| HER2           | Breast, Ovarian, Lung, Gastric, Colorectal, Glcoma, Pancreatic | I/II  | China    | Recruiting | NCT02713984 |
| MSLN           | Lung Adenocarcinoma, Ovarian, Peritoneal Carcinoma, Mesothelomas | I     | UPENN    | Active, not recruiting | NCT03054298 |
| MSLN           | Cervical, Pancreatic, Ovarian, Mesothelioma, Lung | I/II  | NCI      | Recruiting | NCT01583686 |
| MSLN           | Breast, Lung, Malignant Pleural Disease, Mesothelioma, Metastases | I     | MSKCC    | Recruiting | NCT02414269 |
| MSLN           | Breast, Endometrial, Mesothelioma, Ovarian, Pancreatic | I     | China    | Recruiting | NCT02580747 |
| MSLN           | Mesothelioma, Pancreatic, Ovaria, Metastatic | I     | UPENN    | Complete  | NCT02159716 |
| MUC1           | Lung Neoplasms Malignant, NSCLC | I/II  | China    | Recruiting | NCT03525782 |
| MUC1           | Hepatocellular Carcinoma, NSCLC, Pancreatic, Triple-Negative Invasive Breast Carcinoma, Malignant Gloma, Colorectal, Gastric | I/II  | China    | Recruiting | NCT02587689 |
| MUC1           | Hepatocellular Carcinoma, NSCLC, Pancreatic Carcinoma Triple-Negative Invasive Breast Carcinoma, Malignant Gloma of Brain, Colorectal, Gastric | I/II  | China    | Recruiting | NCT02839954 |
| PD1            | Gastric, Lung, Liver | I     | China    | Recruiting | NCT02862028 |
| PD1            | NSCLC | I     | China    | Not yet recruiting | NCT03330834 |
| PSMA/MUC1/CD80/86 | Lung | I     | China    | Recruiting | NCT03198052 |
| ROI1           | Breast (including triple negative), Leukemias (ALL, CLL, mantle cell), NSCLC | I     | NCI      | Recruiting | NCT02706392 |
| VEGF Receptor 2 | Melanoma, Renal, Metastatic | I/II  | NCI      | Complete  | NCT01218687 |

Key: Carcinoembryonic Antigen (CEA), Epidermal Growth Factor (EGFR), Fibroblast Activation Protein (FAP), Ganglioside (GD2), Glypican-3 (GPC3), Human Epidermal Growth Factor 2 (HER2), Mesothelin (MSLN), Transmembrane Glycoprotein Mucin 1 (MUC1), Program Cell Death Protein 1 (PD1), Programmed Cell Death Ligand 1 (PD1), Prostate Stem Cell Antigen (PSCA), Vascular Endothelial Growth Factor (VEGF).
increased expression of PD-1 in the tumor microenvironment, another group at the First Affiliated Hospital of Guangdong Pharmaceutical University is testing MUC-1 CAR-T cells with PD-1 knockout in a randomized Phase I/II study of NSCLC (NCT03525782). There are three trials currently targeting immunomodulatory antigens in NSCLC, including PD-1 (NCT02862028), PD-L1 (NCT03330834), and a combination of PSCA, MUC1, PD-L1 or CD80/CD86 (NCT03198052). No published preclinical data exists of CAR-T cells that target PD-L1 or CD80/CD86. Since PD-1, PD-L1, and CD80/86 are expressed on normal immune cells, the possible off-tumor toxicities need to be carefully considered and reviewed.

FAP is an integral membrane gelatinase that controls fibroblast growth and epithelial-mesenchymal interactions that is activated in fibroblasts in 90% of epithelial cancers, including MM. The University of Zurich is recruiting MM MPE patients for a phase 1 single dose FAP CAR-T cell (NCT01722149) therapy. Local administration of anti-FAP (scFv F19) CAR-T cells, with CD28, CD28, and 4-1BB costimulatory domains, in combination with PD-1 inhibition provided transient tumor control and improved survival in a humanized mouse model of MM and also provided 1 year stable disease in a first-in-man clinical trial in MM with MPE. Given limited therapeutic responses to checkpoint blockade in MM, further clinical evaluation of combinations of FAP-specific CAR-T cells and checkpoint blockade is warranted to improve the T-cell repertoire to generate a therapeutic immune response.

Given the risk of normal tissue toxicity associated with CAR-T therapy targeting less restricted TSAs, new strategies to minimize CAR-T cytotoxicity are needed. Early approaches included use of kill switches that induce CAR-T cell apoptosis in case of severe toxicity. Unfortunately, these cell-suicide systems are irreversible and do not control T cell activation or expansion. A switch-controlled approach was recently developed to enable better control of reactivity and safety of CAR-T therapy for NSCLC. CAR-T cells targeting fluorescein isothiocyanate (FITC), were generated in conjunction with an intermediate antigen switch composed of folate bound to FITC, thereby binding the folate receptor α (FRα) chain, a cell surface protein that is expressed in over 70% of lung adenocarcinomas. This established a pseudoimmunological synapse with FITC-CAR T cells and cells expressing either FRα or FRβ, which is also highly expressed on TAMs in the tumor microenvironment. Potent antigen-specific and dose-dependent in vitro efficacy was demonstrated against NSCLC and macrophage cell lines. Further preclinical testing is required to determine the efficacy targeting both the tumor and tumor microenvironment in NSCLC. These bifunctional switches enable greater control of CAR-T cell antigen specificity and activity, which will help to greatly improve patient safety profiles during ACT treatment.

Conclusion

MPEs represent a unique and understudied tumor microenvironment. Current treatment frequently involves palliative drainage of effusions, providing the opportunity to longitudinally access the evolution of neoplastic cells and the subsequent dynamics of innate and adaptive immunity in response to the disease. Nevertheless, even though immune cells are identified, host immunity has failed to contain malignancy. Given the urgency in this population with a short-expected survival time, only a small temporal window is available for gaining and sustaining therapeutic benefit. Further examination of the microenvironment of MPEs may provide insight into the mechanisms of tumor-mediated immunosuppression, and specifically, how these events change over time. As highlighted above, advances in our understanding of tumor immunology in conjunction with technological innovations have yielded novel immune-based treatments resulting in unprecedented clinical success, particularly in hematopoietic malignancies, melanoma, and lung cancer. Serial evaluation of response to CAR-T therapy, novel application alone or in combination with immunomodulators such as IL-2 and IL-15, employing factors to repolarize immunosuppressive leukocytes, oncolytic virotherapy, and T cell checkpoint inhibitors, alone or in rationally designed combinations may identify correlates of effective anti-tumor immunity and subsequent tumor immune escape mechanisms. This is remarkably possible in the setting of MPEs where serial evaluation of both the tumor and immune cells are realized. Notably, repeat evaluation of MPEs during immunotherapy may identify clinically actionable targets present within the tumor, immune, and/or stromal compartments, allowing for the development of more effective patient-specific treatments. Evaluation of locoregional IL-2 alone or with cisplatin installation in conjunction with checkpoint inhibitors would seem to be an early interesting and useful strategy.

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The authors declare no conflict of interests.

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