New and emerging therapeutic options for malignant pleural mesothelioma: review of early clinical trials

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Abstract: Malignant pleural mesothelioma (MPM) is a rare tumor that is challenging to control. Despite some benefit from using the multimodality-approach (surgery, combination chemotherapy and radiation), survival remains poor. However, current research produced a list of potential therapies. Here, we summarize significant new preclinical and early clinical developments in treatment of MPM, which include mesothelin specific antibody and toxin therapies, interleukin-4 (IL-4) receptor toxins, dendritic cell vaccines, immune checkpoint inhibitors, and gene-based therapies. In addition, several local modalities such as photodynamic therapy, postoperative lavage using betadine, and cryotherapy for local recurrence, have also shown to be effective for local control of disease.

Keywords: MPM, new targeted, systemic, local therapies

Introduction
Malignant pleural mesothelioma (MPM) is a rare asbestos-induced malignancy with an estimated incidence of approximately 2,180 new cases diagnosed in the United States in 2013. Approximately 30% of US cases are diagnosed among veterans, and 20% are seen in women. Worldwide, nearly 80% of mesothelioma deaths occur in ten countries, with the United Kingdom, United States, and Japan being in the top three. While peak incidence of MPM in the US has been reached, worldwide it is expected to continue to increase over the next several decades. Median survival ranges from 9–18 months and correlates with stage.

Currently established therapy
Chemotherapy
Pemetrexed and cisplatin combination therapy was established as a standard treatment for mesothelioma patients who are not surgical candidates after a landmark multicenter randomized Phase III trial of 456 patients. The trial demonstrated a nearly 3-month survival benefit, with median survival of 12.1 months versus 9.3 months for patients treated with cisplatin alone. Tumor response was seen in 41.3% of the 226 pemetrexed and cisplatin treated patients and 16.7% of the 222 patients receiving cisplatin alone.

Surgical resection
The theoretical goal of surgical resection is to achieve complete tumor removal (R0 resection), which in reality is virtually impossible. A more realistic goal is to achieve an R1 resection with only microscopic residual disease.

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While the Mesothelioma And Radical Surgery (MARS) feasibility trial showed that few patients qualified for surgery and the outcomes did not support extrapleural pneumonectomy (EPP), surgery is offered to select patients in high volume centers with specific interest in mesothelioma, and it may offer a survival advantage. EPP was formerly thought to produce better survival than pleurectomy and decortication (P/D) but as more evidence accumulates P/D actually may offer a better survival. Indirect evidence supporting this comes from work by Flores et al, who observed overall survival of only 10.2 months in patients who did not undergo any surgery and 14.0–15.8 months in those who underwent resection.

Radiation

Radiation has been used for either gross tumor (palliative intent) or for adjuvant local control in the postoperative setting in an attempt to control the residual microscopic disease that is nearly universally present following any surgical resection. The ability to administer effective doses of radiation to the large surface area of the pleural space, particularly following P/D with the lung in place, is quite challenging. Consequently, the broadest application of postoperative radiation has been in patients following EPP since the ipsilateral lung is removed. However, the risk for toxicity to the adjacent organs (heart, spinal cord and liver, esophagus) remains. Despite the theoretically lower risk of pulmonary toxicity, radiation following EPP was associated with a nearly 20% incidence of severe pneumonitis in the contralateral lung in at least one study.

Several groups have described use of radiation in patients after ipsilateral lung preservation with P/D with an acceptable toxicity profile (summarized in Table 1). Thus newer techniques, such as tomotherapy, which are able to deliver a circumferential focused radiation field closely following the contour of the chest wall and lung periphery, potentially limiting toxicity to the lung parenchyma, allow postoperative radiation doses after P/D to be nearly the same as after EPP: 45–50 centigray.

The role of preoperative radiation was evaluated in the Surgery for Mesothelioma After Radiation Therapy (“SMART”) trial, which was a feasibility study only. Five fractions of radiation were given to the hemithorax 1 week prior to EPP, with no acute pulmonary toxicities noted in 26 patients but with half the patients developing postoperative complications. Although survival data was reported and an enhanced immune response speculated, more studies are needed before any conclusions can be made regarding impact.

Multimodality therapy

Currently, specialized mesothelioma centers employ multimodality approaches, including surgical resection, chemotherapy, and radiation, with survival in excess of 20 months depending on stage. Reports from the Society of Thoracic Surgeons Database and the European Organisation for Research and Treatment of Cancer strongly suggest that multimodality therapy in highly specialized centers is associated with less morbidity and mortality.

Hyperthermic intraoperative chemotherapy

Experimental data suggests that heating chemotherapeutic agents increases entry into tumor cells. Clinically, most data come from Phase I–II studies evaluating systemic toxicity of intrapleural drug (most commonly cisplatin) administration in highly selected patients. Median survival of patients

Table 1 Studies evaluating toxicity of radiation in intact lung

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Radiation dose</th>
<th>Pulmonary toxicity</th>
<th>Median survival, months</th>
<th>Overall survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minatel et al11,12</td>
<td>28a</td>
<td>5,000 cGy in 25 fractions</td>
<td>17.8%: Grade 2 in 3 patients; Grade 3 in 2 patients</td>
<td>33</td>
<td>2 years – 70%; 3 years – 49%</td>
</tr>
<tr>
<td>Rosenzweig et al13</td>
<td>36a</td>
<td>4,680 cGy (range 4,140–145,040 cGy)</td>
<td>20%: Grade 3 in 5 patients; Grade 4–5 in 2 patients</td>
<td>26</td>
<td>1 year – 75%; 2 years – 53%</td>
</tr>
<tr>
<td>Bolukbas et al14</td>
<td>29a</td>
<td>5,040 cGy</td>
<td>No report of radiation toxicity specifically; note that it was well tolerated</td>
<td>30</td>
<td>1 year – 69%; 2 years – 50%; 3 years – 31%</td>
</tr>
<tr>
<td>Cho et al15</td>
<td>25</td>
<td>2,500 cGy in 5 fractions + 500 cGy boost</td>
<td>None at 1 week when patients underwent EPP</td>
<td>NR</td>
<td>3 years – 58%</td>
</tr>
</tbody>
</table>

Notes: *Twenty patients underwent pleurectomy/decortication and eight patients had biopsy only prior to radiation. Survival is reported for 20 patients who underwent pleurectomy/decortication and radiation. **Twenty patients underwent pleurectomy and decortication and 16 patients had no surgery prior to radiation. *Only five patients underwent 5,040 cGy of radiation; 29 additional patients received 2,100 cGy in three fractions to the incision and chest tube sites.

Abbreviations: cGy, centigray; EPP, extrapleural pneumonectomy; N, number of patients; NR, not reported.
treated with this methodology ranges from 9–20 months and there may be a trend toward prolonged disease-free intervals in those treated with higher doses. A retrospective review by Sugarbaker et al. reported a longer survival and progression free interval in early-stage patients treated with hyperthermic intraoperative chemotherapy. However, lack of randomized trials raises a question whether this method represents significant benefit.

New and future therapies

Successful treatment of mesothelioma will depend on improved understanding of the biology of mesothelioma. Clinicaltrials.gov listed 192 registered trials (Phases I–III) worldwide evaluating multiple therapeutic approaches in a variety of settings. A large number of these trials focus on novel agents, which have arisen primarily from our expanded knowledge of molecular signaling and immune response. Several classes of targeted therapies have emerged from preclinical work and are being evaluated. These focus on following broad mechanisms:

- Tyrosine kinase inhibitors
- Antibody conjugated toxins
- Immune checkpoint inhibitors
- Gene therapy
- Tumor vaccines.

Tyrosine kinase inhibitors

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR) is expressed by a variety of epithelial malignancies, and activation of the pathway interferes with apoptosis, uncontrolled cell proliferation, and angiogenesis. EGFR overexpression in mesothelioma samples was reported by several authors, and inhibition of EGFR-dependent signaling pathway in mesothelioma cell lines also leads to decreased cell survival.

Several clinical trials based on these findings have been conducted but, disappointingly, did not show improved survival (summarized in Table 2). Furthermore, the level of EGFR overexpression did not correlate with clinical outcomes. Additional data showed that mutations found in patients with other cancers may not be the same in malignant mesothelioma tumors, or alternatively the frequency of mutation may be too low in mesothelioma patients, resulting in the lack of clinical response in non-selected patients.

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF) is produced by a variety of tumors, including pleural mesothelioma, and stimulates neovascularization of tumors in addition to normal angiogenesis. Elevated levels of VEGF and its receptor have been detected by immunohistochemistry in the tissue specimens of patients with mesothelioma and as free circulating molecules. Higher levels may be reflective of more-advanced disease and were associated with shorter survival in both studies. In vitro studies demonstrated that increased mesothelioma cell proliferation occurred when treated with VEGF and that significant inhibition of cell growth occurred when this pathway was blocked. As a result, interference with this pathway potentially could lead to successful therapy.

VEGF antibody binds the receptor and inhibits its activation. Bevacizumab has been approved to treat advanced colorectal, renal cell, and gastrointestinal stromal cancers. Numerous clinical trials (Table 3) evaluated the effect of VEGF inhibitors alone and in combination with chemotherapy in MPM. Unfortunately, the results of these trials have been disappointing. Overall survival ranged from 4–15 months and this difference was likely due to the highly variable design of individual trials. For instance, several trials required failure of first-line therapy

<table>
<thead>
<tr>
<th>Agent/author</th>
<th>Study design</th>
<th>N</th>
<th>Median survival (months)</th>
<th>Overall 1-year survival</th>
<th>Response and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gefitinib</td>
<td>Phase II</td>
<td>43</td>
<td>6.8a</td>
<td>32%</td>
<td>I complete response; I partial response; EGFR expression does not predict response</td>
</tr>
<tr>
<td></td>
<td>No prior systemic chemo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erlotinib</td>
<td>Phase II</td>
<td>63</td>
<td>10</td>
<td>43%</td>
<td>None had response; no correlation between EGFR expression and response</td>
</tr>
<tr>
<td></td>
<td>No prior systemic chemo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erlotinib and bevacizumab</td>
<td>Phase II</td>
<td>24</td>
<td>5.8</td>
<td>24%</td>
<td>No responses observed; 12 with stable disease</td>
</tr>
<tr>
<td></td>
<td>Prior systemic chemo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Note:** In this study, survival varied from 8.1 months in patients with high EGFR levels and 3.6 months in patients with low EGFR expression.

**Abbreviations:** EGFR, epidermal growth factor receptor; N, number of patients.
Table 3 Summary of published clinical trials evaluating blockade of VEGF

<table>
<thead>
<tr>
<th>Agent/author</th>
<th>Study design</th>
<th>N</th>
<th>Regimen</th>
<th>Median survival (months)</th>
<th>Overall 1-year survival</th>
<th>Study conclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab/Ceresoli et al</td>
<td>Phase II, No prior treatment</td>
<td>76</td>
<td>Carbo + pem and bevacizumab</td>
<td>15.3</td>
<td>62.6%</td>
<td>26 partial responses; Serum VEGF levels did not correlate with survival; OS was increased but predetermined end points were not reached</td>
</tr>
<tr>
<td>Sorafenib/Papa et al</td>
<td>Phase II, Prior platinum + pem chemo</td>
<td>53</td>
<td>Sorafenib</td>
<td>9</td>
<td>NR</td>
<td>3 partial responses, PFS 36%</td>
</tr>
<tr>
<td>Bevacizumab/Dowell et al</td>
<td>Phase II, No prior treatment</td>
<td>52</td>
<td>Cis + pem and bevacizumab</td>
<td>14.8</td>
<td>NR</td>
<td>40% with partial response; Failed to demonstrate improved survival</td>
</tr>
<tr>
<td>Bevacizumab/Kindler et al</td>
<td>Phase II, double blind, randomized, No prior treatment</td>
<td>55</td>
<td>Cis + gem</td>
<td>15.6</td>
<td>58.6%</td>
<td>Partial response in 24.5% and 21.8% of groups, respectively</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Cis + gem + bevacizumab</td>
<td>14.7</td>
<td>57.0%</td>
<td>No significant differences between the groups; Survival correlated with VEGF plasma levels</td>
</tr>
<tr>
<td>Vatalanib/Jahan et al</td>
<td>Phase II, No prior treatment</td>
<td>47</td>
<td>Vatalanib</td>
<td>10</td>
<td>44.7%</td>
<td>3 partial responses; No correlation between serum VEGF levels and response; No further studies are warranted using this as a single agent</td>
</tr>
<tr>
<td>Dasatinib/Dudek et al</td>
<td>Phase II, Prior pem chemo regimen</td>
<td>43</td>
<td>Dasatinib</td>
<td>26.1 weeks (-6 months)</td>
<td>25.6%</td>
<td>No complete response; 2 (4%) partial responses did not meet criteria; not effective</td>
</tr>
<tr>
<td>Cediranib (AZD2171)/Campbell et al</td>
<td>Phase II, Chemo-naive and prior chemo</td>
<td>50</td>
<td>Cediranib</td>
<td>4.4</td>
<td>15%</td>
<td>5 partial responses; Chemo-naive patients had longer overall survival; high toxicity noted</td>
</tr>
<tr>
<td>Sunitinib/Nowak et al</td>
<td>Phase II, With or without prior systemic cis + pem</td>
<td>53</td>
<td>Sunitinib</td>
<td>6.1 (19 months since diagnosis of mesothelioma)</td>
<td>NR</td>
<td>6 partial responses; Some correlation between VEGF, mesothelin levels and response</td>
</tr>
<tr>
<td>Sunitinib/Laurie et al</td>
<td>Phase II</td>
<td>17</td>
<td>Prior chemo + sunitinib</td>
<td>8.3</td>
<td>NR</td>
<td>1 partial response; Not effective; no further studies are warranted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sunitinib without prior chemo</td>
<td>6.7</td>
<td>NR</td>
<td>4 partial responses</td>
</tr>
<tr>
<td>Cediranib/Garland et al</td>
<td>Phase II, Prior platinum chemo</td>
<td>47</td>
<td>Cediranib</td>
<td>9.5</td>
<td>36%</td>
<td></td>
</tr>
<tr>
<td>Sorafenib/Dubey et al</td>
<td>Phase II, Chemo-naive and prior chemo</td>
<td>50</td>
<td>Sorafenib</td>
<td>13.2 (prior chemo)</td>
<td>57%</td>
<td>3 partial responses; ERK1/2 levels did not correlate with response to agent; lower ERK1/2 levels correlated with improved overall survival; No further studies warranted</td>
</tr>
<tr>
<td>Enzastaurin/Mukohara et al</td>
<td>Phase I, Prior therapy</td>
<td>19</td>
<td>Enzastaurin</td>
<td>NR</td>
<td>NR</td>
<td>1 mesothelioma patient had radiologic improvement</td>
</tr>
</tbody>
</table>
before initiation of VEGF antibody treatment, while other trials allowed it to be given as first-line therapy. The only study\(^{35}\) with a truly prospective randomized allocation to either standard chemotherapy (cisplatin and pemetrexed) or standard chemotherapy with addition of bevacizumab showed no difference in disease progression or overall survival.

Toxicities of TKIs were usually well tolerated, and only one trial concluded that it was excessive and the agent should not be used.\(^{36}\) Table 4 summarizes ongoing trials, but results have been uniformly disappointing and it is unclear whether these agents will assume significant clinical roles.

**Additional molecular targets**

In addition to a large number of kinase inhibitors, several other specific molecular agents are being investigated. Some of these agents influence a common pathway downstream of the EGFR pathway, while other exert action via different mechanisms. For example, histone deacetylase inhibitors (belinostat and vorinostat) exert their action through modification of histones, thus controlling gene transcription.\(^{37}\)

A clinical trial evaluating belinostat in 13 patients was not promising, having found belinostat to be ineffective as a single second-line regimen in patients with MPM.\(^{38}\) As a result, a planned trial of vorinostat combined with chemotherapy has been halted.

Bortezomib is a selective inhibitor that acts via downregulation of nuclear factor-κB and promotes apoptosis. Despite having favorable preclinical results, bortezomib was associated with significant toxicity and lack of expected response in early clinical trials.\(^{39}\) Additional investigational strategies evaluated the role of arginine depletion, effect of everolimus, role of HSP90 inhibitors, ranpirnase, and more. These active trials are summarized in Table 5.

**Therapy targeting cell-surface receptors**

**Mesothelin**

Mesothelin is a 40 kDa cell-surface differentiation glycosylphosphatidylinositol-anchored glycoprotein present on normal mesothelial cells and overexpressed on the surface of mesothelioma as well as ovarian and pancreatic adenocarcinoma cells. It is shed into pleural fluid and released into the serum in 71% of mesothelioma, 67% of ovarian, and nearly all pancreatic cancer patients, but also in normal volunteers.\(^{40,41}\) Mesothelin overexpression, occurring more prominently on epithelioid tumors, may serve to alter cell adhesion and/or invasion.\(^{42}\)
Antimesothelin antibodies
MORAb-009 (amatuximab) is a chimeric IgG1 kappa murine monoclonal antibody with high affinity for human mesothelin. Following receptor binding, this highly specific antibody is rapidly internalized and induces antibody-dependent cellular cytotoxicity and inhibits cellular adhesions via interaction with MUC16 in a receptor density-dependent manner. Preclinical studies in nude mice suggested that MORAb-009 combined with chemotherapy (gemcitabine or paclitaxel) was more effective than either chemotherapy agent alone. An initial Phase I clinical trial demonstrated that MORAb-009 is well tolerated, with a maximum tolerated dose of 200 mg/m², and that eleven of 24 patients exhibited stable disease. A Phase II clinical trial evaluating the combination of MORAb-009 with cisplatin and pemetrexed in patients with pleural mesothelioma has completed recruitment but results have not been published.

BAY 94-9343 (anetuman ravtansine) is a fully human antimesothelin antibody coupled via a reducible disulfide linker to DM4, a microtubule-targeting toxophore that shows highly selective cytotoxicity against cells with high levels of mesothelin expression with an additional potent bystander effect. Preclinical studies showed a dose-dependent and receptor-dependent 94% reduction of tumor growth with BAY 94-9343 compared to 70% with cisplatin and pemetrexed chemotherapy. This drug is now being evaluated in an ongoing Phase I trial (Clinicaltrials.gov NCT01439152).

CRS-207 vaccine
CRS-207 is a genetically modified *Listeria monocytogenes* attenuated vaccine expressing mesothelin. Mesothelin acts as an antigen and stimulates activation of T-cells upon exposure to CRS-207. A Phase I trial, including five mesothelioma patients, determined the maximum tolerated dose to be 1×109 colony-forming units with a favorable safety profile. Mesothelin-specific CD8+ T-cell response was induced in six out of ten evaluable subjects but did not correlate with clinical response. Currently, an ongoing Phase I trial (Clinicaltrials.gov NCT01675765) is evaluating *Listeria* vaccine in combination with chemotherapy in patients with MPM.

SS1P immunotoxin
SS1P is an immunotoxin consisting of an antimesothelin antibody variable fragment linked to a cytotoxic fragment of *Pseudomonas* exotoxin A. A Phase I trial including 16 patients with mesothelioma showed that SS1P was well tolerated up to 25 µg/kg/day × 10 days with modest clinical activity and minor responses, and that two mesothelioma patients had symptomatic improvement. Continuous infusion showed no advantage over bolus dosing. A significant number of patients developed neutralizing antibodies after one cycle and were not able to receive additional therapy. In a subsequent study, Hassan et al attempted to abrogate the production of neutralizing antibodies by inducing an immunosuppressive state with pentostatin and cyclophosphamide. Interestingly, three of ten patients achieved a partial response, but two patients (one with stable and one with progressive disease) experienced dramatic tumor reduction with subsequent chemotherapy. Durable responses were correlated with high serum SS1P levels following the second dose and with multiple doses of therapy. The median overall survival was 8.8 months with a median follow-up of 12.7 months. A Phase I trial (Clinicaltrials.gov NCT01445392) of SS1P infusion...
Depletion of extracellular arginine interferes with vascular endothelium

Table 5 Active and recruiting trials investigating specific molecular therapies in MPM

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target</th>
<th>Mechanism</th>
<th>Goals of study</th>
<th>Trial ID</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>Histone deacetylase inhibitor</td>
<td>Prevents gene transcription by maintaining chromatin condensed</td>
<td>Randomized Phase II: progression after first-line chemo</td>
<td>NCT01353482;</td>
<td>Merck</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase I–II: combination with chemo</td>
<td>withdrawn</td>
<td></td>
</tr>
<tr>
<td>Ganetespib</td>
<td>HSP90 inhibitor</td>
<td></td>
<td>Phase II: bortezomib and oxaliplatin after failure of chemo</td>
<td>NCT00996385</td>
<td>University College, London/UK</td>
</tr>
<tr>
<td>Bortezomib</td>
<td>Proteasome inhibitor</td>
<td></td>
<td>Phase II: effect of ADi-Peg 20 alone</td>
<td>NCT01279967</td>
<td>Columbia University/USA</td>
</tr>
<tr>
<td>ADI-Peg20</td>
<td>(arginine deiminase)</td>
<td>Depletion of extracellular arginine interferes with tumor metabolism while tumor cells frequently down regulate ability to synthesize arginine</td>
<td>Phase I: ADI-Peg20 combination with chemo</td>
<td>NCT02029690</td>
<td>Barts and The London NHS Trust Polaris</td>
</tr>
<tr>
<td>Everolimus</td>
<td>Target of rapamycin</td>
<td>Independently interferes with distal steps of EGFR pathway</td>
<td>Phase II: single drug</td>
<td>NCT00770120</td>
<td>NCI</td>
</tr>
<tr>
<td>Onconase/ranipamase</td>
<td>Inhibits NF-κB pathway</td>
<td></td>
<td>Phase III: doxorubicin +/- onconase</td>
<td>NCT00003034;</td>
<td>NCI</td>
</tr>
<tr>
<td>NGR-TNF</td>
<td>Vascular endothelium</td>
<td>Recombinant TNF fused with a peptide recognizing endothelial cell-surface receptors</td>
<td>Phase II: intravenous TNF given</td>
<td>NCT01358084</td>
<td>MolMed SpA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Phase III: together with second-line chemo</td>
<td>NCT01098266</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: chemo, chemotherapy; EGFR, epidermal growth factor receptor; ID, identification number; MPM, malignant pleural mesothelioma; NCI, National Cancer Institute; NF-κB, nuclear factor kappa B; TNF, tumor necrosis factor.

Interleukin-4 receptor

IL-4 acts as a growth factor for T helper 2 cells and induces immunoglobulin class switching in allergic responses. Several studies showed that in addition to some subsets of immune cells, high affinity IL-4 receptors also are present on a variety of human tumors including mesothelioma. Clinically, high levels of IL-4 receptor expression have been shown on a variety of human tumors including mesothelioma. IL-4 receptor expression levels were noted in biopsies of sarcomatous histology specimens, which have a significantly worse prognosis compared to those with a more typical mesotheliomatous histology. These IL-4 receptors, therefore, represent potential clinical targets.

Cytotoxic T lymphocyte antigen-4

The CTLA-4 (CTLA-4) is vital for maintaining host immune tolerance by antigen-presenting cells, thus raising the activation threshold for T lymphocytes. Systemic administration of a circularly permuted recombinant CTLA-4 receptor (CTLA-4Ig) has been shown to significantly prolong the survival of mice in a murine pancreatic model. Moreover, systemic administration of an agonistic anti-CTLA-4 antibody (Basiliximab) has been shown to significantly enhance the antitumor efficacy of IL-2 therapy in a murine melanoma model.

Immune checkpoint inhibitors

CTLA-4 and PD-1 (programmed death-1) are two immune checkpoints that dampen inflammatory responses and mediate immune tolerance toward normal tissue. Because most immune checkpoints are initially blocked by ligand-receptor interactions or recombining forms of CTLA-4 and PD-1, they can be readily blocked by antagonist antibodies or recombining forms of CTLA-4 and PD-1. For example, the CTLA-4 receptor sequesters cognate ligands/receptors. Cytotoxic T lymphocyte antigen-4 (CTLA-4) is a member of the immunoglobulin superfamily and is expressed on the surface of T lymphocytes. It is a negative regulator of T lymphocyte function and is a potent inhibitor of the T cell receptor (TCR) signaling pathway.

The CTLA-4 receptor is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. The CTLA-4 receptor is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is also expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway.

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The CTLA-4 receptor is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway. It is expressed on the surface of T lymphocytes and is a potent inhibitor of the T cell receptor (TCR) signaling pathway.
CTLA-4 blocking antibody as monotherapy or combined with therapeutic tumor-cell vaccination induced regression of established melanoma and colon tumors in mice.\textsuperscript{57,58}

A Phase II trial evaluating anti-CTLA-4 antibody (tremelimumab) in 29 patients with chemotherapy-resistant advanced mesothelioma (28 pleural and 1 peritoneal) was recently reported by Calabrò et al.\textsuperscript{59} Objective clinical responses were observed in only two of 29 patients. However, disease stabilization was noted in nine patients (31%), all with epitheliod histology. Overall survival rates were 48% at 1 year and 37% at 2 years. Currently, there are two active clinical trials investigating the administration of tremelimumab in patients with pleural mesothelioma (ClinicalTrials.gov NCT01655888 and NCT01843374).

Programmed death receptor-1
Programmed death receptor is found on the surface of T-cells and its stimulation leads to T-cell deactivation, thus allowing escape from the immune system surveillance in the presence of otherwise antigenic substrate.\textsuperscript{60} Activation of this receptor occurs by a programmed death ligand 1 (PD-L1), which exists within the tumor microenvironment on the surface of tumor cells.\textsuperscript{61}

Currie et al.\textsuperscript{62} demonstrated PD-L1 to be present on murine mesothelioma cells in vivo. Interestingly, upregulation of PD-L1 expression occurred in response to increased concentrations of interferon (IFN)-γ and T-cells in tumor draining lymph nodes, supporting the hypothesis that this is an important pathway of tumor-mediated local immunosuppression.\textsuperscript{60} The effect of PD-L1 blockade on different subpopulations of T-cells produced opposing effects on tumor progression and suggested that tumor-derived immune suppression is mediated by specific subsets of T-cells. Mansfield et al.\textsuperscript{63} noted that PD-L1 expression occurred in approximately 40% of 106 mesothelioma specimens (all sarcomatoid tumors) and higher expression was correlated with worse prognosis (5.0 months versus 14.5 months).

Several trials are currently evaluating role of inhibition in this pathway using different agents (lambrolizumab and nivolumab) in cancers other than MPM.

Gene therapy
Multiple genetic abnormalities have been identified in mesothelioma, and a variety of genetic manipulation strategies have been employed in preclinical studies.\textsuperscript{63} Several types of gene therapies have shown particular promise and are discussed below.

Suicide gene therapy
This approach utilizes engineered viruses that deliver transgenes encoding enzymes that metabolize prodrugs into toxic metabolites capable of killing tumor cells. Multiple viral vectors have been studied. A clinical trial of intrapleural Adenovirus herpes simplex thymidine kinase/ganciclovir\textsuperscript{64} enrolled 34 patients and reported minimal morbidity and a dose-dependent median survival as high as 15 months at the highest viral titers. Some patients experienced prolonged survival, suggesting induction of antitumor immunity in addition to the acute viral-mediated cytotoxicity.\textsuperscript{64}

Cytokine gene therapy
Another strategy involves administration of viral vectors encoding specific cytokine gene(s) that may exert a direct cytotoxic effect on tumor cells or may alter the immunologic response(s) to the tumor. Although early trials of direct intrapleural administration of interleukin-2 (IL-2) showed a nearly 50% response rate and a 28-month median survival in responders,\textsuperscript{65} subsequent interest has centered on gene therapy with IFN, which play a key role in activation of the immune system and have direct antitumor cytotoxic/cytostatic effects. Several clinical trials (summarized in Table 6) evaluated

<table>
<thead>
<tr>
<th>Study</th>
<th>Type of therapy</th>
<th>N</th>
<th>Survival, months</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterman et al\textsuperscript{66}</td>
<td>Intrapleural adenovirus</td>
<td>34:</td>
<td>MS – 15</td>
<td>Neutralizing antibody developed</td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{66}</td>
<td>Herpes simplex suicide gene</td>
<td>21 high dose</td>
<td>MS – 10</td>
<td>Suggests that response may be due to immunologic stimulation by tumor antigens</td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{66}</td>
<td>1 dose</td>
<td>13 low dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{66}</td>
<td>Intrapleural adenoviral vector with IFNβ gene \times 1 dose</td>
<td>8</td>
<td>NR</td>
<td>Activation of NK cells and increase in levels of anti-mesothelin antibody in some patients</td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{67}</td>
<td>2 doses</td>
<td>10</td>
<td>OS &gt; 18 months\textsuperscript{a}</td>
<td>Neutralizing antibody production noted with lower subsequent pleural IFN levels</td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{68}</td>
<td>Intrapleural adenoviral vector with IFNα2b gene \times 2 doses</td>
<td>9</td>
<td>NR</td>
<td>Strong activation of NK cells</td>
</tr>
<tr>
<td>Sterman et al\textsuperscript{68}</td>
<td>(OS ranged from 1–22 months with 3 patients alive)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: \textsuperscript{a}Includes seven additional patients with malignant effusions from ovarian, breast, or lung carcinomas.

Abbreviations: IFN, interferon; MPM, malignant pleural mesothelioma; MS, median survival; N, number of patients; NK, natural killer; NR, not reported; OS, overall survival.
Table 7 Current investigations in gene therapy

<table>
<thead>
<tr>
<th>Gene therapies</th>
<th>Agent</th>
<th>Route of administration</th>
<th>Status</th>
<th>Study ID</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytokine-based</td>
<td>Interferon</td>
<td>Intrapleural</td>
<td>Recruiting</td>
<td>NCT01212367</td>
<td>University of Pennsylvania/NCI</td>
</tr>
<tr>
<td></td>
<td>Interferon with chemotherapy</td>
<td>Intrapleural</td>
<td>Unknown</td>
<td>NCT01119664</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td>Suicide gene</td>
<td>Herpes simplex virus</td>
<td>Intrapleural</td>
<td>Recruiting</td>
<td>NCT01721018</td>
<td>Virtu Biologics Limited</td>
</tr>
<tr>
<td></td>
<td>Measles virus</td>
<td>Intrapleural</td>
<td>Recruiting</td>
<td>NCT01503177</td>
<td>Mayo Clinic/NCI</td>
</tr>
<tr>
<td>Autologous modified T-cells with receptor to:</td>
<td>Fibroblast activation protein</td>
<td>Intrapleural</td>
<td>Not yet recruiting</td>
<td>NCT01722149</td>
<td>University of Zurich</td>
</tr>
<tr>
<td></td>
<td>Mesothelin</td>
<td>Intravenous</td>
<td>Recruiting</td>
<td>NCT01355965</td>
<td>University of Pennsylvania</td>
</tr>
</tbody>
</table>

Abbreviations: ID, identification number; NCI, National Cancer Institute.

adenoviral-mediated IFN (α and β) therapy in patients with MPM. Survival ranged from 1–22 months with some long-term survival, but neutralizing antibodies did limit ability to administer repeated treatments.

Gene-modified lymphocytes

Genetically engineered autologous T lymphocytes may increase antigen recognition or alter the immunosuppressive tumor microenvironment through production of cytokines. Carpenito et al reported that antimesothelin-engineered T-cells mediated specific cytolysis of mesothelin-expressing cells and produced significant tumor regression in animals. Fibroblast activating protein and chemokine receptor-2 also have been evaluated in vitro as possible additional candidates for T-cell genetic modifications.

Currently ongoing clinical trials involving gene therapies are summarized in Table 7.

Immunotherapy and vaccines

Dendritic cell vaccines

Successful cancer immunotherapy requires effective antigen presentation. Antigen-exposed autologous dendritic cells remain the most potent antigen presenting cell. Preclinical in vitro and in vivo data supports the use of dendritic cell vaccines as a valuable strategy in mesothelioma. Calretinin, mesothelin, and Wilm’s tumor-1 have been used as candidate antigens, and measurable specific immune responses were shown to these antigens in early clinical trials, although no responses were seen. In a pilot Phase I study, Hegmans et al exposed autologous dendritic cells ex vivo to autologous tumor antigens purified from pleural effusions or biopsy samples. This vaccine strategy was well tolerated and produced three partial responses with overall median survival of 19 months, which is encouraging.

Currently, several manufactured mesothelioma vaccines are being evaluated in Phase I–II clinical trials and are summarized in Table 8.

Direct physical cytotoxic therapies

Photodynamic therapy

Photodynamic therapy (PDT) was originally investigated by Pass at the National Cancer Institute in the 1980s. PDT recently has been used by Friedberg et al following a meticulous P/D. In a Phase I–II experience, the median disease-free progression was 15 months and the overall survival was over 40 months. Due to this encouraging data, a Phase II trial (NCT NCT02153229) is ongoing and a randomized Phase III trial now is being planned, as is further investigation into the basic science of PDT (NCT02106559).

Table 8 Active trials investigating vaccine-based strategy

<table>
<thead>
<tr>
<th>Vaccine antigen</th>
<th>Additional therapies combined with vaccine</th>
<th>Trial ID</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT-1 (randomized)</td>
<td>GM-CSF</td>
<td>NCT01265433</td>
<td>Memorial Sloan-Kettering Cancer Center</td>
</tr>
<tr>
<td>WT-1</td>
<td>GM-CSF</td>
<td>NCT01890980</td>
<td>MD Anderson Cancer Center</td>
</tr>
<tr>
<td>ST4 tumor-associated antigen expressed by modified vaccinia virus (TroVax)</td>
<td>Chemotherapy</td>
<td>NCT01569919</td>
<td>Wales Cancer Trials</td>
</tr>
<tr>
<td>PA-1-STK compound – modified ovarian carcinoma vaccine</td>
<td>Cancyclovir</td>
<td>NCT00006216</td>
<td>University of Louisiana, NCI</td>
</tr>
<tr>
<td>Allogeneic tumor cell</td>
<td>Cyclophosphamide and celecoxib</td>
<td>NCT 01143545</td>
<td>NCI</td>
</tr>
<tr>
<td>Mesothelin (CRS-207)</td>
<td>Chemotherapy</td>
<td>NCT01675765</td>
<td>Aduro BioTech Inc</td>
</tr>
</tbody>
</table>

Note: Includes malignant mesothelioma, lung, esophageal, and thymic cancers.

Abbreviations: GM-CSF, granulocyte/macrophage colony-stimulating factor; ID, identification number; NCI, National Cancer Institute; WT-1, Wilm’s tumor-1.
Heated therapy
Sugarbaker has advocated in favor of intraoperative hyperthermic chemotherapy; however, evidence supporting the use of hyperthermia is quite limited. No controlled trials exist and a recent report by Cameron and Hou suggested that temperatures required for a clinically meaningful effect were 43°C–45°C, far above those currently used at most centers, and that hyperthermia and chemotherapy were additive and not synergistic.

Iodine–povidone (betadine) lavage
A Phase I–II trial of intraoperative hyperthermic (41°C) iodine–povidone lavage following surgery (either P/D or EPP) was recently reported by Lang-Lazdunski et al. Mechanism of action is likely generation of reactive oxygen intermediate species leading to cellular necrosis. Although the treatment was tolerated well and the surgical outcomes were acceptable, convincing evidence of efficacy is still lacking.

Cryotherapy
Cryotherapy for the treatment of cancer has been used for decades. Abtin et al recently reported the use of percutaneous cryoablation in 24 mesothelioma patients for control of limited recurrent local disease following P/D. The treatment was well tolerated, had a >90% control rate, and was associated with a median survival of 11.4 months following first therapy (36.1 months following surgery). Cryotherapy also may enhance immune responses through enhanced natural killer cell activity, T-cell responses and systemic IFN production. In an animal model of prostate cancer, tumor cryotherapy with simultaneous anti-CTLA-4 immunotherapy produced enhanced immune-mediated protection against tumor rechallenge at both primary and distant tumor sites.

Summary
MPM remains a challenging tumor to control. Despite some benefit from surgery and combination chemotherapy (cisplatin/pemetrexed), survival remains poor. However, the list of potential new therapies is long and the number of clinical trials is impressive (nearly 200). With all the ongoing research, progress is only a matter of time. Although the low prevalence of this disease makes enrollment in clinical trials challenging, more than 1,000 patients over the last decade have participated in the clinical trials covered by this review. In the future, it is critical that clinicians treat this disease with equipoise and that patients be placed in randomized prospective clinical trials in order to truly determine optimal therapy for these patients.

Disclosure
The authors report no conflicts of interest in this work.

References


