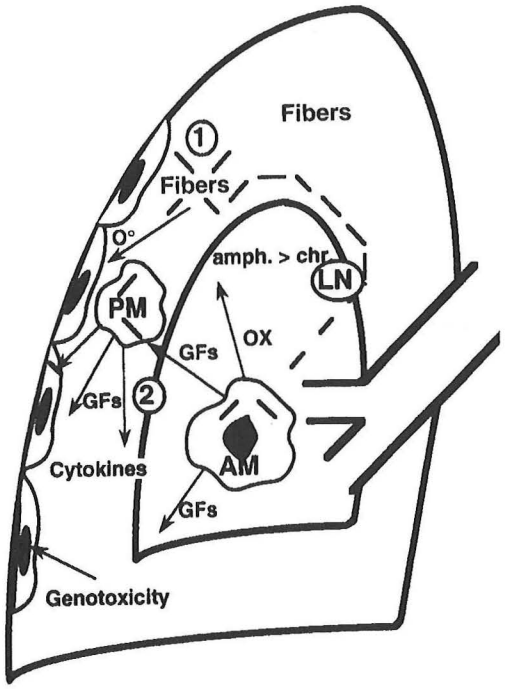
MALIGNANT PLEURAL MESOTHELIOMA



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HISTORY

In the first half of the twentieth century, malignant pleural mesothelioma (MPM) was considered to be a rare neoplasm, and some even debated its existence as a primary tumor (1). In 1931, Klemperer and Rabin acknowledged the controversy regarding the histiogenesis and classification of primary pleural tumors (2). The confusion was reflected in the multitude of names found in the literature for this tumor: endothelioma, endothelial carcinoma, lymphangiitis proliferans, sarcocarcinoma, and mesothelioma. Subsequently, the authors concluded that many of these were actually cases of tumor metastatic to the pleura, especially if there were tumor present in another viscera that could be considered the primary (2). There remained, however, cases of diffuse pleural neoplasms that completely encased the lung. Histologically, these tumors contained islands of epithelial cells, in addition to fibroblastic elements. Noting that much of the difficulty with classification had been due to too much emphasis on the histologic picture and a lack of appreciation of the origin of this tumor, Klemperer and Rabin recommended all diffuse neoplasms arising from the pleura be termed "mesotheliomas". This effectively established mesothelioma as a unique tumor entity.

The more familiar history of mesothelioma, however, did not begin until 1960 when Wagner et al published an important paper implicating asbestos exposure in the development of mesothelioma (3). They described a region of the Northwest Cape Province in South Africa covering about 8000 square miles where crocidolite (Cape Blue Asbestos) was mined. The earliest mining was done by families; men quarried the rocks and women and children 'cobbed' (separated) fiber from stone. With the approach of World War II, the demand for asbestos increased and much of the mining and processing was taken over by large companies. Wagner noted that while mesothelioma was rarely encountered in South Africa, in a period of just four years, he and his colleagues identified thirty-three cases of MPM. Further investigation revealed that twenty-three were born on asbestos fields, nine worked near a mine or mill, and only one had no history of asbestos exposure. While there had been case reports decades prior, this study provided the strongest evidence to date of a relationship between asbestos and mesothelioma. Subsequently, numerous case-control studies were published confirming these findings (4, 5, 6, 7, 8). In this review, we will address the changing epidemiology, etiologies, presentation and diagnosis, current treatments, and new directions in the therapy of MPM.

EPIDEMIOLOGY

Before the 1950s, MPMs were very uncommon tumors. In fact, in reviewing forty-seven thousand autopsies at the Massachusetts General Hospital from 1896-1917, no cases were found (9). Subsequently, about a hundred cases were identified between 1947 and 1990. The incidence has significantly increased and currently stands at about two to three thousand cases annually in the United States (10). These projections in terms of incidence rates are from the Surveillance, Epidemiology, and End Results (SEER) program, which

provides population based, tumor specific data on all histologically proven cancers in specific geographic regions. Only about 10% of the U.S. population is represented by these geographic sites. Since these sites do not specifically reflect the geography of asbestos use, and because mesothelioma is not identified as a separate tumor entity, the true incidence is difficult to determine. Moreover, it is associated with a latency of 30 to 45 years between first exposure and development of tumor. Prior to 1950 before the use of asbestos was widespread, the incidence of mesothelioma among males and females was about the same. SEER data now shows that age-specific rates climb most above 60 years of age and two-thirds of MPM are between 50 and 70 years of age, with the incidence two to six times higher in males (11, 12, 13). The age-adjusted rates for the period 1995-1999 for white males and females are 2.04 and 0.4 per hundred thousand, respectively (13).

Similar rising incidence rates have been noted around the world. Describing this as the "European Mesothelioma Epidemic", Peto et al analyzed epidemiologic data from France, the Netherlands, Germany, Italy, Switzerland and Britain (14). For the period 1995-2029, they projected that the number of men dying would double over the next twenty years, with about two hundred and fifty thousand deaths over the next thirty five years. Due to extensive use of asbestos in the past, Australia is similarly experiencing a rising incidence of MPM. Leigh et al analyzed the incidence rates between 1945 and 2000 (15). Between 1945 and 1979 there were just 658 cases. However, between 1980 and 2001, there were 6291 notifications to the National Mesothelioma Registry. The authors project the total number of cases to reach eighteen thousand by 2020. At 35.5 cases per million in 1997, this represented the highest rate in the world.

Epidemiologic studies have also shown occupational risks associated with MPM. In a case control study from McDonald et al, 344 males from across North America were examined. The greatest relative risk (RR) was associated with insulation work (RR = 46), and this was followed by employment in asbestos manufacture (RR = 6.1), heating trades (RR = 4.4), shipyards (RR = 2.8), and construction (RR = 2.0) (8). In a recent review from Jemal et al, lung cancer and mesothelioma rates were examined in 49 shipyard counties in the U.S. from 1970-1994 (16). While mortality was much lower for mesothelioma than lung cancer, rates were higher for both in counties where shipyards were major employers, compared to coastal nonshipyard counties. Similarly, in their review of mesothelioma in Britain, Peto et al found the highest proportional mortality to be among metal plate workers, which was a category included among shipyard workers (17). In recent years, more asbestos exposure may be occurring among "downstream" users rather than in direct asbestos manufacture, such as repair of asbestos-containing products (15, 18). The geographic variation in incidence rates generally parallels the locations of asbestos mining or asbestos-using industries (3, 16, 19, 20, 21). Most of the literature suggests that mesothelioma is becoming a more common malignancy. Taking the 30 to 40 year latency into account, the epidemiology and chronology has paralleled the use of asbestos in western countries, and the rising incidence is not expected to peak for 10 to 20 years.

ETIOLOGY

The etiology of MPM has historically been most closely linked to asbestos exposure and this remains the single most important proven cause. There are likely some other potential etiologies that will be mentioned briefly before discussing the current opinion regarding the most important causes of mesothelioma.

First, irradiation has been associated with the development of MPM. There are reports of patients developing peritoneal mesothelioma after radiation for testicular carcinoma, cervical cancer, or administration of thorium dioxide (22, 23). Malignant pleural mesothelioma has been reported in young adults who received radiation during childhood for Wilm's tumor (24). Pleural mesothelioma in proximity to prior irradiation fields was reported in four patients treated 10 to 31 years previously (25).

The use of iatrogenic pneumothorax for treating tuberculosis has been associated with a small number of mesothelioma cases. Roviaro and colleagues reviewed 35 cases of MPM and found three had developed in the setting of calcified post-tuberculosis fibrothorax (26). The authors speculated that there might be a relationship between chronic irritation or inflammation of the pleura and the development of mesothelioma.

Organic chemicals, including polyurethane, polysilicone, ethylene oxide, N-methyl-N nitrosurethane diethylstilbestrol and mineral oil have been shown to cause mesothelioma in rodents (27). Nonasbestos fibers such as glass fibers have been linked to mesothelioma in animals, as well (28). Recent investigations have focused on the epidemic of MPM in Cappadocia, a region of Central Anatolia, Turkey. In this region characterized by natural caves and volcanic formations, there are two villages (Karain and Tuzkoy) where 50% of deaths are caused by mesothelioma (29). Early investigators looking for asbestos found it nearly everywhere in Cappodocia as a component of the volcanic terrain. However, since the remarkably high incidence of MPM was found only in the two villages, it was felt that asbestos alone could not explain this. Subsequently, a nonasbestos mineral fiber, erionite, was found particularly in these villages. Erionite was located in surrounding volcanic tuffs and was used extensively in most of the buildings and homes (30). This mineral was found in the lungs of villagers, and when injected intrapleurally into animals causes mesothelioma. It is likely that erionite is also capable of causing mesothelioma in humans.

Further investigation into the uniquely high incidence of mesothelioma within certain families of these two villages led to another observation: mesothelioma developed mostly in certain houses, called "houses of death" (30). In fact, pedigree analysis suggested that the predisposition was inherited in an autosomal dominant pattern (31). The specific gene has not been identified, however. Two genes that have been reported to affect risk in asbestos-related mesothelioma are glutathione-S-transferase M1 (GSTM1), which is important for detoxification of carcinogens, and N-acetyltransferase 2 (NAT2), which is

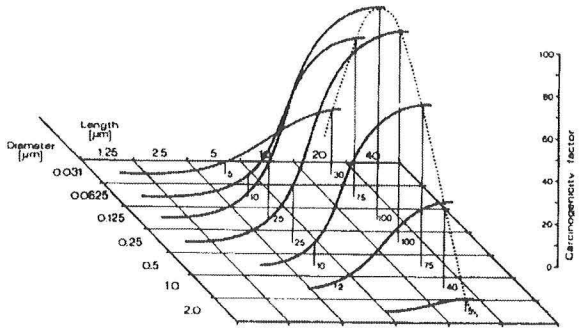
involved with biotransformation of aromatic amines. Null genotype GSTM1 and slow acetylator NAT2 each increased the risk of developing mesothelioma two-fold, and together resulted in a seven-fold increase risk compared to GSTM1+/NAT2+ individuals (32). So, it appears that a number of factors may be contributing to the rising incidence of mesothelioma, including irradiation, and exposure to chemicals, asbestos and nonasbestos fibers. Within individuals, the risk may be modified by an inherited genetic predisposition.

ASBESTOS AS A CARCINOGEN

In the latter half of the twentieth century, the history of asbestos use became the history of mesothelioma. However, before its relationship to disease was appreciated, it was regarded as safe, and it was handled like cotton or wool. Even before Wagner established the relationship between asbestos and mesothelioma, cases of fibrosis were reported in the early part of the century. They were lost among the more prevalent tuberculosis, however. Eventually, as exposure increased with growing use of asbestos, the association with pulmonary fibrosis (asbestosis) and subsequently with non-small cell lung cancer, became clear (33, 34). While tobacco smoke produces a greater increase in the incidence of squamous cell and small cell cancer, asbestos workers (most of whom were smokers) had a higher proportion of adenocarcinomas, suggesting a shift back to the pattern in nonsmokers. In addition to asbestosis and primary lung cancers, other manifestations of asbestos exposure became apparent, including pleural plaques and benign pleural effusions.

Among the fibrous mineral dusts that cause disease, asbestos is the most important. Long, thin, respirable fibers with length:diameter ratios of greater than 10 are particularly dangerous. These elongated particles are poorly cleared from the lung and are carcinogenic if they remain long enough. Asbestos is not a specific mineralogic term, but rather refers to a group of compound silicates that have crystallized as thin particles. All types result from the leaching by water of siliceous minerals and recrystallization in the interstices of the parent rock. It is mined throughout the world, including Russia, Canada, the United States, Australia, Finland, and the Mediterranean basin (34). It is ubiquitous in the environment, having been found in most drinking water, the air, and in parenteral medication (35). Based on physical shape of the fibers, asbestoses are divided into two categories: long and curly serpentines, the main one being chrysotile asbestos; and the thin rod-like amphiboles, which include crocidolite, amosite, tremolite, and anthophyllite. All are relatively inert to chemical attack and poor conductors of heat, accounting for their industrial use. Often, different fibers are admixed with each other in the raw state. Chrysotile, or "white" asbestos, accounts for 90% of the commercially used asbestos worldwide, whereas the amphiboles constitute the remaining 5-10%. Crocidolite, or "blue" asbestos, is primarily mined in South Africa and Western Australia, and is particularly acid resistant.

Serpentine and amphibole fibers have different biologic and carcinogenic properties, specifically with regard to the development of mesothelioma (36). Few cases of pleural mesothelioma have been associated with chrysotile asbestos, and these are probably related to contamination by amphiboles. The amphiboles, and particularly crocidolite, appear to be the most carcinogenic of the fibers. The primary determinant of this difference in carcinogenicity between serpentines and amphiboles is the physical dimension of the fiber. Longer fibers are believed to have greater toxicity, as do fibers within a certain range of diameters (37, 38). The length, diameter and aspect ratio most likely are determinants of persistence of fibers within the lung. Short fibers are more easily removed by lung macrophages to airways or lymphatics, while fibers in the 10μm to 20μm range are retained. Additionally, serpentine chrysotiles are more easily broken down under acidic conditions to individual fibrils, as in the phagolysosome. In contrast, the long thin durable rod-like crocidolite fiber is resistant to phagocytosis by macrophages, and can persist within the lung for years.



It is commonly thought that the genotoxicity of asbestos is in part a result of the generation of reactive oxygen species. These reactive species can be generated by cells exposed to the fibers or by the fibers themselves. Asbestos fibers, especially the iron-rich crocidolite fibers, spontaneously produce oxidants by redox reactions occurring extracellularly on the fiber surface. Surface Fe II reduces molecular oxygen to superoxide which is converted to hydrogen peroxide and, ultimately, to hydroxyl radicals. In addition, the frustrated phagocytosis of asbestos fibers by alveolar macrophages is another source of chronically generated oxidants (39). Phagocytic cells themselves produce these radicals after internalization of asbestos. Along with reactive oxygen species, reactive nitrogen species, such as peroxynitrate, have been demonstrated in the alveolar macrophages from asbestos-exposed rodent lungs (40). Both reactive oxygen and reactive nitrogen species can damage DNA. For example, the production of hydroxyl radicals in cells treated with asbestos can result in the formation of premutagenic bases such as 8-hydroxydeoxyguanosine (8-OHdG), which can induce G to T transversions. Using rats exposed to crocidolite, Unfried et al demonstrated that G to T transversions were the most common mutation type found, supporting the role of reactive oxygen species in vivo in crocidolite mutagenesis (41).

The radicals generated in these reactions may also affect cellular signaling pathways. Upregulation of transforming growth factor (TGF)-α in asbestosis suggests epidermal growth factor may be a mediator of disease since TGF-α binds EGF receptor. Increased

expression of EGFR has been documented on human mesothelial cells exposed to asbestos (42). Additionally, asbestos can cause autophosphorylation and activation of EGFR of mesothelial cells suggesting its role in autocrine and paracrine behavior (43).

A variety of chromosomal abnormalities have been described in MPM. In tissue cultures, asbestos can interfere with chromosomal segregation by interacting with the mitotic spindle leading to aneuploidy (36). *In vitro* studies have demonstrated the acquisition of numerical and structural abnormalities after exposure to asbestos (44). In a series of 20 MPMs karyotyped by Murthy et al, loss of chromosome 22 was the most common numerical change. Loss of genetic material in specific chromosomal regions was the most common overall alteration. In particular, deletions at 1p, 3p, 9p and 6q were commonly noted. These have been described in other tumors, and 9p21 region is known to be the location of two tumor suppressor genes, p16/CDK4 and $p14^{ARF}$. These tumor suppressor genes collectively affect Rb and p53 dependent pathways suggesting they may be critical targets of 9p21 deletion. Current opinion is that the biopersistance of the amphiboles implicates them as the primary cause of mesothelioma, and crocidolite is the most dangerous. Chronic production of reactive oxygen and nitrogen species, intra- and extracellularly, is tied to direct DNA damage and altered cell signal pathways, and asbestos may directly cause missegregation and structural damage to chromosomes.

SV40 AND MESOTHELIOMA

Recent interest has focused on the role of the SV40 virus as a potential etiologic agent in malignant mesothelioma. SV40 (simian vacuolating virus 40) was first identified in 1960 by Sweet and Hilleman and is included among the polyomaviruses (45). These viruses contain circular double stranded DNA, and are small, icosahedral and nonenveloped. In addition to a nontranslated region containing the origin of replication, promoter, and enhancers, the genome contains an early region expressed after viral entry into the cell, and a late region. The early region encodes three replication proteins: Tag, tag, and 17KT. The late region encodes primarily for viral capsid proteins when viral DNA replication is underway. Tag (large T antigen) is the principal early protein and is multifunctional. It binds viral DNA at specific sites, unwinds it, and along with cellular DNA polymerase and topoisomerase, allows replication (46). The susceptibility to lysis and transformation caused by SV40 depends on cell type. Hamster cells are nonpermissive in that they do not allow viral replication. SV40 DNA remains episomal and daughter cells lose the DNA after a few divisions. Rarely, the viral DNA will become integrated into the host genome, causing malignant transformation (46). Monkey cells and human epithelial cells, though, are permissive and allow many viral particles to be produced leading to cell lysis. Therefore, transformation usually does not occur. Mesothelial cells seem to be uniquely sensitive to SV40-induced transformation. While mesothelial cells are infected and allow viral replication, few viral particles are produced and cell lysis is uncommon. Viral DNA remains episomal and nearly all cells express Tag, the key viral protein implicated in malignant transformation (46). SV40 was initially identified in African green monkey kidney cells used during polio vaccine

testing, however, it had previously gone unrecognized when cultured in rhesus monkey kidney cell cultures. This led to its discovery as a contaminant of the inactivated Salk vaccine, and the live attenuated Sabin vaccine, which were both prepared from rhesus monkey cell cultures. Soon after SV40 was reported to be oncogenic in hamsters, the U.S. government required that all newly produced polio vaccine be free of SV40 (47). Previously produced vaccine was not removed from the vaccine program, however, and contaminated poliovirus vaccine was probably widely used in the U.S. from 1955 through 1963 (48). Additionally, contaminated vaccine was used in Europe, the former USSR, Mexico, Japan and Central America (46). Data from surveys conducted during the mass immunization program, which was initially directed at younger persons and pregnant women, showed that about 90% of people younger than 20 years of age had received at least one vaccination. It is estimated that in the U.S. alone, 98 million people received at least one dose of contaminated vaccine. Adenovirus vaccines may have been another source of exposure since they were contaminated, as well (49). The exact extent of exposure in the population is difficult to determine because not all batches of polio vaccine were tested, and infected batches were not evenly distributed geographically. The current prevalence of SV40 infection is unknown, but serologic evidence suggests it is less than 20% (50).

In hamster models, SV40 can cause several types of tumors and is dependent on the route of inoculation. Both intrapleural and systemic administration cause mesothelioma, and systemic administration can cause lymphoma and osteosarcoma. Intracranial inoculation leads to ependymomas and other brain tumors (46). The types of human tumors associated with SV40 are similiar to those seen in the hamster. Bergsagel et al found SV40-like DNA sequences in choroid plexus tumors and ependymomas (51). In another series, about one-third of osteosarcomas demonstrated evidence of SV40 (54). Two studies have identified SV40 sequences in non-Hodgkin's lymphomas and with similar incidences, about 43% of cases studied, while other investigators found it far less commonly (53, 54, 55). The incidence of SV40 among the most common cancers (colon, lung, breast, prostate) is said to be low although one group found Tag sequences in 29% of bronchogenic carcinomas (53, 56). Noting that SV40 caused mesothelioma when inoculated intrapleurally in hamsters, Carbone et al looked for evidence in human mesothelioma and found it in 29 of 48 tumors (57). The discovery of SV40 in 40% - 50% of mesotheliomas has since been documented in numerous reports, including a multi-institutional review confirming these findings (58). Interestingly, geographic variation has been noted (59, 60, 61). Evidence of SV40 has not been found in cases of mesothelioma from Turkey or Finland, and this may be tied to the distribution of contaminated polio vaccine.

THE ROLE OF SV40 IN CARCINOGENESIS

Evidence suggests that the oncogenic activity of SV40 is at least in part due to the interaction of the large T antigen (Tag) and the tumor suppressor gene products pRb and p53. The RbI gene encodes the nuclear phosphoprotein pRb, and is mutated in many

tumors. pRb regulates progression through G1 cell cycle phase. It is phosphorylated in late G1-S and G2-M, but hypophosphorylated in GO-1. The hypophosphorylated state is associated with inhibitory activity while the phosphorylated form promotes proliferation. This regulation is due to the binding of pRb to members of the E2F transcription factor family. Hypophosphorylated pRb preferentially binds E2F, thereby sequestering it and repressing its transcription function. In the phosphorylated form, its affinity for E2F decreases, releasing E2F to activate gene transcription for cyclins and other genes (62). The Rb proteins, including pRb, p107 and p130, undergo cell cycle dependent hyperphosphorylation and this is mediated by cyclin dependent kinases. The cdk inhibitors $p21^{WAF1/C1P1}$ and $p16^{INK4A}$ inhibit the cdk/cyclin D interaction, thus blocking the phosphorylation of pRb. SV40 Tag preferentially binds to hypophosphorylated pRb allowing E2F-initiated cell cycle progression to proceed unchecked. Additionally, the loss of 9p21-22 has been described in mesothelioma (63). The tumor suppressor gene $p16^{INK4A}$ is located in this region, and as a cdk inhibitor, also suppresses the phosphorylation of pRb.

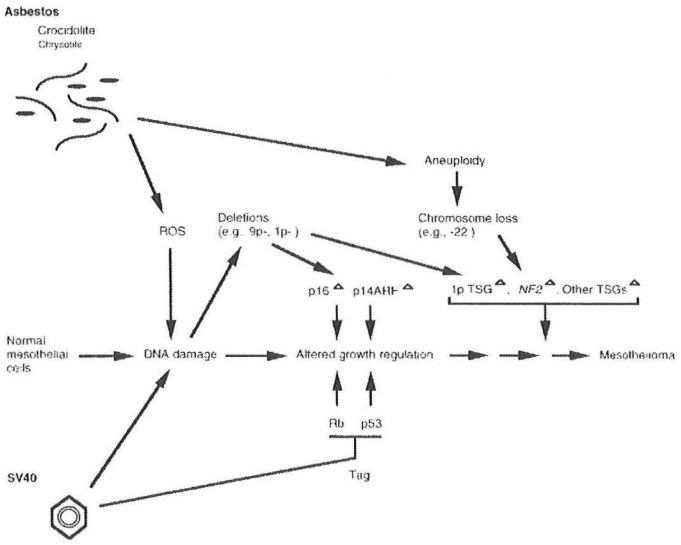
Acting in concert with the pRb pathway is p53. As a negative regulator of cell proliferation, p53 responds to stressors that cause genotoxicity, including DNA damage, hypoxia, or the presence of an activated oncogene (64). In the normal resting cell, the level of p53 is low and the pathway inactive due to degradation. In the face of cellular stress such as DNA damage, p53 is activated to become a transcription factor. This activation leads to cell cycle arrest and either repair or apoptosis. The p53-mediated arrest of proliferation that operates in the G1/S phase occurs through transcriptional activation of genes encoding $p21^{WAF1/C1PI}$ and other factors. The p21 gene in particular produces a protein that inhibits cyclin dependent kinases, which phosphorylate pRb, p107, and p130 (62). So in response to increased p53, mediated by p21 expression, pRb remains hypophosphorylated and bound to E2F, diminishing E2F-mediated transcription of genes promoting proliferation. Control of p53 is regulated by degradation mediated by MDM2 protein, which is increased by p53 in an autoregulatory fashion. Conversely, $p14^{ARF}$ binds MDM2 and prevents p53 degradation. The gene encoding $p14^{ARF}$ is located at the same locus as p16. Homozygous loss of 9p21-22 could then collectively affect both p53 and pRb pathways. The action of SV40 Tag is to bind p53, inactivating it and preventing it from acting as a transcription factor for genes that are critical for cell cycle arrest and apoptosis (64).

Tag has other actions as well. The angiogenic vascular endothelial growth factor (VEGF) is expressed by and stimulates growth of mesothelioma cells. Catalano et al showed that Tag expression increased VEGF (65). The disruption of the Tag/p53 complex by the drug menadione leads to a decline in VEGF levels suggesting Tag in part mediates the increase in VEGF. Finally, Tag has been shown to induce telomerase activity in normal mesothelial cells, which may set the stage for transformation when other SV40-induced alterations have occurred (66).

Other growth factors, oncogenes, tumor suppressor genes and signaling pathways are likely involved. Preliminary evidence, however, suggests a model in which the central

action of Tag on the Rb family is to release cells from growth control and drive them into cycle. In response, the p53 pathway is activated and Tag then acts to directly inhibit the growth-arresting effect of p53. The result is the simultaneous inactivation of p53 and pRb to induce transformation.

Ultimately, the development of mesothelioma may result from an interaction between asbestos and SV40 virus. Mayall et al examined eleven mesotheliomas, seven of which revealed fiber evidence of asbestos exposure. Of these, five were positive for SV40 sequences. No SV40 was found in the mesotheliomas that had no asbestos exposure (67). A current model suggests cooperation between asbestos and SV40. In addition to promoting missegregation of chromosomes and inducing chronic production of DNA-damaging reactive oxygen species, asbestos also activates EGFR which ultimately induces the transcription factor AP-1 leading to mitosis and apoptosis. Mitosis may be favored in the setting of SV40 infection owing to the interaction between Tag and p53 and Rb family proteins, allowing more time for acquisition of further DNA damage (29, 46). Asbestos may also suppress the immune system allowing abnormal cells to evade surveillance.



CLINICAL PRESENTATION

The median age at presentation is about 60 years, reflecting the long latency period. The most common presenting symptoms and signs are those associated with pleural effusion. The median time to diagnosis is about 3 months, but up to 25% will have symptoms for more than 6 months before seeking medical attention. Typically, patients present with gradual onset of nonpleuritic chest pain, which is often located in the lower posterolateral thorax (68). About 40% of patients have dyspnea, but only about 10% have an associated cough. Rarely, patients may present with acute onset of dyspnea and chest pain and this

is usually associated with spontaneous pneumothorax. About 30% of patients will have fever, weight loss, and fatigue (69, 70, 71).

Symptoms	Examination
Chest pain	Adenopathy
Dyspnea	Hepatomegaly
Fever	Clubbing
Night sweats	↓ Breath sounds
Weight loss	Dullness
Cough	Scoliosis
	Chest wall mass

The pleural effusions gradually accumulate and the pleura thickens to form a rind that encases the lung. The tumor infiltrates the fissures and fixes the lung to the diaphragm and intercostal muscles. Progressive local invasion can lead to dysphagia, chest pain, cord compression, plexopathy, Horner's syndrome or SVC syndrome. Pericardial involvement can be associated with EKG changes, arrhythmias and conduction disturbances. Penetration through the diaphragm allows tumor to spread easily throughout the peritoneal cavity. In addition to nodal spread, hematogenous metastases to adrenals, spine, brain, and liver may occur.

Examination usually reveals decreased or absent breath sounds associated with an effusion. Percussion is muted and fremitus is decreased. Occasionally, adenopathy, hepatomegaly or clubbing may be seen. With more advanced disease, scoliosis of the affected hemithorax may occur. Subcutaneous nodules are seen in association with needle tracks from thoracentesis or at thoracotomy scars. Disease may progress to the contralateral pleural space with expected physical findings, or to peritoneum leading to ascites and abdominal distension (69).

At presentation, chemistries and complete blood count may be unremarkable (70). Thrombocytosis and leukocytosis are common, as is an elevated erythrocyte sedimentation rate. Evidence of disseminated intravascular coagulation and Coomb's positive anemia has been described. Other nonspecific laboratory abnormalities may include hypergammaglobulinemia and eosinophilia. There are no specific serum markers for mesothelioma (68). Examination of the pleural fluid may reveal that it is viscous due to the large amounts of hyaluronic acid. High levels of hyaluronate are found more commonly in mesothelioma than in any other process.

Laboratory	Pleural Fluid
Thrombocytosis	glucose <50 mg/dl
Leukocytosis	protein >3.4 g/dl
Coombs (+)	pH < 7.20
DIC	LDH/serum > 0.6
Elevated ESR	cellular

About half the fluids are bloody and most are exudative with protein concentration greater than 3.4g/dl, and elevated LDH ratio > 0.6 (70, 72). In about one-third of patients, the pleural fluid glucose is less than 50 mg/dl and the fluid pH below 7.20 (73). The fluid is typically cellular containing a mixture of normal mesothelial cells, malignant mesothelial cells, lymphocytes and polymorphonuclear leukocytes (74). Cytologic findings are seldom adequate for making a diagnosis, however. In one series, malignant cells were found in the pleural fluid of only 32% of patients, and the diagnosis of mesothelioma, based on cytology alone, could be made in only 10% (75). And while repeated CT-guided procedures increase the diagnostic yield of closed pleural biopsies, seeding of the needle tracks is known to occur, resulting in development of chest wall masses. When pleural mesothelioma is suspected, it is appropriate to proceed directly to video-assisted thoracoscopy (VATS). VATS can effectively drain effusions and provide adequate tissue for histology and immunohistochemical staining (76).

RADIOLOGIC EVALUATION

Plain chest films are usually the first diagnostic test performed in patients presenting with symptoms of MPM. Roentgenograms show evidence of asbestos exposure such as plaques or calcifications in the diaphragm in only about 20% of cases, however (69). The typical finding on plain film is a unilateral pleural effusion. In about half of cases, it presents as a lobular pleural mass invading the pleural space and fissures (12). The mediastinum may be shifted and lateral films may show nodular abnormalities or fluid in the major fissure. Most will show diffuse pleural thickening rather than a discreet solitary pleural mass. Metastatic parenchymal nodules are common at autopsy, but less commonly described on plain chest films (77). Mediastinal widening and enlargement of the cardiac silhouette suggests pericardial invasion, and bone destruction is indicative of chest wall invasion (78).

Computed tomography has become important in the evaluation of MPM. In addition to showing evidence of asbestos exposure such as pleural plaques, the majority of patients will demonstrate effusion and nodular pleural thickening, often predominant in the lung base (71, 78). The major fissure may be markedly thickened from fibrosis, tumor, and fluid, and the volume of the hemithorax is frequently decreased. CT can demonstrate intrapulmonary nodules, as well as invasion of chest wall. CT scan may also reveal mediastinal invasion, and it remains the most common modality for evaluating mediastinal adenopathy. Finally, CT scanning is useful in revealing distant metastatic disease. Although distant hematogenous dissemination to remote viscera was once thought uncommon, autopsy experience refutes this (79). From one-third to one-half of patients may have distant metastases discovered at post-mortem examination, including opposite lung, liver, kidney, adrenal, and bone (80).

In patients with potentially resectable disease, MRI has been recommended to assist in local staging. Heelan et al compared MRI and CT images in patients with MPM. They

found the two modalities to be nearly equivalent in terms of diagnostic accuracy, including N1 and N2 nodes. MRI was more accurate, however, at evaluating diaphragm invasion (CT-55% vs. MR-82%), and endothoracic fascia (CT-46% vs. MR-69%). The current opinion is that in potentially resectable pleural mesothelioma, MRI may provide additional information in terms of assessing foci of invasion (81).

Recently, positron emission tomography (PET) has been used to evaluate MPM. Using ^{18}F fluorodeoxyglucose (FDG), PET takes advantage of tumor cells increased glucose metabolism to identify malignant lesions. PET was recently compared to CT scan in 28 patients with mesothelioma. FDG uptake was higher in mesothelioma than benign pleural disease, and was superior at identifying mediastinal nodal involvement (84). PET will likely be most useful for staging in the preoperative setting.

HISTOLOGIC EVALUATION

An older male with a remote history of asbestos exposure presenting with dyspnea and a pleural effusion typifies the picture of mesothelioma. A firm diagnosis, however, rests heavily on the histologic interpretation of the pathologist. Adequate tissue is required for histologic evaluation, but fine needle aspirates and core biopsies frequently fail to obtain sufficient material (69). Two series have found needle biopsy results to be diagnostic in only 25% and 39%, respectively (12, 82). Repeated needle biopsy attempts lead to tumor developing in the needle track in up to 20% of cases (83). Currently, VATS is the procedure of choice for obtaining adequate tissue, and is as effective as open thoracotomy but less invasive (76).

In addition to the challenge of procuring adequate biopsy material, difficulty is encountered at the level of histologic evaluation because MPM demonstrates a variety of cytoarchitectural appearances. Tumors may appear epithelial or mesenchymal with a range of intermediate forms, and vary from well differentiated to anaplastic. The three main categories based on morphology are monophasic epithelioid mesothelioma, sarcomatous/fibroblastic mesothelioma, and the biphasic mesothelioma, which contains both epithelial and fibroblastic appearing elements. The frequency varies depending on the series, but generally the epithelial type is most common (50% - 70%), followed by biphasic (20% - 25%) and sarcomatous (7% - 20%) (68, 79). The primary challenges encountered in the cytologic diagnosis of mesothelioma are distinguishing reactive mesothelial cells from mesothelioma, and differentiating mesothelioma from carcinoma. Nuclear pleomorphism, macronuclei, and cell-in-cell engulfment favor malignant over reactive mesothelia. Multinucleation, cell-to-cell opposition, and absence of acinus-like structures and balloon vacuolation help distinguish mesothelioma from adenocarcinoma. Since these assessments are frequently inconclusive, ancillary testing is commonly employed.

Histochemistry helps distinguish between the acid mucins (mainly hyaluronic acid) of epithelial mesotheliomas, and the neutral mucins in adenocarcinomas. Mucicarmine and

diastase–PAS stain neutral mucins and therefore are positive in most adenocarcinomas. (85). In constrast, Alcian blue and colloidal iron stain acid mucins which are present in both MPM and adenocarcinomas. Hyaluronic acid, however, is the primary acid mucin in mesothelioma. Preincubation with hyaluronidase digests hyaluronic acid and subsequently leads to an absence of staining in about half of mesotheliomas.

Immunohistochemical techniques also may aid in the diagnosis. Among the targets of immunoreactivity are intermediate filaments. This includes keratins, which are present in most epithelial cells and mesotheliomas. Antibodies to specific cytokeratins such as CK5 help distinguish epithelial mesothelioma from adenocarcinoma. Coexpression with vimentin is another feature that may aid in this distinction (86, 87).

Glycoproteins that are generally positive in adenocarcinomas but not mesothelioma include CEA, Leu-M1 and Ber-EP4 (85, 88, 89, 90). Most recently, thyroid transcription factor-1 (TTF-1) has been used effectively to distinguish between metastatic and primary pulmonary adenocarcinomas. It also consistently has distinguished between pulmonary adenocarcinoma and mesothelioma (91). Finally, electron microscopy reveals epithelial mesotheliomas to possess numerous long branching microvilli and tonofilaments, in contrast to the short blunt microvilli in adenocarcinomas (85).

STAGING

In addition to small numbers of patients, the lack of an accurate and uniformly accepted staging system has impeded implementation of prospective clinical trials. The earliest staging system was introduced by Butchart et al (92). This remains a popular system, but is based on only 29 patients and does not stratify survival based on stage. The lack of uniformity and number of staging systems led the International Mesothelioma Interest Group (IMIG) to hold a consensus meeting in June 1994, resulting in a new international TNM staging system for MPM (93). The 'T' stage description is designed to explicitly describe the extent of disease within the affected hemithorax. Nodal and metastatic stagings are the same as for bronchogenic lung cancer, except that N3 is considered stage IV.

The accurate staging for all solid tumors is critical for several reasons. First, it provides a universally understood system for description of extent of disease. Secondly, subgroups of patients with similar prognosis can be identified based on extent of disease. Finally, accurate staging distinguishes subsets of patients who may be appropriate candidates for specific therapies. In the case of MPM, the IMIG staging system clearly emphasizes local (T) staging, which is optimally determined by invasive procedures such as thoracoscopy. A major goal of this staging system is to identify the small group of patients who may be candidates for resection. The validation of this most recent staging system will probably require several years of application in prospective trials.

PROGNOSIS

The determination of prognostic factors is useful in oncology because it allows the physician to give his/her patients a prognosis which may help them make plans for the future. Additionally, it may assist in selecting patients for specific treatments or trials, and it complements staging as a descriptor with respect to a uniform system of communication. Three recent chemotherapy trials have reported median survivals of 6 months, 9.5 months, and 10.6 months (94, 95, 111). Occasionally, patients may survive for a number of years, but generally most series show that median survival is between 4 and 18 months (96). A variety of prognostic factors have been evaluated including histology, gender, age, weight loss, chest pain, and performance status, among others. Two studies have specifically addressed this issue. The first from the Cancer and Leukemia Group B (CALGB), reviewed 337 patients treated over ten years on various treatment regimens. Median survival for all patients was 7 months. Predictors of poorer outcome included age greater than 49 years, nonepithelial histology, chest pain, weight loss, low hemoglobin, thrombocytosis, leukocytosis, elevated LDH, and poor performance status (97). A second study from the European Organization for Research and Treatment of Cancer (EORTC) examined data on 204 patients with MPM treated on trials over a 10-year period. Median overall survival was 8.4 months. Similar predictors of worse outcome were poor performance status, leukocytosis, sarcomatous histology, and male gender (98). It was concluded that these features along with anemia and thrombocytosis were the most important negative prognostic features (96).

Poor Prognostic Features
Male gender
Age >60
Sarcomatous/mixed histology
WBC > 15K
Platelets > 400K
Hemoglobin < 14
ECOG > 0

A more recent publication mirrored these findings. Ceresoli et al reviewed 121 cases of MPM seen between 1986 and 1999. Median overall survival was 10.5 months. Lower overall survivals were associated with poor performance status, nonepithelial histology and stage > 1. Interestingly, patients treated with any therapy had longer survivals than patients treated with supportive care only, but only among these with nonsarcomatous histology (99).

TREATMENT

Although malignant mesothelioma is very uncommon in comparison to bronchogenic carcinoma, its incidence is believed to be increasing. Because of the difficulty in diagnosing it, especially at an early stage, and because of its poor median survival, the approach to treatment has often been nihilistic. In general, three modalities are available for the treatment of solid tumors: irradiation, surgery, and chemotherapy. All of these modalities have been employed in the treatment of MPM, and the role of each will be reviewed.

RADIOTHERAPY

As a single modality, radiotherapy is probably the least effective. Data on its use as a single modality are minimal; more often it is used in combined modality regimens. The difficulty with using radiation is primarily related to the toxicity associated with the field required for administration. Unlike other thoracic tumors, mesothelioma is a diffuse process requiring a much larger field. Because the entire ipsilateral pleural surface is at risk, the entire ipsilateral hemithorax must be treated. Dose-limiting thoracic structures include spinal cord (45 Gy), heart (45 Gy), lung (20 Gy) and esophagus (45-50 Gy) (100). In one review, only 1 of 23 patients receiving radiation for mesothelioma achieved palliation if the dose was lower than 40 Gy. Most cases required more than 50 Gy. The complications associated with radiation for mesothelioma include nausea and vomiting, radiation hepatitis, esophagitis, myelitis, myocarditis and pneumonitis with further deterioration of lung function. As a single modality, it may occasionally be useful for palliating symptoms or controlling effusions, but it is generally felt to have little impact on disease control or survival (101).

Radiotherapy may be more effective when used in the adjuvant setting to reduce local recurrence after surgery. Rusch et al delivered 54 Gy postoperatively to 54 patients undergoing extrapleural pneumonectomy. Among 37 patients with recurrent disease, only 7 were local or locoregional (102). Finally, prophylactic radiation has been shown to effectively reduce tumor seeding at sites of thoracentesis, incisions, and thoracoscopy. Boutin et al randomized 40 patients to receive either 21 Gy after VATS, or to no radiation. Those receiving radiation experienced no metastases at their incisions, while 40% of patients in the nonirradiated arm developed tumor seeding at the incision site (103).

SURGERY

The indications for surgical intervention are to obtain a diagnosis, for resection of early stage disease, and for palliation of late stage disease. Generally, three surgical procedures have been employed for the treatment or palliation of MPM: pleurodesis, pleurectomy/decortication (P/D), and extrapleural pneumonectomy (EPP). There are no randomized studies comparing these procedures.

Pleurodesis by thoracotomy or thoracoscopy is performed for palliation without cytoreduction. Loculations can be disrupted and the effusion can be drained. Historically, sclerosing agents have included tetracycline, doxycycline, bleomycin, and talc. This procedure is most appropriate for patients with comorbid conditions that preclude more aggressive surgery, or who may be more advanced and clearly unresectable. Moreover, advocates of pleurodesis as a primary surgical procedure argue that more aggressive procedures such as P/D or EPP, as single modalities, have not provided any improvement in median survival (100).

The cytoreductive procedures are pleurectomy/decortication (P/D) and extrapleural pneumonectomy (EPP). Both may be performed with curative intent, although most often gross tumor is left behind with P/D. There is debate regarding which procedure is most appropriate. P/D consists of removal of all grossly involved parietal and visceral pleura, and may include parts of diaphragm and pericardium, as well. It is less technically demanding and physiologically more tolerable for patients since it does not remove the lung (100, 104). As a form of complete resection, it is feasible only in patients with very early stage disease. It generally can be done with very low mortality (<5%) and is very effective at controlling pleural effusions, but probably has little impact on survival (100, 68). Since mesothelioma frequently invades tissues, complete resection is difficult with P/D and local recurrence rates are high. Moreover, postoperative radiation is limited by the presence of the lung.

Extrapleural pneumonectomy is a more aggressive procedure that involves the en bloc resection of the parietal and visceral pleura with the enclosed lung, pericardium, diaphragm, and mediastinal lymph nodes. EPP is a more effective cytoreductive procedure than P/D and allows for higher doses of postoperative radiation. However, it requires a higher degree of technical skill, and the patient must have better cardiac and pulmonary reserve. Potential complications include bronchial leaks, empyema, vocal cord paralysis, chylothorax, and patch failure. Not surprisingly, the mortality rate is high, almost 30% in the original series from Butchart et al (92). A more recent series, though, documented a mortality rate of about 4% in 183 patients (105). There has been no randomized study comparing P/D with EPP as single modality therapies, and there are no specific criteria for selecting between these two surgeries. Generally, the skill and philosophy of the surgeon, the bulk and local extent of disease, and the performance status of the patient are the most important factors affecting the choice of procedure.

In an effort to improve local control, other modalities have been combined with surgery. Rusch et al followed extrapleural pneumonectomy with 54 Gy of radiation to the involved hemithorax. Among patients with stage I and II disease (22%), the median survival was 34 months, but only 10 months for those with stage III and IV. This appeared to shift recurrences in early stage patients away from locoregional, but the majority of patients with more advanced disease experienced no improvement in median survival (102). P/D followed by radiation has also been examined. In a report from Mychalasak et al, 41 patients were treated perioperatively with either iodine 125 or iridium 192 brachytherapy, and four to six weeks post-operatively, received 45 Gy of external beam radiation. When

an update of this series was reported, the median survival was 12.6 months, not significantly different from other therapies (106). In the setting of P/D, postoperative radiation is limited by the remaining lung. Chemotherapy has been investigated as an alternative to radiation after P/D. Rusch et al treated 28 patients with intrapleural cisplatin and mitomycin after P/D, and subsequently 23 received systemic chemotherapy with the same drugs. The median survival for those receiving all therapy was 17 months. Unfortunately, two patients developed renal failure after intrapleural chemotherapy and 80% of relapses were still local (107). The role of intrapleural therapy is limited to early stage disease because the pleural space disappears progressively with the advancing rind of tumor. Moreover, the intracavitary approach is limited by the shallow penetration of chemotherapy. Cisplatin is probably the best-studied drug in this respect. While studies have shown higher peak levels are achieved with intracavitary than with intravenous cisplatin, intrapleural administration has not been very successful (101).

Another local treatment strategy is intrapleural photodynamic therapy (PDT). This involves light-activated sensitization of malignant cells after selective uptake of a hematoporphyrin derivative. This sensitizer is then activated by 630 nm light, which then interacts with molecular oxygen to produce reactive oxygen species. In a study from the National Cancer Institute, patients were randomized after surgery to receive PDT or not, followed by post-operative chemotherapy. While the study showed this therapy could be safely delivered, there were no differences in median survival or recurrence pattern (108).

Finally, among the most aggressive therapies is the trimodality approach favored at Brigham and Women's Hospital. In their program, extrapleural pneumonectomy is the preferred surgical procedure because it provides maximum cytoreduction. Patients are carefully selected based on meticulous staging to establish resectability, and preoperative assessment of systemic comorbidities. After surgery, patients receive chemotherapy for several cycles, and subsequently are treated with radiation given concurrently with chemotherapy. The best results are achieved in patients with stage I disease, epithelial histology, and negative lymph nodes, with a median survival of 51 months. Unfortunately, no patients with sarcomatous histology or positive mediastinal lymph nodes survived 5 years (105).

CHEMOTHERAPY

The role of systemic chemotherapy in MPM is an area of active investigation. Over the years, numerous drugs have been tested, but most have led to response rates of less than 20%. Since MPM is an uncommon disease, the trials have typically been small and therefore lacking in statistical significance. Moreover, trials performed prior to the routine use of CT scanning were compromised with respect to the accurate determination of response. Similarly, the lack of a universal staging system has hindered interpretation of data regarding the impact of chemotherapy. Most studies of chemotherapeutic agents in MPM have begun with single agents in order to determine if there is activity. A variety

of drug classes have been investigated and include anthracyclines, alkylating agents, plant alkaloids, platinum analogs, antimetabolites and other newer agents.

Historically, anthracyclines have been considered to have activity in mesothelioma. Doxorubicin is the most commonly used anthracycline and in a number of studies, has generally achieved response rates of 10% - 20% (109). Among other anthracyclines, detorubicin had the highest response rate at 26%, however no further reports of the drug have been published since 1985 (110). Other related DNA intercalating agents, including mitoxantrone, amsacrine, and diaziquone have demonstrated no significant activity in mesothelioma (109).

The alkylating agents, including cyclophosphamide and ifosfamide, have been studied. In reviewing a number of studies, cyclophosphamide was described as having a composite activity of 13%, however in trials with adequate numbers of patients, there were no responses (101). Ifosfamide has been studied in various doses and schedules with a broad range of responses. The composite response rate, however, is very similar to cyclophosphamide at 12% (109).

The plant-derived agents include the vinca alkaloids vindesine, vincristine, and vinblastine. None of these drugs have shown significant activity in mesothelioma. An exception may be the newest vinca alkaloid, vinorelbine. In a phase II trial, vinorelbine given weekly led to an overall response rate of 24% and median survival of 10.6 months (111). The podophyllotoxin, etoposide, has been given orally and intravenously and yielded few responses (101).

The platinum analogs, along with the anthracyclines, have generally been considered to have some modest activity in MPM. The two commonly used agents, cisplatin and carboplatin, have both been tested. Using standard doses and schedules for cisplatin, the response rates have usually been less than 20% (110). Using a weekly administration schedule, however, Planting et al documented a 36% response suggesting there may be a dose response relationship (112). The experience with carboplatin has generally been similar with response rates falling below 20% (110).

Among the classes of drugs that have been suggested to have some activity, along with anthracyclines and platinum analogs, are the antimetabolites. This view was supported by a trial from Solheim et al in which methotrexate given at a dose of 3 grams/m^2 every 10 days to 63 patients with MPM yielded an overall response rate of 37%. Less impressive was the median survival of 11 months and the significant toxicity (113). Two other antifolates have been evaluated in mesothelioma also. Trimetrexate has been investigated by the CALGB and yielded an unimpressive response rate and median survival of 12% and 9 months, respectively (114). The same group studied edatrexate and achieved a response rate of 25%. However, when leucovorin was added to ameliorate toxicity, the response rate fell to 16% (115).

Several newer agents, some of which are active in bronchogenic carcinoma and other solid tumors, have been studied in mesothelioma, as well. Among the newer plant alkaloids and natural products are the taxanes, paclitaxel and docetaxel. Neither of these drugs appears to have significant activity (110). The camptothecins are topoisomerase I inhibitors and are active in a variety of tumors. However, neither topotecan nor irinotecan, the two commonly used members of this drug class, have been active in mesothelioma. The nucleoside analog gemcitabine is another antimetabolite drug that has been tested in MPM. As a single agent, it had a response rate of 7% and a median survival of 8 months (116). Temozolomide is a new alkylating agent that has demonstrated activity in primary brain tumors and metastatic malignant melanoma. In a study including 27 patients, the response rate was only 4% and it was concluded to be inactive in MPM (117). A unique antitumor agent, ranpirnase, was assessed recently in patients with MPM and led to a response rate of 5%, with 43% of patients having stabilization of disease. The authors noted that the stable and responding group had a prolonged median survival of 18 months (118).

COMBINATION CHEMOTHERAPY

It is commonly observed among many types of tumors that combination chemotherapy is more active than single agent therapy. Not surprisingly, this is true with regard to MPM. Overall, the response rates using combinations of the previously described drugs provide responses that more often range from 20% - 30%, and occasionally a higher response is reported. Unfortunately, a commensurate improvement in median survival cannot be demonstrated. Most combinations have been based on an anthracycline such as doxorubicin, plus an alkylator or platinum, or cisplatin plus an antimetabolite or plant alkaloid. The most recently published among these and notable for its response rate is cisplatin/gemcitabine. Byrne et al achieved a response rate of 48% with this combination (95). Unfortunately, the impressive response was not reproduced by other investigators, and was not matched by an improvement in median survival.

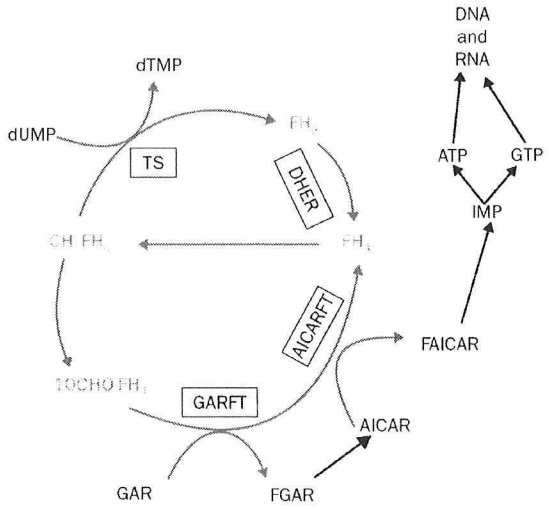
FOCUS ON ANTIFOLATES

Clinicians have often regarded malignant mesothelioma as a chemoresistant neoplasm. Among the few agents described above with modest activity was the antimetabolite, methotrexate, and renewed interest in the treatment of mesothelioma has fueled studies of several other antimetabolites. Because of the presence of pleural effusions and the frequent older age of this patient population, high-dose methotrexate with its associated toxicities is less appealing.

Several other antimetabolites have been evaluated. The few trials employing 5-fluorouracil, a thymidylate synthase inhibitor, do not allow for any conclusion regarding efficacy. Capecitabine, like 5-fluorouracil, is a fluoropyrimidine that is currently approved for use in colon and breast cancer. A multicenter trial from CALGB testing this

orally active agent has been completed but has yet to be reported (119). The other antifolates, trimetrexate and edatrexate, were described above (114,115). Trimetrexate yielded a response rate of only 12%, and when leucovorin was added to edatrexate to attenuate its toxicity, the response rate fell from 25% to 16%.

Two new antifolates have recently demonstrated activity in MPM. Raltitrexed is an antifolate thymidylate synthase inhibitor approved in Europe for the treatment of metastatic colon cancer (119). In the United States, a second antifolate has emerged as the most important new chemotherapy agent for the treatment of mesothelioma in over a decade. Pemetrexed (Alimta, Eli Lilly) is a recently developed novel antifolate that has shown promising activity in mesothelioma, alone and in combination with platinum compounds.



Generally, antifolates act by interfering with the binding of natural folate cofactors to biosynthetic enzymes. Fully reduced natural folates from the diet carry one-carbon compounds needed for the *de novo* synthesis of purines and pyrimidines. The folate requiring enzymes are 1) thymidylate synthase (TS), 2) glycinamide ribonucleotide formyl transferase (GARFT) and aminoimidazol carboxamide ribonucleotide formyl transferase (AICARFT), and 3) dihydrofolate reductase (DHFR). TS, GARFT and AICARFT are important in generating nucleotides for DNA and RNA synthesis. DHFR functions to regenerate tetrahydrofolate. Inhibiting any of the enzymes interferes with the synthesis of nucleotides, which are needed by dividing cancer cells. Most antifolates inhibit just one of these enzymes: methotrexate is a DHFR inhibitor, and 5-FU is primarily an inhibitor of TS. Raltitrexed is also a TS inhibitor. In a recent trial from Fizazi et al, the combination of raltitrexed and oxaliplatin induced responses in 25% of patients (120). Pemetrexed is unique in that while it is primarily an inhibitor of TS, it is also active against DHFR, GARFT and AICARFT (121). This multitargeted antifolate thus inhibits several steps in the folate cycle. Additionally, pemetrexed is a substrate for folylpolyglutamate synthase. Intracellular polyglutamation of pemetrexed leads to increased retention within the cell and prolonged cytotoxic effect (122). In phase I trials, pemetrexed was given as a single agent in various schedules. In all of these trials, the

main dose-limiting toxicity has been neutropenia. Other side effects include reversible renal and hepatic dysfunction, asthenia, rash, and mucositis. Interestingly, studies on mice indicated that diets deficient in folate were associated with more toxicity and lower antitumor activity (123). This observation was extended to human data. Niyikiza et al noted an association between worse myelosuppression and homocysteine levels, as a marker of folate deficiency, in patients treated with pemetrexed (124). When used a single agent in 62 previously untreated patients with MPM, 14% had a partial response and 55% had stable disease when treated on an every-three-week schedule (125). When combined with cisplatin in a phase I study, the response rate among mesothelioma patients was 45% (126). Similar results were reported by Calvert et al in a phase I trial combining carboplatin and pemetrexed with a preliminary response rate of 50% (127). These results led to the largest randomized study ever performed in patients with malignant pleural mesothelioma. In this international phase III single blind trial, 456 patients were randomized to either pemetrexed + cisplatin, or to placebo + cisplatin. Preliminary results were presented in the plenary session meeting of the American Society of Clinical Oncology May 2002. Vogelzang et al reported a response rate of 41% in the combination arm vs. 17% response in patients receiving cisplatin alone. Survival was improved by almost 3 months (128). Notably, the protocol was revised when severe toxicity (and two deaths) was observed on the treatment arm. Subsequently, folic acid and B12 were given to the study participants resulting in a significant diminution in toxicity. In a single arm study, Shin et al demonstrated the importance of folic acid and B12 supplementation. Of 64 patients with MPM enrolled in their study with every-3-week pemetrexed, the first 21 did not receive folic acid and B12 supplementation. The 43 patients who were supplemented had less neutropenia, tolerated more chemotherapy and had higher response rates (129). Ongoing randomized trials that will further define the efficacy of these new antifolates include a 240 patient trial comparing pemetrexed with best supportive care in pretreated patients, and a trial comparing raltitrexed + cisplatin to cisplatin alone. With the approval of pemetrexed for treatment of mesothelioma anticipated, it is expected to become the standard treatment for unresectable disease.

NEW DIRECTIONS IN THERAPY

Vascular endothelial growth factor (VEGF) is an autocrine growth factor in a variety of solid tumors, and patients with mesothelioma appear to have higher VEGF levels than other tumors (130). Vascular endothelial growth factor therefore represents an attractive target for therapy of MPM. SU5416 (Semaxanib, Sugen) is a selective inhibitor of the VEGF receptor tyrosine kinase, flk-1. Preliminary analysis of a study from the University of Chicago suggests this drug has antitumor activity (131). Also targeting VEGF is bevacizumab (Avastin, Genentech), a recombinant humanized anti-VEGF monoclonal antibody which has demonstrated activity in non-small cell lung cancer and appears to increase response rates when used conjunction with chemotherapy (132). There is currently a randomized trial underway that is comparing a combination with known activity in mesothelioma, cisplatin/gemcitabine, with or without bevacizumab. Plasma VEGF levels will be correlated with response (133).

Epidermal growth factor receptor has been shown to be over expressed in mesothelioma, primarily in the epithelial and biphasic histologies (134). ZD1839 (Iressa, Astra Zeneca) is an oral selective EGFR tyrosine kinase inhibitor. The CALGB has recently completed a trial in 40 previously untreated patients with time-to-progression as the primary endpoint. A final report is anticipated soon.

Platelet derived growth factor appears to be another autocrine growth factor for mesothelioma cells (135). Normal mesothelial cells express PDGF-α receptors but few β receptors. Mesothelioma cells, however, express primarily PDGF-β receptors and express increased levels of PDGF-β as well. The location of the gene encoding for the β chain of the PDGF receptor is at 22q13, and abnormalities at this site are one of the more frequently noted in mesothelioma. Imitanib (Gleevec, Novartis) is an oral selective inhibitor of the tyrosine kinase associated with BCR-abl, c-kit, and PDGF. This represents another intriguing target for therapy and trials testing Gleevec in patients with mesothelioma are planned. Other proposed trials include using suicide gene therapies and anti-sense therapy directed at Tag. In the meantime efforts will continue to be directed at understanding the role of SV40 in mesothelioma and in other tumors, and optimizing treatment for the majority of patients who present with advanced disease.

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